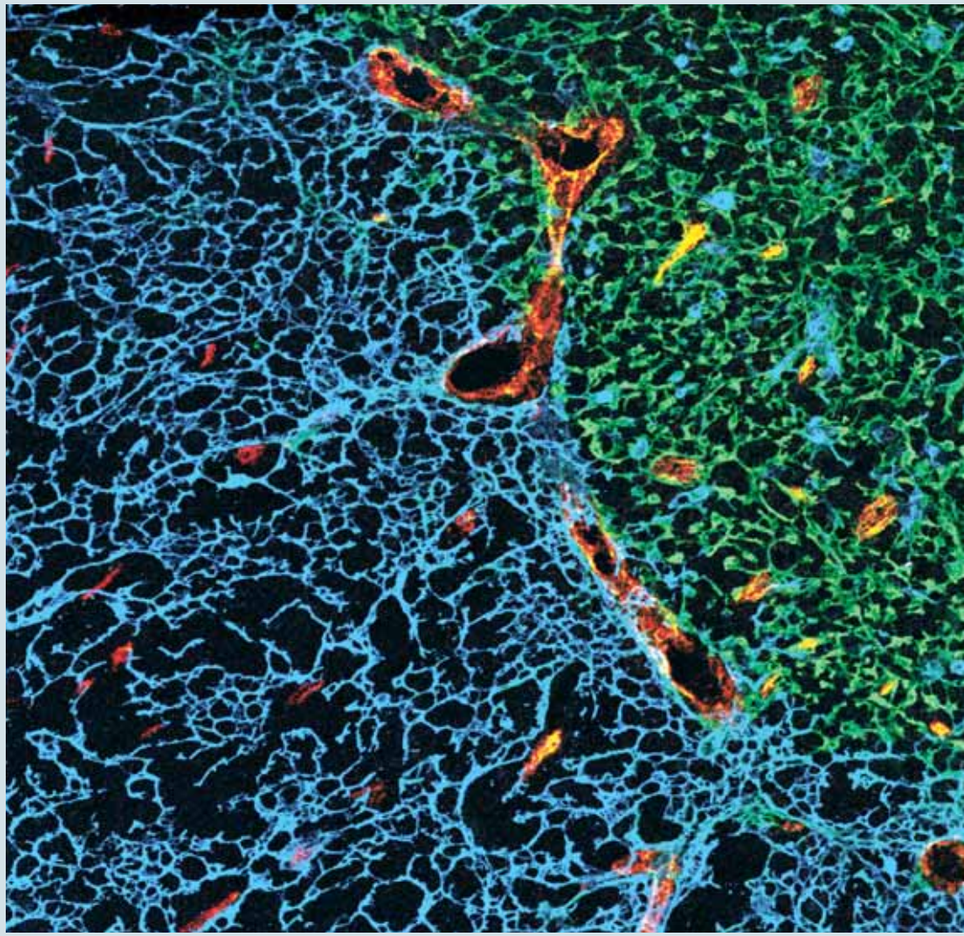
Departement
Biomedizin
Basel

DBM 2005–2007

Department of Biomedicine



Departement
Biomedizin
Basel

DBM 2005–2007

Department of Biomedicine

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Contents

Preface 6
Department of Biomedicine (DBM), Organization Chart 2007 8
Key Data 2007 9
Managerial Committees of the Department of Biomedicine 10
The Sites housing the Department of Biomedicine 12
Research Groups of the Department of Biomedicine 14
Newly Appointed Professors 2005–2007 16

Focal Area Neurobiology 18
- Cellular Neurobiology 20
- Clinical Neuroimmunology 22
- Developmental Neurobiology and Regeneration 24
- Functional Neuroanatomy 26
- Molecular Neurobiology Neural-immune interactions 28
- Molecular Neurobiology Synapse Formation 30
- Molecular Neurobiology Synaptic Plasticity 32
- Neurobiology 34
- Neuro-Oncology 36
- Neurosurgery 38

Focal Area Cell Plasticity and Tissue Repair 40
- CardioBiology 42
- Cell and Gene Therapy 44
- Clinical Pharmacology 46
- Clinical Pharmacology 48
- Dermatology 50
- Developmental Genetics 52
- Endocrinology 54
- Experimental Hematology 56
- Gastroenterology 60
- Gynecological Endocrinology 62
- Hepatology 64
- Inner Ear Research 66
- Metabolism 68
- Musculoskeletal Research 70
- Myocardial Research 72

Impressum

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Ocular Pharmacology and Physiology 74
Pneumology 76
Prenatal Medicine and Gynecological Oncology 78
Signal Transduction 80
Tissue Engineering 82
Vascular Biology 84

Focal Area Oncology 86
Cancer- and Immunobiology 88
Cell migration and Neuritogenesis 90
Childhood Leukemia 92
Experimental Oncology 94
Extracellular Matrix Adhesion 96
Human Genetics 98
Medical Oncology 100
Molecular Genetics 102
Oncology Surgery 104
Tumor Biology 106

Focal Area Immunology 108
Clinical Immunology 110
Developmental and Molecular Immunology 112
Developmental Immunology 114
Experimental Critical Care Medicine 116
Experimental Immunology 118
Experimental Immunology 120
Immunobiology 122
Immunonephrology 124
Infection Biology 126
Infectious Diseases 128
Molecular Diagnostics 132
Molecular Nephrology 134
Pediatric Immunology 136
Transplantation Immunology and Nephrology 138
Transplantation Virology 140

Other Research Topics
Perioperative Patient Safety 142
Integrative Biology 144

Research Groups associated with the DBM
Rheumatology 146

DBM Publications 2005–2007 148
Index 166

Preface

The Department of Biomedicine (formerly called “Department of Clinical and Biological Sciences/ DKBW”) is now in its eighth year. The concept of bringing together clinicians and basic scientist to create synergies and to advance the studies on human disease is bearing fruit, and is well documented in this report.

Good research needs a lot of money. Since January 1, 2007, a new agreement between the Kanton Basel-Stadt and Kanton Basel-Landschaft (“Staatsvertrag”) provides the basis for financing the clinical curriculum and for supporting research carried out at the University Hospitals in Basel. As a consequence, the DKBW-Council (DKBW Rat) that supervised the activities of the Department since it’s creation in 2000 was dissolved in November 2007. From now on, the Department will be overseen by the Medical Faculty and a new Advisory Board.

I am convinced that the Department of Biomedicine will be a key player within the Faculty of Medicine. It can rely on motivated physicians and scientists, willing and eager to carry out research that connects the bench to the bedside and vice-versa. This continuous dialogue between clinical and basic research is essential as it provides the basis for successful biomedical research. Still, one major condition has to be fulfilled: as a small Medical Faculty, we must concentrate our efforts on a limited number of cutting edge directions in medical research.

I’m confident that this concept can deliver its promise to develop a first class research institution with an outstanding international reputation.

Prof. Dr. André P. Perruchoud
Head of the Council (DKBW-Rat),
Dean of the Faculty of Medicine

Departement Biomedizin Basel

The Department of Biological and Clinical Sciences was created in the year 2000, with the idea to create a department that unites the entire laboratory research of the Faculty of Medicine. The intention was to abolish the barriers and intensify the interactions between the “pre-clinical” and “clinical” research units and to promote excellence in bio-medical research. The founding members in the year 2000 were the University of Basel, represented by the head of the University Council, the University Hospital Basel, represented by the head of the Department of Health of Basel-City, and the University Children’s Hospital Basel.

To define the direction, in which future investments should be made, the Faculty of Medicine designated five key (focal) research areas, of which four, Oncology, Immunology, Neurobiology and Cell Plasticity and Tissue Repair, are represented in the Department of Biomedicine.
By providing a bridge between basic science and clinical medicine, the Department of Biomedicine is an important component in the University of Basel’s strategic plan for Life Sciences. To reflect the tight connection between basic and clinically oriented research, we adopted a new name, the Department of Biomedicine, which since December 2007 replaces our previous name.

Key to the success of the Department of Biomedicine is the willingness of our scientists and clinicians to communicate and to strive for excellence. Several core facilities have been established, two of them as a joint venture between our Department and the “Biozentrum” from the Faculty of Natural Sciences; these provide access to key technologies, such as genomic micro-arrays and knockout mice. The Department’s research groups obtain a large proportion of their research funds from competitive grants by foundations in Switzerland, the EU and other countries.
More than 60% of the members are supported by third party funds.

This report summarizes the activities of the 58 research groups of the Department of Biomedicine during the period of 2005-2007.
The reports are grouped thematically according to the four focal areas.
Each research group has selected their most relevant publications from this period and a complete list of all publication can be found on page 148.

I strongly believe that a single Department, which unites the entire laboratory research of the Faculty of Medicine provides an attractive and stimulating environment for young scientists and physicians interested in biomedical research. This new structure positions us well for future challenges and opportunities.

Prof. Dr. Radek Skoda
Head of the Department of Biomedicine

Department of Biomedicine Organization Chart 2007

HEAD OF DEPARTMENT
Prof. R. Skoda

CHIEF OPERATING OFFICER
Dr. C. Möller

CENTRAL SERVICES

Controlling:	G. Brunner
Human Resources:	H. Hoyermann
Departmental Assistant:	M. Bernasconi

EXECUTIVE COMMITTEE

Chair:	Prof. R. Skoda
University of Basel:	Prof. B. Bettler Prof. G. Christofori Prof. Ch. Moroni Prof. R. Zeller
University Hospital:	Prof. A. Urwyler Prof. W. Holzgreve Prof. M. Heim Prof. G. Spagnoli
Univ. Children's Hospital:	Prof. G. Holländer
Chief Operating Officer:	Dr. C. Möller

5 RESEARCH UNITS
Operating Managers
Information Techniques
Controlling
Office
Animal Facility

58 RESEARCH GROUPS

FOCAL AREAS

Oncology:	Prof. G. Christofori Prof. R. Herrmann
Immunology:	Prof. A. Rolink Prof. G. Holländer
Cell Pl. and Tissue Repair:	Prof. R. Zeller Prof. A. Gratwohl
Neurobiology:	Prof. B. Bettler Prof. L. Kappos

The Department of Biomedicine is currently led by the Head of the Department Prof. Radek Skoda. The Chief Operating Officer and the staff of the Central Services support the Head of the Department in all administrative issues. Together with the Executive Committee (group of 11), the Head of the Department defines the overall strategy. The Executive Committee is composed of 4 representatives from the pre-clinical Institutes of the University, 4 representatives form the divisions of the University Hospital and one representative from the University Children's Hospital.

Key Data 2007

Research groups	57
Diagnostics services and others	5
Full professors	16
Associate professors	23
Assistant professors (Titular-, SNF-, and tenure track-assistant professors)	17
Employees total (of these 60% are paid by third-party funds)	521
Space	12'644 m^2

Budget 2007

Personnel	CHF	22'411'630
Supplies		7'479'303
Income (total)		- 6'513'182
Investments (equipment)		2'352'814
Total	CHF	25'730'565
Overhead costs		13'529'663
Total	CHF	39'260'228
Third-party funds	CHF	20'514'322

Managerial Committees of the Department of Biomedicine

Council of the Department
(DKBW-Rat)

until November 2007

Prof. Dr.
André P. Perruchoud
Head of the Council,
Dean of the Faculty of Medicine,
until July 2007

Lic. oec. Rita Ziegler
Director University Hospital Basel,
until December 2007

Guido Speck
Department of Health
(Sanitätsdepartement Kanton Basel-Stadt)

Dr. Hanspeter Meister
Head of Administration
University of Basel

Prof. Dr. Walter E. Haefeli
University of Heidelberg,
Germany

Prof. em. Dr. Werner Arber
University of Basel

Dr. Konrad Widmer
Director University Children's Hospital Basel,
until October 2007

Executive Committee
(Departementsleitung)

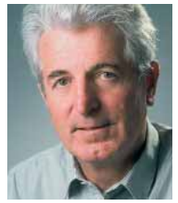
Prof. Dr. Radek Skoda
Head of the Department

Prof. Dr. Gerhard Christofori

Prof. Dr. mult.
Wolfgang Holzgreve

Prof. Dr. Bernhard Bettler

Prof. Dr. Markus Heim



Prof. Dr. Christoph Moroni

Prof. Dr. Giulio Spagnoli

Prof. Dr. Rolf Zeller

Prof. Dr. Georg Holländer

Prof. Dr. Albert Urwyler

Dr. Caroline Moeller-Dossenbach
Chief Operating Officer

The Sites housing the Department of Biomedicine

1 Institute of Anatomy
Pestalozzistrasse 20, 4056 Basel

2 Department of Biomedicine (ZLF)
Hebelstrasse 20, 4031 Basel

3 Pharmazentrum (7th floor)
Klingelbergstrasse 50/70, 4056 Basel

4 Department of Biomedicine
Mattenstrasse 28, 4058 Basel

5 Institute for Medical Microbiology
Petersplatz 10, 4003 Basel


1


2

3


4


5

Research Groups of the Department of Biomedicine

grouped according to location and focal area

Department of Biomedicine
Hebelstrasse 20

Prof. Radek Skoda

- Experimental Immunology
  Prof. Gennaro De Libero
- Transplantation Immunology and Nephrology
  Prof. Ed Palmer
  Prof. Jürg Steiger
- Immunonephrology
  Prof. Jürg A. Schifferli
- Immunobiology SNF-Förderprofessur
  Prof. Christoph Hess
- Experimental Critical Care Medicine SNF-Förderprofessur
  Prof. Urs Eriksson
- Gastroenterology
  Prof. Christoph Beglinger
- Ocular Pharmacology and Physiology
  PD Dr. Peter Meyer
- Myocardial Research SNF SCORE
  Dr. Gabriela Kuster Pfister
- Infection Biology
  Prof. Regine Landmann
  Prof. Manuel Battegay
- Infectious Diseases
  Dr. Andrej Trampuz
  Prof. Manuel Battegay
- Molecular Nephrology
  PD Dr. Barbara Biedermann
  Prof. Reto Krapf
- Experimental Hematology
  Prof. Radek Skoda
  Prof. Aleksandra Wodnar-Filipowicz
- Medical Oncology
  Prof. Christoph Rochlitz
- Hepatology
  Prof. Markus H. Heim
- Pneumology
  Prof. Michael Roth
  Prof. Michael Tamm
- Dermatology
  Prof. Peter Itin
- Clinical Pharmacology
  Prof. Jürgen Drewe
  Prof. Stephan Krähenbühl
- Prenatal Medicine and Gynecologic Oncology
  Prof. Sinuhe Hahn
  Prof. Wolfgang Holzgreve
- Gynaecological Endocrinology
  Prof. Christian DeGeyter
- Endocrinology
  Prof. Alex N. Eberle
- Clinical Immunology SNF SCORE
  PD Dr. Marten Trendelenburg
- Childhood Leukemia G. von Meissner Professur
  Prof. Jürg Schwaller
- Oncology Surgery
  Prof. Giulio C. Spagnoli
  Prof. Michael Heberer
- Tissue Engineering
  Prof. Ivan Martin
  Prof. Michael Heberer
- Cell and Gene Therapy
  Dr. Andrea Banfi
  Prof. Michael Heberer
- Cardiobiology
  Prof. Marijke Brink
  Prof. Peter Buser
- Vascular Biology
  Prof. Edouard Battegay
- Metabolism
  Prof. Ulrich Keller
  Prof. Beat Müller
- Perioperative Patient Safety
  PD Dr. Susan Treves
  PD Dr. Thierry Girard
- Neuro-Oncology
  Prof. Adrian Merlo
- Signal Transduction
  Prof. Therese J. Resink
  Prof. Paul Erne
- Signal Transduction
  Prof. Daniel Bodmer

Department of Biomedicine
Mattenstrasse 28

Prof. Georg Holländer

- Developmental Genetics
  Prof. Rolf Zeller
  Prof. Aimée Zuniga
- Pediatric Immunology
  Prof. Georg A. Holländer
- Developmental and Molecular Immunology
  Prof. Antonius Rolink
- Developmental Immunology SNF-Förderprofessur
  Prof. Daniela Finke
- Tumor Biology
  Prof. Gerhard Christofori
- Molecular Genetics
  Prof. Primo Schär
- Cancer- and Immunobiology
  Prof. Mathias Wymann
- Cell migration and neuritogenesis SNF-Förderprofessur
  Prof. Olivier Pertz
- Human Genetics
  PD Dr. Karl Heinimann
- Extracellular Matrix Adhesion
  Prof. Gertraud Orend

Department of Biomedicine
Pestalozzistrasse 20

Prof. Rolf Zeller

- Musculoskeletal Research
  Prof. Magdalena Müller-Gerbl
- Functional Neuroanatomy
  Prof. Cordula Nitsch
- Developmental Neurobiology and Regeneration
  Prof. Josef Kapfhammer
- Integrative Biology
  Prof. Daniel Haag
- Molecular Neurobiology Neural-immune interactions
  Prof. Uwe Otten
- Cellular Neurobiology SNF-Förderprofessur
  Prof. Suzanna Atanasoski

Department of Biomedicine
Klingelbergstrasse 50/70

Prof. Bernhard Bettler

- Molecular Neurobiology Synaptic Plasticity
  Prof. Bernhard Bettler
- Molecular Neurobiology Synapse Formation
  Prof. Hans-Rudolf Brenner
- Neurobiology
  Prof. Nicole Schaeren-Wiemers
  Prof. Andreas J. Steck
- Clinical Neuroimmunology
  Prof. Ludwig Kappos
  Prof. Raija Lindberg
- Neurosurgery
  PD Dr. Oliver Hausmann

Department of Biomedicine
Petersplatz 10

Prof. Christoph Moroni

- Experimental Oncology
  Prof. Christoph Moroni
- Experimental Immunology
  Prof. Peter Erb
- Transplantation Virology
  Prof. Hans H. Hirsch
- Molecular Diagnostics
  PD Dr. Thomas Klimkait

Research groups associated with the Department of Biomedicine

- Rheumatology Felix Platter Spital
  Prof. Alan Tyndall
- Brain Aging and Mental Health UPK Basel
  PD Dr. Anne Eckert
  Prof. Franz Müller-Spahn

Routine Diagnostics and other Services

Institute for Medical Microbiology

- PCR/HIV Laboratory
  Prof. Hans H. Hirsch
- Serology/Virology Laboratory
  Dr. Ingrid Steffen

University Children's Hospital Basel

- Genetic Counselling and Diagnostics
  Prof. Peter Miny

Institute of Anatomy

- Histology
  Prof. Konstantin Beier
- Anatomy Museum
  Prof. Magdalena Müller-Gerbl

Legend:

- Focal Area Neurobiology
- Focal Area Cell plasticity and tissue repair
- Focal Area Oncology
- Focal Area Immunology
- Associated Research-Groups
- Specialities
- Services

Newly Appointed Professors 2005–2007

Prof. Dr. Suzana Atanasoski, born 1968 in Frauenfeld, Switzerland, studied biochemistry at the ETH Zurich. After research activities at the University Hospital of Zurich and at the Cold Spring Harbor Laboratory, USA, she obtained her PhD in 1996. From 1997, she worked as a postdoctoral fellow at the ETH Zurich on molecular aspects of Schwann cell biology. Since 2006 she holds a professorship from the Swiss National Science Foundation and chose the Department of Biomedicine as host institution. Her work focuses on the molecular and cell biology of neural and oligodendrocyte progenitor cells during development and following injury.

Dr. Andrea Banfi, born in 1972 in Cremona, Italy, studied Medicine at the University of Genoa, where he obtained also his specialization in Clinical Oncology in 2000. Between 2000 and 2004 he worked in the Department of Molecular Pharmacology of Stanford University, USA, first as a postdoctoral fellow and then as a staff scientist. At the end of 2004 he was appointed group leader at the University Hospital in Basel, within the Institute for Surgical Research and Hospital Management. His work focuses on the mechanisms that regulate blood vessel growth and cell and gene therapy approaches to induce therapeutic angiogenesis.

Prof. Dr. Gennaro De Libero, born 1957 in Guardia Sanframondi, Italy, studied medicine at the University of Pisa, where he graduated in 1982. He also obtained a PhD in Microbiology in 1985. After two years at the Max Planck Institut für Immunbiologie in Freiburg, he was member of the Basel Institute of Immunology. In 1990 he became group leader at the Department of Research, Kantonsspital Basel. He habilitated in 1997, was appointed Professor of Immunology in 2001 and Professor of Tumor Immunology in 2007. His work focuses on the T cell-mediated immune response in cancer and in infectious, autoimmune and inflammatory diseases.

Prof. Dr. Peter Itin, born 1955 in Liestal, Switzerland, studied Medicine in Basel. 1989/1990 he studied the biological effects of vitamin D to skin cells in the Mayo Clinic. Since the beginning of his medical career he was interested in genodermatoses. Two major research projects started 15 years ago with one big Swiss family suffering from Erythrokeratodermia figurate variabilis and another large family with Nägeli-Franceschetti-Jadassohn syndrome. The aim was in a first step to better delineate the phenotype of these rare diseases and then finding the gene locus and the responsible genes in collaboration with other groups. As a consequence subsequent projects were conducted and helped establishing a network between Europe and the USA. Translation from basic research into clinic are focused in the fields of cell biology and wound healing, skin changes in systemic diseases incl. HIV and Pediatric Dermatology.

Dr. Gabriela Kuster Pfister, born in 1969 in St. Gallen, Switzerland, studied medicine at the University of Zurich, where she graduated in 1995. Upon completion of her clinical training in Internal Medicine and in Cardiology in Lucerne, Berne and Basel, she was a postdoctoral fellow in the Myocardial Biology Unit of the Whitaker Cardiovascular Institute at Boston University Medical Center, USA (2002-2005). In 2006, she obtained a SCORE grant from the Swiss National Science Foundation and joined the Department of Biomedicine at the University Hospital in Basel. Her work focuses on the molecular mechanisms of myocardial remodeling and repair, specifically the role of reactive oxygen species in these processes.

Prof. Dr. Ivan Martin, born 1969 in Santa Margherita Ligure (GE), Italy, studied Biomedical Engineering at the University of Genova where he obtained his PhD in 1996. Between 1996 and 1999 he was a postdoctoral associate at Harvard/MIT. He joined the Department of Surgery and Biomedicine at the University of Basel in 1999 as Director of the Tissue Engineering Research Group. In 2007 he was appointed Associate Professor for Tissue Engineering. His work focuses on the development of bioreactors for automated and controlled manufacturing of cartilage, bone and osteochondral grafts, based on autologous cells and 3D scaffolds.

Prof. Dr. Peter Meyer, born in 1961 in Rheinfelden, Switzerland, studied human medicine at the University of Basel. He practised internal medicine and ophthalmology in which he habilitated in 2001. Since 1996 Peter Meyer acts as head of the department of ophthalmic pathology at the University of Basel. His work is focussed on the pathology of the optic nerve and retina (main topic: glaucoma) and basic science research in ocular blood flow.

Prof. Dr. Magdalena Müller-Gerbl, born 1958 in Kenzingen, Germany, studied Medicine at the University of Freiburg i.B, where she graduated 1984. In 1985 she obtained her PhD in Medicine. From 1985 to 1989 she worked in the Anatomical Institute in Freiburg i.B.. In 1989 she moved tot he Anatomical Insitute in Munich, where she habilitated in 1992. In 1998 she was appointed Pro fessor of Anatomy. In 1999 she worked as a guest lecturer at the Harvard Medical school in Boston. Her scientific work focuses on the functional anatomy oft he locomotor apparatus with special emphasis on the form-function relationsship in human joints and their application in vivo.

Prof. Dr. Olivier Pertz, born 1971 in Herisau, Switzerland, studied biology at the University of Lausanne. In 1999, he obtained his PhD in Biophysical Chemistry at the Biocenter of the University Basel. Between 2000 and 2007, he was a postdoctoral fellow at the Department of Cell biology at the Scripps Research Institute in La Jolla, California, and then a staff scientist in at the Cancer center of the University of California San Diego. In 2007, he became group leader at the Institute of Biochemistry and Genetics on a SNF professorship position. His work focuses on spatio-temporal signaling to the cytoskeleton during cell migration and axonal guidance.

Prof. Dr. Jürg Schwaller, born 1964 in Solothurn, Switzerland, obtained his medical degree from the University of Berne. After training in clinical pathology (University of Zürich), he was research fellow in hematology-oncology at University of Berne followed by a post-doctoral fellowship at Harvard Medical School in Boston (1996-1999). Back in Switzerland he became head of the molecular biology laboratory at the department of clinical pathology, University of Geneva. End of 2004 he was appointed as research professor supported by the Gertrude Von Meissner Foundation heading the childhood leukemia group. The goal of his work is to understand molecular mechanisms underlying acute leukemia in order to delineate new targeted therapeutic strategies.

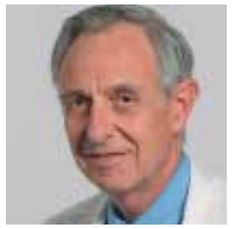
PD Dr. Andrej Trampuz, born in 1966 in Slovenia, studied medicine at the University of Ljubljana, Slovenia where he obtained his MD degree in 1993. After his clinical training in internal medicine (1994-1998) and infectious diseases (1999-2001) at the University Hospital Basel, he become a postdoctoral research fellow (2001-2004) at the Mayo Clinic in Rochester, Minnesota, USA. In 2005 he obtained a clinical staff position in the Division of Infectious Diseases at the University Hospital Basel. In 2006, he became a group leader and habilitated in infectious diseases research at the Department of Biomedicine. His work focuses on microbial biofilms and novel diagnostic and treatment approaches for implant-associated infections.

PD Dr. Marten Trendelenburg, born in 1968 in Homburg-Saar/D, studied Medicine at the Universities of Saarland/D and Lausanne/CH. He started his specialization in internal medicine at the University Clinic Mannheim/D, followed by a research fellowship and further clinical training at the University Hospital in Basel/CH. From 2001-2003, he served as a research fellow in the Department of Rheumatology at Hammersmith Hospital, Imperial College, London. Back in Basel he received a second specialization in immunology and currently practices as senior consultant for internal medicine. His research group focuses on the role of complement and autoantibodies in systemic autoimmunity.

DBM Focal Area Neurobiology

Focal Area Coordinators

Prof. Dr. B. Bettler
Department of Biomedicine
Institute of Physiology
University of Basel



Prof. Dr. A. J. Steck
Department of Biomedicine
and Department of Neurology
University Hospital Basel

Understanding the molecular events underlying diseases of the nervous system and exploiting this knowledge for improving treatment are among the major challenges in the life sciences. In view of the increasing social and financial burden generated by an ageing population, the Department of Biomedicine (DBM) has defined the neurosciences as one of its focal areas. The Focal Area Neurobiology of the DBM complements parallel efforts at the Biozentrum and at the FMI. The Focal Area Neurobiology of the DBM is part of the Basel Neuroscience Program (BNP), which comprises more than 400 neuroscientists from 40 different laboratories associated with the University, the University Hospitals, the FMI and the Basel Life Science Industry. Research is conducted at all levels - from molecules to behavior – thus providing outstanding research opportunities and an excellent platform for a strong educational program. The BNP offers weekly research seminars and lecture series at the graduate and postgraduate levels, covering all aspects of basic and clinical neuroscience. The BNP is part of a trinational educational and collaborative network with the neuroscience programs at the Universities of Freiburg (Germany) and Strasbourg (France).

A major aim of the Neuroscience groups at the DBM is to take advantage of the unique expertise in the neurosciences present in the Basel area to pursue translational research projects. As a consequence of these efforts, basic and clinical neuroscientists have successfully raised grant support for translational research projects from the Swiss National Science Foundation, the Swiss Cancer League and various private foundations. The focus of these projects is on neuroinflammatory, neurodenenerative, psychiatric, neuro-onological and neuro-muscular disorders.

To promote the rapid translation of research results into clinical practice the DBM Focal Area Neurobiology organizes the Annual Basel Neuroscience Symposium “From Bench to Bedside”. The one-day event provides a platform for exchange of ideas and is regularly attended by more than 150 local neuroscientists, including basic and clinical researchers from Novartis, Roche, Actelion, Santhera Pharmaceuticals, FMI and the University.

Neurobiology
Development
Regeneration
Neural stem cells
Cell cycle
Ski

Cellular Neurobiology

Prof. Dr. Suzana Atanasoski
Department of Biomedicine
Institute of Physiology
University of Basel

Group Members
Constanze Baranek (PhD student)
Lionel Nobs (PhD student)
Nicoleta Sustreanu (PhD student)
Nicolas Boileau (technician)*

* left during report period

Molecular Mechanisms in Neuro-development and Neurodegeneration

Neural stem cells are a focus of strong public and scientific interest, since with the discovery of neurogenesis in the adult brain we can envision novel strategies for the treatment of neurodegenerative diseases. The characterization of neural stem cells during brain development and repair will play a fundamental role in the rational design of therapeutic procedures. With our projects, we expect to obtain considerable insights into the expression and function of key candidate genes, using molecular and cellular techniques, and conditional and inducible gene ablation in the mouse.

The central nervous system (CNS) develops from self-renewing, multipotent neural stem cells. These differentiate into neural progenitor cells, which eventually give rise to neurons, astrocytes, and oligodendrocytes. A central issue in neural stem cell biology is to understand the roles of regulatory pathways in stem cell maintenance, proliferation, and differentiation. The identification of neural stem cells in the adult CNS suggested a capacity of these cells for self-repair after brain injury. However, there are profound differences between embryonic and adult neural progenitor cells with respect to the markers they express, their location in the brain, and their proliferative capabilities. This raises the question of how proliferation is controlled in embryonic and adult neural progenitors, and which signaling pathways influence maintenance and cell division in these two cell populations. TGFβ has potent effects on cell proliferation and differentiation of various progenitor cells. It is involved in the regulation of cell cycle proteins, and recent findings have identified the proto-oncogene Ski as an inhibitor of the TGFβ pathway (Fig. 1). Thus, our goal is to investigate the role of Ski and specific cell cycle proteins as part of the mechanisms by which maintenance and proliferation of embryonic and adult neural and oligodendrocyte progenitors are controlled.

In the peripheral nervous system, we have identified the proto-oncogene Ski as a crucial player in the regulation of Schwann cell proliferation and myelination. We found that Ski overexpression inhibited TGF-mediated proliferation of Schwann cells, myelination was blocked in myelin-competent cultures derived from Ski-deficient mice (Fig. 2), and genes encoding myelin components were downregulated in the absence of Ski (Fig. 3). We showed that Ski links proliferation and differentiation in postnatal Schwann cells, thereby contributing to the understanding of the molecular mechanisms that control nerve development, regeneration, and neuropathies. We then extended these studies to examine proliferation during development and following nerve injury. Our findings showed that certain distinct components of the cell cycle machinery that regulate Schwann cell proliferation during development differ fundamentally from those activated following nerve injury or in peripheral neuropathies.

To achieve a comprehensive understanding of the role of these molecules and pathways in the CNS we are taking advantage of cultures of neural and oligodendrocyte progenitor cells isolated from embryonic and adult brain or derived from embryonic stem cells. Such cultures provide a good test system, in that the regulation of progenitor cell proliferation and differentiation can be manipulated by extracellular cues and by genetic means. These experiments are running in parallel with in vivo studies using appropriate animal models. The goal of our projects is to improve the understanding of functional differences between embryonic and adult neural progenitors, and to identify the intrinsic differences between dividing oligodendrocyte progenitor cells during development and following injury. These studies may lead to a better understanding of the aberrant differentiation observed in demyelinating diseases such as Multiple Sclerosis. Further, knowledge of how neural progenitor cells can be maintained in a proliferative state or induced to differentiate into distinct cell types may be of potential medical use in regenerative repair or cell replacement therapies.

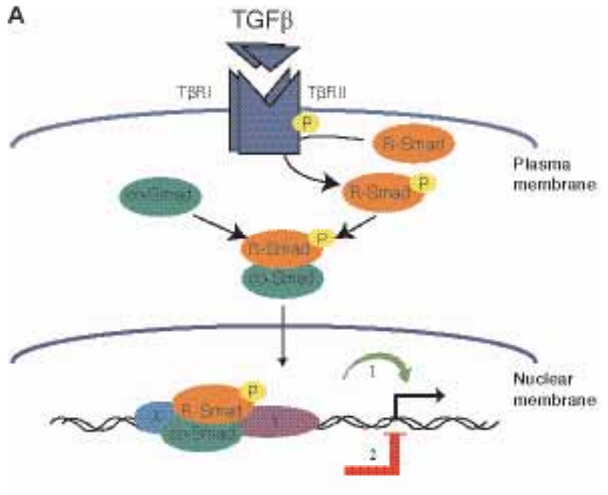


Fig. 1: Ski-mediated repression of TGFβ signaling.
(A) TGFβ binding to type II and type I receptors leads to receptor activation. R-Smads are phosphorylated, associate with the co-Smad Smad4 and translocate to the nucleus. Together with interacting transcription factors (X and Y), Smads can bind specific gene promoters and either activate (1) or repress (2) transcription of the target genes, depending on the biological context.
(B) Ski represses TGFβ-induced gene expression through direct interactions with the Co- and R-Smads, thereby modulating gene transcription.

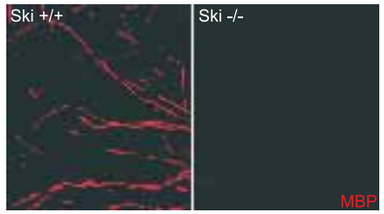


Fig. 2: Absence of myelin formation in dorsal root ganglia (DRG) of Ski-deficient mice. Expression of the myelin marker MBP in control (Ski+/+) and mutant (Ski-/-) DRG explant cultures. Note the complete absence of myelin in Ski-deficient samples.

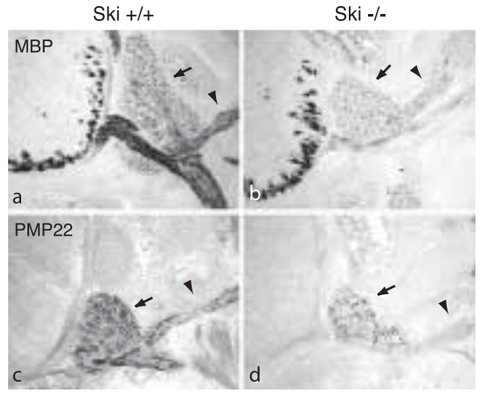


Fig. 3: In situ hybridization analysis of glial markers on transverse sections of E19.5 embryos. Expression of MBP (a, b) and PMP22 (c, d) in wt and Ski-deficient embryos revealed that myelin-gene related markers are strongly downregulated in the DRG (arrows) and peripheral nerves (arrowheads) of the mutant (b, d) compared to control embryos (a, c).

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- Atanasoski, S., Boller, D., De Ventura, L., Kögel, H., Böntert, M., Young, P., Werner, S., and Suter, U. (2006). The cell cycle inhibitors p21 and p16 are required for the regulation of Schwann cell proliferation. Glia 53, 147-157.
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- Atanasoski, S., Shumas, S., Dickson, C., Scherer, S. S., and Suter, U. (2001). Differential Cyclin D1 requirements of proliferating Schwann cells during development and after injury. Mol. Cell. Neurosci. 18, 581-592.

Multiple sclerosis
Expression profiling
Prognostic markers
Antibodies
Treatment response
Immunomodulation

Clinical Neuro-immunology

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Molecular Analysis of Multiple Sclerosis

Multiple Sclerosis (MS) is a disease that combines the complexities of structure and function of the Central Nervous System with the complexity of our innate and adaptive Immune System. Inflammation, demyelination, degenerative and even hypoxic damage mechanisms and repair, contribute to the disease phenotype. Patients display variable symptoms and courses and poorly predictable response to therapies. Our research focuses on the molecular and immunological analysis of MS in close collaboration with a clinical and a neuroimaging research group. Taking advantage of its active and often leading role in international multicentre therapeutic and diagnostic MS studies, the Clinical Research Group – based on a large multidisciplinary MS Clinic – provides access to biological samples from well characterized and systematically followed patients with different clinical courses and treatments. The Neuroimaging Group (MS-MRI Evaluation Center, Prof. EW Radü; Tissue Characterization, Prof. A. Gass) allows for better characterization of disease course and in vivo pathology of the disease as well as measurement of therapeutic response. With our research we aim to get a better understanding of altered physiological pathways in MS pathogenesis and to provide new insights into the mode of action of currently available and newly developed treatments, which ultimately could offer a basis for the development of novel therapeutic strategies.

1. Gene expression profiling in multiple sclerosis (MS) – Identifying diagnostic/prognostic markers and therapeutic targets for multiple sclerosis
   Our earlier large-scale transcriptional analysis of brain tissue from secondary progressive multiple sclerosis (spMS) patients provided molecular evidence of a continuum of dysfunctional homeostasis and inflammatory changes in spMS lesions and NAWM, and supported the concept of MS as generalized, as opposed to a focally restricted disease of the CNS. In extension of these studies, our RNA expression profiling of peripheral blood of MS patients with different clinically defined disease courses: relapsing-remitting (rr), secondary progressive (sp) and primary progressive (pp)MS indicates that it is possible to distinguish various disease courses based on expression patterns in peripheral blood.
   We have also investigated the effects of natalizumab, a humanized monoclonal antibody to ∝4 integrins, on gene expression profiles in blood to define markers for treatment efficacy and to identify responders and non-responders (Fig. 1). Our molecular analysis demonstrates that it induces an array of changes in the regulation of almost all subtypes of blood cells including B-cells and neutrophils. These findings provide more insights into additional mechanisms of action of natalizumab and possible predictability of adverse events.
   We have initiated two large scale collaborative genetic and transcriptomic studies of well defined patient populations: Gene MSA (Genetic associations in MS), a 3 Centre study conducted together with the University of San Francisco and Amsterdam Free University and financially supported by GSK, Medical Genetics. 1000 MS and 1000 healthy controls have been recruited and characterized by yearly thorough clinical and MRI investigations (baseline, year 1 and 2). The Sentrix® HumanHap550 BeadChip platform from Illumina has been used for genome-wide SNP analysis. Correlation of genetic and phenotypic data is ongoing. BEST PGx, an investigator initiated European multicentre study lead by our clinical group and conducted in cooperation with Bayer Schering (Berlin), investigates the value of RNA expression profiling and pharmacogenetics in predicting treatment response to IFNB1b in patients with early relapsing MS. Both studies provide the opportunity to relate transcriptomics results with data from DNA analysis and the phenotypic characterization by clinical and state of the art imaging tools.

2. Myelin (anti-MOG and anti-MBP) antibodies as possible prognostic markers for early Multiple Sclerosis (MS)
   Approximately 70-90% of relapsing remitting MS patients present with a clinically isolated syndrome (CIS). Since not all patients will have a second episode defining conversion to Clinically Definite MS (CDMS), it is important to establish prognostic markers that provide more accurate information for patients about their individual risk of developing MS and guide treatment decisions. It has been suggested that myelin (anti-MOG and anti-MBP) antibodies are a useful tool for predicting early conversion to CDMS. In the setting of a large therapeutic trial in CIS we evaluated the importance of anti-MOG and -MBP antibodies as prognostic markers for conversion to Multiple Sclerosis in large well-defined patient cohort (n=462). Our results showed no association between anti-myelin antibodies and progression to multiple sclerosis (Fig. 2).

3. Lymphocyte subpopulations in patients with multiple sclerosis treated with FTY720
   The novel oral immunomodulator FTY720 functionally antagonizes the S1P1 receptor on lymphocytes, and, as a consequence, egress of lymphocytes from secondary lymphatic organs. FTY720 has shown clinical and MRI efficacy in a 6-month, placebo-controlled phase-II clinical trial in patients with MS (Fig. 3).
   We have shown that in FTY720-treated patients, CD4+ and CD8+ T-cell counts are reduced compared to the other groups. The reduction related to a selective depletion of naïve (CCR7+ CD45RA+) and of central memory (CCR7+ CD45RA-) T cells (TCM), and resulted in a relative increase of peripheral effector memory (CCR7- CD45RA- (TEM) and (CCR7- CD45RA+ (TEMRA) T cells. We are extending our studies on monocyte-derived dendritic cells and characterization of the effector memory T cell subset more in detail. This project will ultimately provide a more detailed insight into the immunological effects of FTY720 and their impact on the immunopathogenesis of MS.

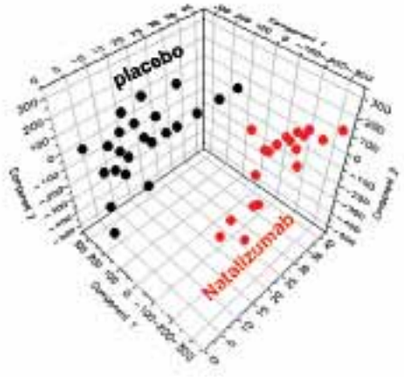
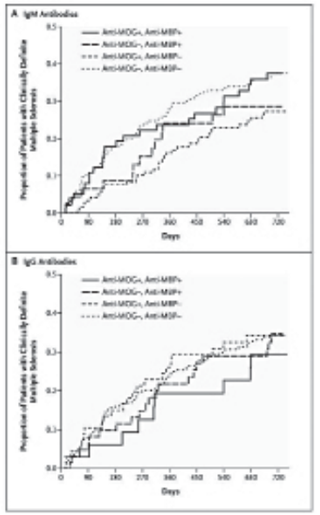
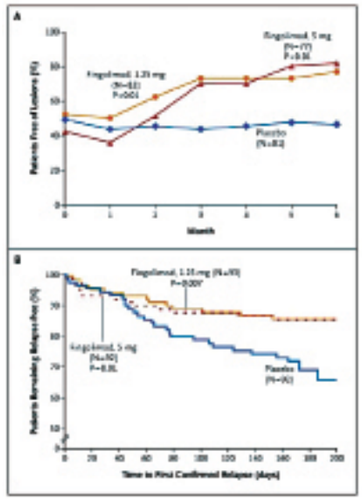


Fig. 1: Multivariate modelling of samples of MS patients treated with natalizumab (●) or placebo (●) based on expression profiles.
VIP (variable importance in the projection) was used to define the genes, which were the most accountable for the separation.





▲ Fig. 3: Proportions of patients, free of gadolinium-enhanced lesions on T1-Weighted MRI at 0 to 6 Months (Panel A) and estimated time to first confirmed relapse. NEJM 355 (2006)

◄ Fig. 2: Kaplan-Meier Curves for the Time to Conversion to Clinically Definite Multiple Sclerosis According to IgM (Panel A) and IgG (Panel B) Antibody Status. The risk of clinically definite multiple sclerosis over a period of 2 years was not influenced by antibody positivity. NEJM 356 (2007)

Selected Publications 2005–2007

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- Kuhle, J., Pohl, C., Mehling, M., Edan, G., Freedman, M.S., Hartung, H.P., Polman, C.H., Miller, D.H., Montalban, X., Barkhof, F., Bauer, L., Dahms, S., Lindberg, R.L.P., Kappos, L., Sandbrink, R. (2007). Lack of Association between Antimyelin Antibodies and Progression to Multiple Sclerosis. N Engl J Med. 356(4), 371-8.
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Cerebellar Purkinje cells
Dendritic development
Activity driven plasticity
Axonal regeneration
Spinal cord
Organotypic slice cultures

Developmental Neurobiology and Regeneration

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The control of Purkinje cell dendritic development and axonal growth in the Central Nervous System

The outgrowth of the dendritic tree is an important step in the differentiation of neurons. Most types of neurons can actually be identified by the morphology and the shape of their dendritic tree. Because the dendritic tree harbours most of the synaptic input of a neuron its growth and shape will determine the synaptic connectivity of the cell. We take advantage of a special culture system which allows growing a thin cerebellar slice in a culture dish. In such cultured slices the dendritic development of Purkinje cells proceeds in a way very similar to the in vivo situation, but allows for simple experimental manipulation of the system (Fig. 1). Using this culture system we have shown that the activity of Protein Kinase C (PKC) is an important regulator of the dendritic growth of Purkinje cells, a major neuron of the cerebellum with a large dendritic tree. Stimulation of PKC activity inhibits Purkinje cell dendritic growth and branching. In contrast, inhibition of PKC activity thus stimulates Purkinje cell dendritic growth and branching. PKC is a signaling molecule which was shown before to determine synaptic function of Purkinje cells, raising the possibility that similar signaling mechanisms are involved in the regulation of synaptic plasticity and dendritic growth. By using different mouse strains deficient for specific isoforms of the Protein kinase C proteins we could attribute the function on dendritic development to the alpha- and gamma-Isoforms of the protein. In further experiments we have explored the role of activity and neurotransmitter receptors for the development of Purkinje cell dendritic trees. The blockade of neurotransmitter receptors and the suppression of signal conduction in the axons had only rather small effects on the development of the Purkinje cell dendritic tree suggesting that there is an intrinsic growth program which can proceed independent of synaptic activity. In contrast, when we stimulated metabotropic glutamate receptors which are signaling via a G-protein coupled pathway the dendritic development of Purkinje cells was severely compromised (Fig. 2). This dendritic growth inhibition via activition of glutamate receptors could be part of a negative feedback loop which protects the Purkinje cells from developing too many excitatory synaptic connections. It is well known that a very strong excitatory stimulation can induce Purkinje cell death in a process called excitotoxicity. In the ongoing projects we are analyzing which cellular signaling pathways are involved in this control of dendritic outgrowth and whether these mechanisms are also involved in Purkinje cell pathology and degeneration.

In a second line of research we are developing an in vitro model for research on axonal regeneration. The regrowth of axons after traumatic or vascular lesions is critical for a functional recovery. Because axonal growth after lesions is strongly determined by the complex environment of the growing fibers it is typically studied in animal experiments. The slice culture model, however, could offer a similar microenvironment to the growing fibers as in the intact animal. Because the spinal cord is the CNS structure most relevant to regeneration research we have developed a slice culture model with spinal cord slices cut in the longitudinal sagittal direction. This novel spinal cord culture model maintains the cellular and organotypic network of the intact spinal cord and a strong bundle of longitudinally running axons develop in these cultures (Fig. 3) which extend within the slice and in contact with the complex spinal cord microenvironment similar to the situation in vivo. These fibers can be lesioned by a transverse cut through the entire culture with a scalpel blade and regrowth of these fibers through the lesion site can be assessed. Our first results show that regeneration of these fibers shows a decline with increasing age of the culture as has been shown before in vivo. The further development of this culture model could add an interesting tool to regeneration research and reduce the need for animal experiments.

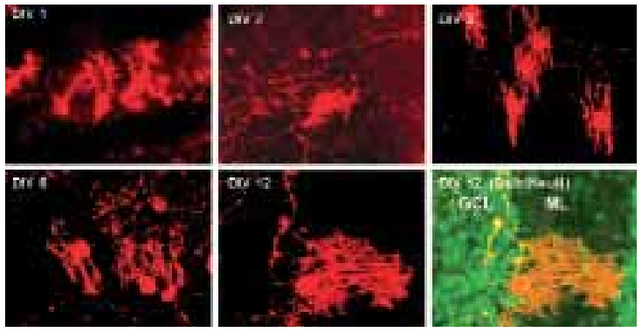

Fig. 1: Development of the Purkinje cell dendritic tree in vitro.
At the time the slice culture is made, the dendrites of the Purkinje cells are still very short and extend in multiple directions (DIV 1 – DIV 3, upper row of images). The Purkinje cell dendritic tree then becomes polarized and starts to rapidly grow and branch (DIV 6) until a well developed dendritic tree is present after 12 days in culture (DIV12) which extends in the molecular layer of the cerebellum (ML) situated above the granule cell layer (GCL).

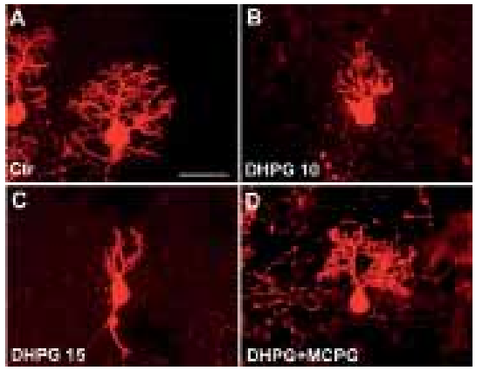

Fig. 2: Inhibition of dendritic tree development by activation of metabotropic glutamate receptors (mGluR). When cerebellar slice cultures were treated with DHPG, an agonist of mGluR, the outgrowth of the dendritic tree is severely compromised (B and C) in a dose-dependant manner compared to untreated control cultures (A). This effect is dependent on mGluR activation, because it can be blocked by co-application of the mGluR antagonist MCPG (D).

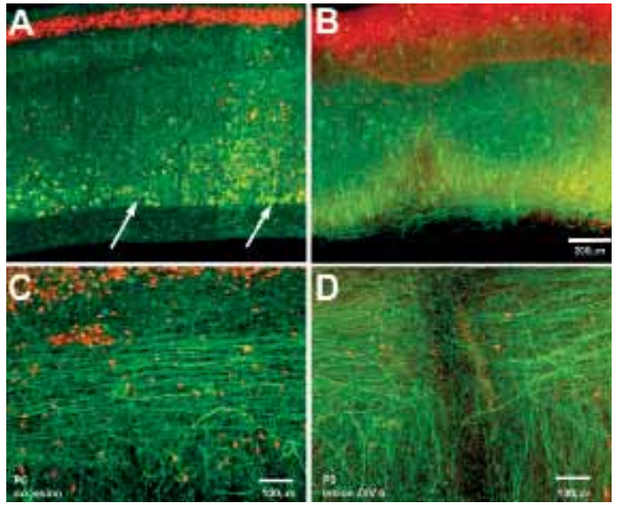

Fig. 3: Spinal cord slice cultures as an in vitro model for axonal regeneration. After a culture period of 12 days a sagittal longitudinal spinal cord slice culture has maintained the typical dorso-ventral polarity of the spinal cord (B). In the ventral domain many motoneuron like cells are present (stained in green). The small neurons of the dorsal horn (stained in red) are present in the dorsal domain of the culture. This distribution well reflects the situation in an age-matched spinal cord in vivo (A). Within the spinal cord culture, many longitudinally running axons are present (C). After a mechanical cut, many of these axons stop at the lesion site, but some can also be seen crossing the lesion (D). This culture model is suitable for evaluating treatments aiming at a stimulation of axonal regeneration within the spinal cord.

Selected Publications

– Kapfhammer, J.P., Xu, H., Raper, J.A. (2007) The detection and quantification of growth cone collapsing activities. Nature Protoc. 2:2005-2011.
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– Adcock, K. H., Metzger, F., Kapfhammer, J.P. (2004) Purkinje cell dendritic tree development in the absence of excitatory neurotransmission and of brain-derived neurotrophic factor in organotypic slice cultures. Neuroscience 127, 137-145.
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– Metzger, F., and Kapfhammer, J.P. (2000) Protein kinase C activity modulates dendritic differentiation of rat Purkinje cells in cerebellar slice cultures. Eur. J. Neurosci., 12, 1993-2005.

Neurodegeneration
Parkinson's disease
Stroke
Blood-brain barrier
Tight junctions
Organotypic slice culture
SDS-electrophoresis

Functional Neuroanatomy

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The regional microenvironment in the brain and its role in neurodegeneration and neuroprotection

The causes and consequences of neurodegeneration: that is the central topic of our research. Why are certain nerve cells and brain areas more vulnerable than others against a noxious exposure as occurring in stroke or in Parkinson's disease e.g? Are there endogenous mechanisms protecting against or aggravating an insult - mechanisms which could be exploited therapeutically? What are the roles of the different tissue elements and cell types next to the neurons in these events? In fact, an intact homeostasis of the microenvironment around the nerve cells and their synapses is the prerequisite for regular functioning of the brain. Its control by non-neuronal cells was the main focus of our research over the last couple of years.

A new model of the blood-brain barrier

Homeostasis of the nervous tissue is guaranteed by the blood-brain barrier (BBB). It consists of endothelial cells sealed by an extensive network of interendothelial tight junctions. Existing BBB models rely on cultures of endothelial cells or co-cultures with astrocytes obtained from rat or pig brain tissue. The drawback of these models becomes evident when considering that the unique BBB phenotype is the result of the continuing influence of the surrounding nervous tissue in its 3-dimensional structural connectivity. In the organotypic cerebral slice culture the organization of the tissue is maintained, but blood vessels dissociate under standard conditions (Fig. 1 A, C). We characterized in explanted mouse brain tissue the conditions for survival and integrity of cerebral blood vessels. In the presence of moderate concentrations of the basic fibroblast growth factor (FGF-2), blood vessels of different diameters persist in culture for up 10 days (Fig. 1 B, D, E, F). The vessels keep their in vivo structural integrity as evidenced by the presence of the tight junction proteins ZO-1, occludin and claudins 3 and 5 (Fig. 2). Thus, in the presence of FGF-2, blood vessels are preserved in the organotypic slice culture of the mouse brain and show features of an intact BBB. We are now using this tool for investigating cerebral angiogenesis and for mechanistic studies of the BBB.

A new SDS Disc electrophoresis method to separate low and high molecular weight proteins

Brain tissue is notorious for its wide range of proteins, both in terms of size as well as in terms of physicochemical characteristics. To analyze the entire profile, different systems had to be employed or, in the case of SDS electrophoresis, two gel types had to be run. By using a multiphasic buffer system which prevents the continuous stacking of SDS as it occurs in the "classical" Laemmli system (Fig. 3 A), we have succeeded to separate in a single gel run of a tissue sample proteins in the range of 3.5 to 250 kDa at low acrylamide concentrations (Fig. 3 B, C). Taurine is used as trailing ion in the cathode buffer and in the resolving zone of the gel, and two counter ions (Tris and imidazole) in the stacking zone. We are presently using this system successfully in the analysis of the different constituents of the BBB and are collaborating with several other research groups.

Osteopontin and Parkinson's disease

Osteopontin is a glycosylated phosphoprotein belonging to the small integrin binding ligand N-linked glycoprotein (SIBLING) family of proteins. The multiple functions of this protein (involvement in oxidative stress and apoptosis, cytokine regulation and chemotaxis, generation of NO and buffering of calcium) and its presence in the brain under physiological and pathological conditions suggest a role in neurodegeneration. In own studies in the ibotenic acid model of excitotoxic neurodegeneration we observed that osteopontin-null mice exhibited an attenuated and reduced loss of nerve cells (unpublished results). We have tested the involvement of this protein in the mouse MPTP model of Parkinson's disease. Osteopontin-null mice are partially protected against the loss of dopaminergic neurons. This, together with data from body fluids of Parkinson patients and post-mortem findings, may argue for a causative role of osteopontin in nerve cell loss in Parkinson's disease.

Fig. 1

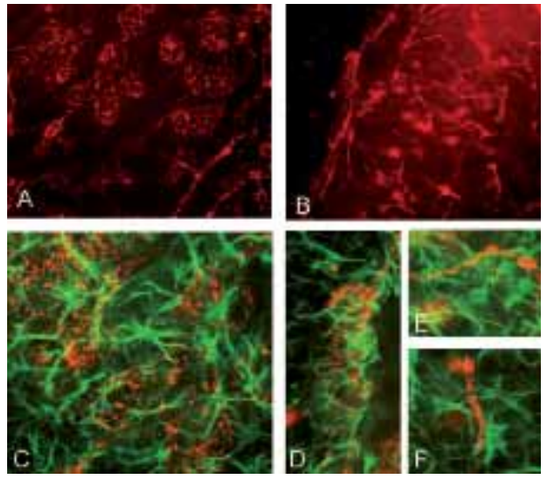


Fig. 2

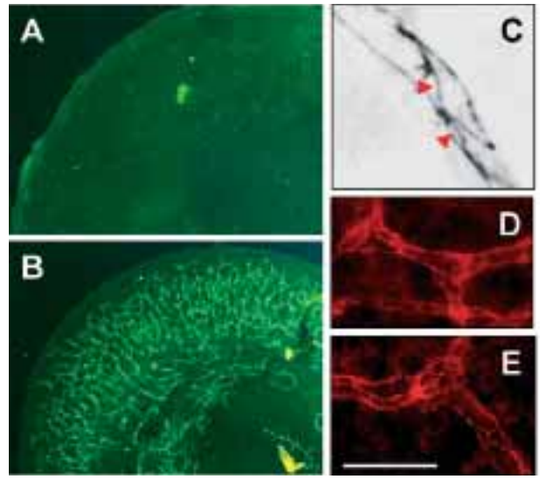
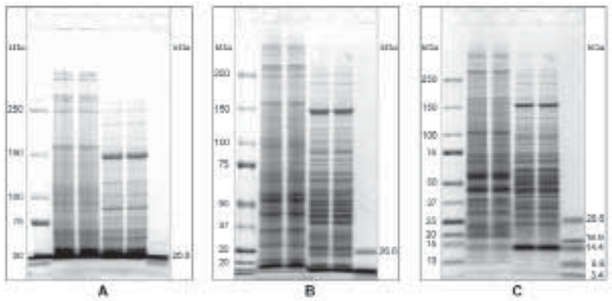


Fig. 3



Selected Publications

– Bendfeldt K, Radojevic V, Kapfhammer J, Nitsch C (2007) Basic fibroblast growth factor modulates density of blood vessels and preserves tight junctions in organotypic cortical cultures of mice - a new in vitro model of the blood-brain barrier. J. Neurosci. 27(12): 3260-3267.
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– Maetzler W, Berg D, Schalamberidze N, Melms A, Schott K, Mueller JC, Liaw L, Gasser T, Nitsch C (2007) Osteopontin is elevated in Parkinson's disease and its absence leads to reduced neurodegeneration in the MPTP model. Neurobiol.Dis. 25(3): 473-482.
– Kaiser A, Kuenzli E, Zappatero D, Nitsch C. (2007) On females' lateral and males' bilateral activation during language production: a fMRI study. Int. J. Psychophysiol. 63: 192-198.
– Nitsch C (2007) Mehrsprachigkeit: Eine neurowissenschaftliche Perspektive. In: «Mehrsprachigkeit bei Kindern und Erwachsenen» (T. Anstatt, ed.) Attempto Tübingen, pp. 47-68.

Interleukin-6
Neuroprotection
Neurodegeneration
Signaling
Transgenic animals
Spinocerebellar ataxia

Molecular Neurobiology Neural-immune interactions

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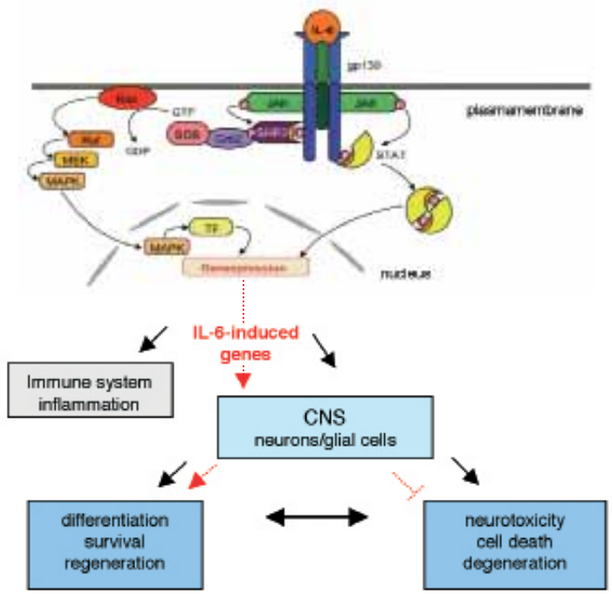
Functional role of interleukin-6 (IL-6)-induced genes/gene products in neuro-protection and -degeneration of brain neurons

Using molecular biological techniques including gene chip arrays and differential screening, we identified two new IL-6 regulated genes/gene products which are involved in neuroprotection. These findings form the basis of two ongoing research projects:

1) Spinocerebellar ataxia (SCA) type 10, an autosomal dominant disesase characterized by cerebellar ataxia, is caused by a novel pentanucleotide (ATTCT) repeat expansion in the SCA10 gene. Although clinical features of the disease are well characterized, little in known so far about the affected SCA10 gene product, ataxin-10 (Atx-10). We have cloned the rat SCA 10 gene and expressed the corresponding protein in HEK 293 cells. Atx-10 has a molecular mass of 55 kDa and belongs to the family of armadillo repeat proteins (März et al. 2004). Atx-10 immunostaining of mouse and human brain sections revealed a predominantly cytoplasmic and perinuclear localization with a clear restriction to olivocerebellar regions. Knockdown of SCA 10 in primary neuronal cells by small interfering RNAs resulted in an increased apoptosis of cerebellar neurons, arguing for a loss-of-function phenotype in SCA-patients.
   Further studies to identify interacting proteins in the brain led to the identification of O-linked β-N-acetylglucosamine transferase (OGT) as a binding partner of ATX-10 (März et al., 2006). Overexpression of Atx-10 in neurons resulted in enhanced glycosylation activity, indicating that Atx-10 serves as a positive effector of OGT function. We postulate that a balanced interplay between intracellular glycosylation and phosphorylation determines CNS neuron survival and that ATX-10 is a major regulator of this dynamic process.

2) Recent studies in our laboratory revealed that Pancreatitis-associated protein I PAP I (also called reg IIIβ) expression, normally observed at high levels in acinar cells during acute pancreatitis, is present at low levels in brain but can be induced by IL-6-type cytokine-mediated inflammation (März et al, in press). Neurons represent the major cell type of PAP I synthesis in rat brain. Moreover, we demonstrated that PAP I dose-dependently protects cerebellar, hippocampal as well as cortical neurons against programmed cell death (apoptosis). These results which are in line with the finding that classical survival pathways including the Akt- and MAP-kinase signaling pathways are activated in neurons by PAP I, strongly suggest that PAP I exerts its neuroprotective function in an autocrine/paracrine fashion. We postulate that neuron-specific PAP I expression in brain plays a key role in neuroprotection. Most recently, PAP I-specific knock-out animals have become available. These PAP I knock-out animals (also designated Reg 2 -/-) have been provided by Prof. S. Hunt and collaborators to our research group and offer the unique chance to unravel the biological neuroprotective function of PAP I in brain neurons. Our research is directed towards elucidation of the mechanisms involved in neuroprotective effects of IL-6-type cytokines against various toxic stimuli using primary CNS neuron cultures from PAP I/ knock-out and wild type animals. Moreover, in vivo studies with these animals will be performed to prove our hypothesis that animals lacking PAP I in brain are more susceptible to damaging stimuli as compared to controls. For these experiments an established ischemia model for CNS neurodegeneration will be used and the extent of infarct areas will be analyzed (in collaboration with Prof. C. Nitsch). In addition, it will be tested whether exogenous application of recombinant PAP I can significantly reverse detrimental effects induced by ischemia.
   Identification of PAP I as a natural defense molecule for brain neurons – which is nearly undetectable under normal conditions, but can be strongly induced by inflammatory signals – may lead to the development of new therapeutic strategies to prevent neuronal damage.



Connection to Clinical Practice

Prof. Dr. André Miserez
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Statins induce differentiation and cell death in neurons and astroglia

Experimental and clinical studies indicate that statins, potent inhibitors of cholesterol biosynthesis, have beneficial effects on neurodegenerative disorders. Using well-defined cultures of CNS neurons and astrocytes, we have analyzed the direct effects of statins on their morphology and survival. Treatment of astrocytes with statins induced a time- and dose-dependent stellation which was followed by apoptosis. Similarly, statins elicited programmed cell death of CNS neurons. Analysis of the cholesterol biosynthetic pathway revealed that lack of mevalonate and of its downstream metabolites mainly geranylgeranyl-pyrophosphate, is responsible for the statin-induced apoptosis of neurons and astrocytes. Interestingly, neuronal cell death was significantly reduced in astrocyte/neuron co-cultures treated with statins suggesting that under these conditions availability of metabolites of mevalonate, e.g. isoprenoids, possibly provided by astrocytes, play a key role in neuronal survival.
Considering pharmacological treatment of clinical syndroms of atherosclerosis as well as various disorders, including inflammatory diseases, sepsis, osteoporosis, cancer, and neurodegenerative diseases with statins, one should be aware that these drugs could be toxic to CNS neurons and glial cells.

Selected Publications

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Neuromuscular Junction
Synaspe Formation
Developmental Neurobiology
Muscle
Agrin

Molecular Neurobiology Synapse Formation

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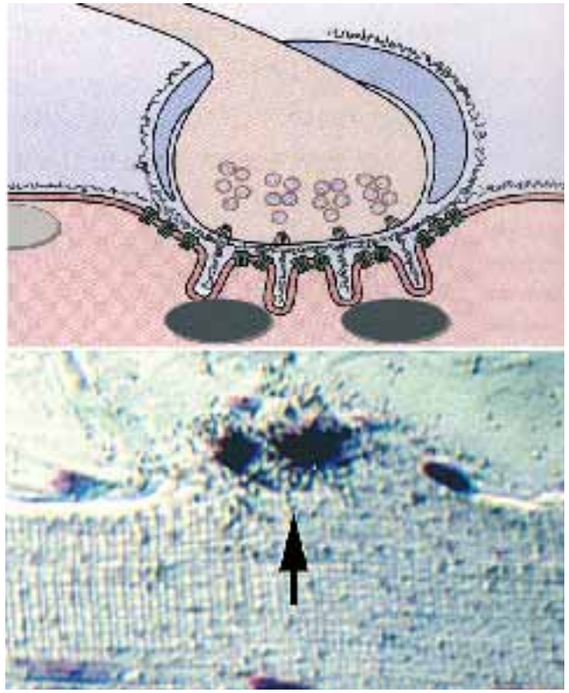
Signaling Mechanisms Regulating the Formation of the Neuromuscular Junction

Skeletal muscles contract only in response to electrical impulses in motor nerves that in turn are driven by the central nervous system. Impulse transmission from motor nerve axons to muscle fibers takes place at specialized sites of contact, the neuromuscular junctions. In response to an invading impulse the nerve terminal releases the transmitter substance acetylcholine (ACh), which in turn activates acetylcholine receptor (AChR) channels concentrated in the muscle membrane at the NMJ, thus eliciting muscle contraction. Diseases of the NMJ are due to malfunctioning of the release process or of AChR number and function. They may cause severe muscle weakness and may ultimately be lethal. In recent years, an increasing number of congenital myasthenic syndromes have been linked to various mutations of genes encoding components that are either regulating NMJ formation or are involved in the impulse transmission itself. Detailed investigation of NMJ formation at the molecular level will thus contribute to understanding the etiology of congenital myasthenic syndromes.

The formation of the NMJ during the development of the motor system involves the differentiation of the motor nerve process into a nerve terminal secreting ACh and the expression of *AChR* genes and the accumulation of the AChR proteins at the site of the contact. Aim of our research project is to understand the molecular interactions exchanged between motor neurons and skeletal muscle fibers that regulate the coordinated differentiation of pre- and postsynaptic elements. Specifically, we investigate the molecular mechanisms by which motor neurons are made to contact muscle fibers, how through this contact they induce the muscle fiber to locally express *AChR* genes and how the fiber reciprocates to induce the differentiation of the motor nerve terminal.

Two key molecules required for NMJ formation are Agrin, a heparansulfate proteoglycan secreted from nerve terminals, and its receptor MuSK expressed by muscle. We found that Agrin/MuSK are sufficient for the induction of a postsynaptic muscle membrane, including the localized expression of the *musk* and *AChR* genes by a subset of nuclei located in the synaptic region of the muscle fiber. Like *AChR* and *musk* gene induction, the recruitment of this small group of muscle nuclei to the NMJ is regulated by Agrin. Using appropriate mouse mutants generated by gene targeting we found that a classical path via neuregulin/ErbB is dispensable for the neural regulation of *AChR* and *musk* genes. The same mutants revealed a novel role for neuregulin/ErbB in maintaining a high AChR concentration by increasing their life time in the synaptic muscle membrane.

Although Agrin/MuSK are sufficient for making a postsynaptic muscle membrane, motor neurons made to secrete Agrin and muscle fibers overexpressing *musk* are not sufficient for synapse formation. Rather, the formation of a neuromuscular contact requires additional muscle factors that are expressed when muscles become electrically inactive, e.g. after nerve lesion. We have recently identified a candidate molecule in the muscle membrane that promotes the outgrowth of nerve processes from synapses. We are currently investigating its mode of function, its potential role in NMJ formation and in the re-innervation of the muscle after the motor nerve is damaged.



Top: Schematic of neuromuscular junction. Synaptic (dark coloured) and extrasynaptic nuclei (light coloured) express different sets of genes under neural control. Subsynaptic apparatus including accumulation of nuclei and their induction to express genes essential for NMJ formation and function are induced by recombinant Agrin.
Bottom: Longitudinal section through skeletal muscle fiber after detection of acetylcholine receptor ε-subunit mRNA (arrow: accumulation of silver grains).

Selected Publications

– Stocksley, M.A., Awad, S.S., Young, C., Lightowlers, R.N., Brenner, H.R. and Slater, C.R. Accumulation of NaV1 mRNAs at differentiating postsynaptic sites in rat soleus muscles. (2005) Mol. Cell. Neurosci. 28, 694-702, 2005
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Anxiety
Depression
Glioma
Neurotransmitter
G-protein coupled receptor
Neural stem cells

Molecular Neurobiology Synaptic Plasticity

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GABA-B Receptors as Drug Targets for the Treatment of Mental Health Disorders

GABA is the major inhibitory neurotransmitter in the brain and as such plays a key role in controlling neuronal activity. GABA mediates its action via two receptor systems, the $GABA_A$ and $GABA_B$ receptors. Unlike $GABA_A$ receptors that are ion channels, $GABA_B$ receptors are G-protein coupled receptors (GPCRs) and signal through second messenger systems. Dysfunction of GABA-mediated synaptic transmission in the brain is a cause or a consequence of various neurological and psychiatric disorders. For example, a hypoactivity of the GABA system was proposed to underlie epilepsy, spasticity, anxiety, stress, sleep disorders, depression, addiction and pain. In contrast, a hyperactivity of the GABA system was associated with schizophrenia. The pharmaceutical industry successfully exploited the GABA system and introduced a variety of drugs, such as e.g. the benzodiazepines and barbiturates, to the clinic. Most of these drugs target the $GABA_A$ system, while progress in developing $GABA_B$ drugs was slow. Baclofen (Lioresal®), the only $GABA_B$ drug on the market, is used as a muscle-relaxant to treat spasticity. Baclofen showed therapeutic potential in mental health indications. However, its main therapeutic effect, muscle relaxation, is a severe side-effect when it comes to psychiatric indications. All attempts to develop $GABA_B$ agonists without muscle-relaxant activity failed, which prohibited a more widespread therapeutic use of $GABA_B$ drugs in man.

To reduce side-effects, a goal in pharmaceutical research is to target drugs selectively to structurally and functionally distinct receptor subtypes. The shortage of clinically successful $GABA_B$ drugs was attributed to this lack of subtype-selectivity. In 1997, our laboratory succeeded in cloning the first $GABA_B$ receptors. Revealing a new principle for GPCRs, we showed that $GABA_B$ receptors are not monomeric proteins but instead consist of two distinct subunits. Dimerization between $GABA_{B1a}$, $GABA_{B1b}$ and $GABA_{B2}$ subunits generates two pharmacologically indistinguishable receptor subtypes in the brain, $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$. These subtypes represent the only means for directing the search for novel $GABA_B$ drugs towards molecularly distinct receptor populations. We have started to address the individual functions of $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors in the brain by generating knock-out mice that selectively express one, but not the other receptor subtype. These mice revealed that $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors differentially contribute to pre- and postsynaptic $GABA_B$ functions. Presynaptically, selectively $GABA_{B(1a,2)}$ receptors assume heteroreceptor function and inhibit the release of the excitatory neurotransmitter glutamate. Postsynaptically, predominantly $GABA_{B(1b,2)}$ receptors localize to the dendritic spines and mediate postsynaptic inhibition through the activation of potassium channels that hyperpolarize the membrane. The mechanisms leading to the differential subcellular localization of $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$ receptors are unknown. Likewise, it is unclear why the two receptors exhibit functional differences in neurons, while they have indistinguishable properties in transfected non-neuronal cells. Our laboratory is currently using genetic, ultrastructural, biochemical and electrophysiological approaches to address these issues. We are especially interested in a family of neuronal proteins that bind to $GABA_B$ receptors and alter their functional properties.

In collaboration with colleagues at Novartis and the FMI we recently established that mice lacking individual $GABA_B$ receptor subtypes exhibit pronounced differences in behavioural tests assessing cognitive performance, anxiety and depression. This is of immediate importance for drug discovery, as this demonstrates that subtype-specific $GABA_B$ drugs will have a novel spectrum of activity in vivo. We therefore aim at establishing high-throughput screening systems to identify subtype-specific compounds and to provide leads for medicinal chemistry programs.

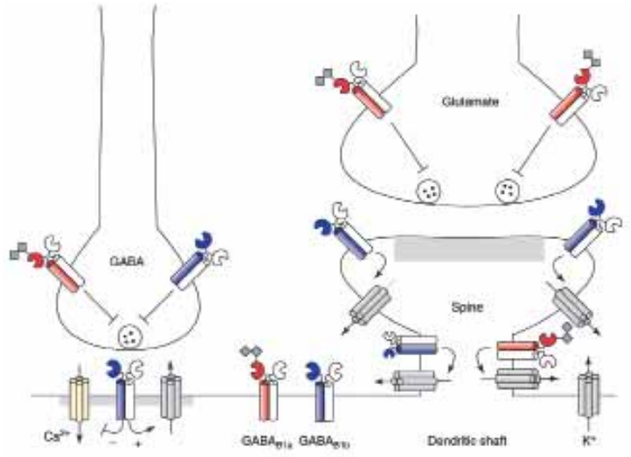

Fig. 1: Functional compartmentalization of $GABA_{B1}$ subunit isoforms
Scheme of a glutamatergic terminal and a GABAergic terminal contacting a dendritic spine and shaft (shaded areas), respectively. A feature that is preserved at all glutamatergic synapses analyzed so far is the predominant association of $GABA_{B1a}$ with the terminal. No consistent picture emerged from the functional analysis of GABAergic terminals. $GABA_{B1a}$ is the only $GABA_{B1}$ isoform present on inhibitory inputs at the apical tuft of cortical layer 5 pyramidal neurons, whereas both $GABA_{B1a}$ and $GABA_{B1b}$ are expressed at inhibitory inputs to CA1 pyramidal neurons and amygdala neurons. $GABA_B$ receptors that are coupled to postsynaptic K^+ channels in CA1 pyramidal neurons and layer 5 cortical neurons mostly involve the $GABA_{B1b}$ subunit. Adapted from Ulrich and Bettler (2007).

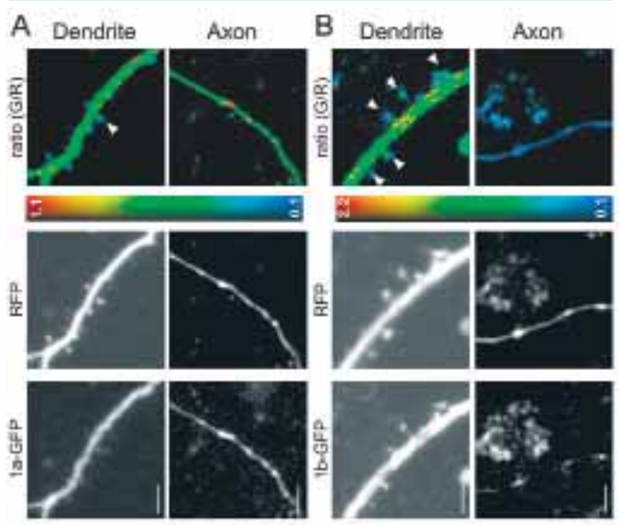

Fig. 2: Expression of $GABA_{B1a}$ and $GABA_{B1b}$ subunits fused to green fluorescent protein (GFP) in organotypic slice culture.
Dendrites and axons in the CA1 region of the hippocampus expressing $GABA_{B1a}$-GFP (A) or $GABA_{B1b}$-GFP (B) in combination with the freely diffusible tdimer2 red fluorescent protein (RFP) are shown. The ratio of green-to-red fluorescence (G/R) is coded in rainbow colors. Scale bar, 5 µm. Predominantly $GABA_{B1a}$-GFP protein is expressed in axons. The axonal expression level of $GABA_{B1a}$ and $GABA_{B1b}$ was normalized to the dendritic expression level. $GABA_{B1b}$-GFP was expressed in the majority of dendritic spines, while $GABA_{B1a}$-GFP was excluded from most spines. Examples of positive spines are indicated by white arrow heads in the G/R ratio images in (A) and (B). Adapted from Vigot et al. (2006).

Connection to Clinical Practice

Prof. Dr. Adrian Merlo
Neurosurgery

Notch2 as s Drug Target for the Treatment of Brain Tumours

Research in the laboratory of Adrian Merlo is focusing on the genetics of malignant brain tumors of glial origin (gliomas). A mapping study in the laboratory showed that chromosomal deletion breakpoints in oligodendrogliomas target the Notch2 gene, consistent with a lack of Notch2 protein expression in these tumour cells. In contrast, Notch2 is highly expressed in astrocytoma cell lines and primary malignant astrocytomas. This suggests that Notch2 acts both as a tumour suppressor gene and an oncogene. To address whether Notch2 can act as an oncogene we generated mice that express a constitutively active form of Notch2 in the brain. To address whether Noch2 can act as tumour suppressor gene we generated mice with a selective deletion of the Notch2 gene in glial precursor cells. We are currently analyzing whether these mice develop brain tumours.

In order to have a homogenous cellular system to study Notch2 signaling pathways we established a neural stem cell culture system (neurospheres). We use this system to test whether gliomas arise from aberrant neural stem cells and to understand the role of Notch2 in cell lineage commitment. We already have achieved ablation of the Notch2 gene in neurospheres and experiments to express a constitutively active form of Notch2 in neurospheres are ongoing.

Selected Publications

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Myelin Biology
Axon-Glia Interaction
Membrane Domains and Trafficking
Multiple Sclerosis
Peripheral Neuropathy
Neuroprotection

Neurobiology

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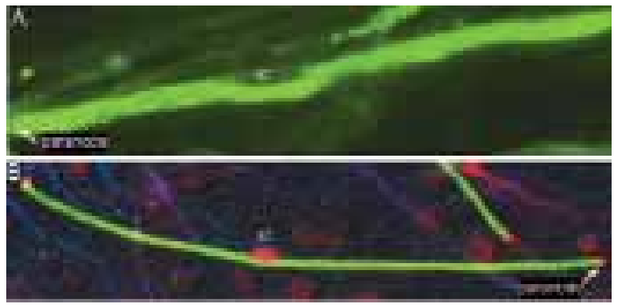
Molecular Mechanisms of Myelin Formation and Maintenance in Health and Disease

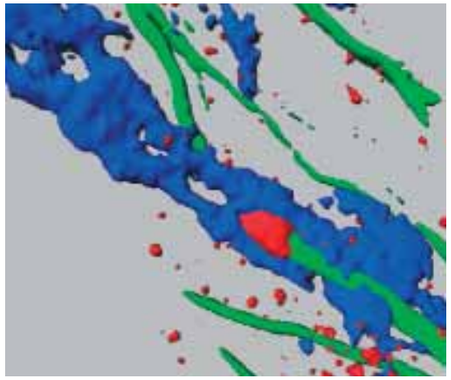
Myelin formation is a solution of higher vertebrates allowing nerve conduction with high velocity. Reciprocal signaling between axon and the myelinating cell is mandatory for myelin formation as well as for its maintenance. Oligodendrocytes, the myelinating cells of the central nervous system (CNS), are maintaining up to 50 internodes of myelin depending on the particular nerve fiber they ensheath. Repair mechanisms after CNS injury (such as traumatic brain injury, stroke and demyelinating lesions in Multiple sclerosis) depend on the regenerative capacity of neurite outgrowth together with remyelination of the nerve fibers. The molecular mechanisms of remyelination are currently of central interest, since only limited remyelination occurs. The question arises whether oligodendrocytes in Multiple Sclerosis (MS) are impaired to fulfill their function, because even earliest changes leading to lesion formation and development are unknown. For this reason, we investigate possible molecular alterations in the so-called normal appearing white matter (NAWM) in MS brain tissues and showed an upregulation of genes involved in maintenance of cellular homeostasis as well as neural protective mechanisms known to be induced upon long-lasting ischemic preconditioning (Graumann et al., 2003). More recent investigations have revealed a differential regulation of inflammation-related genes in MS NAWM (Zeis et al., 2007). In this study, we found pro- as well as anti-inflammatory genes to be upregulated suggesting that NAWM in MS is in a subtle balance between inflammation and neuroprotection. A key finding of our study was the upregulation of a major anti-inflammatory transcription factor STAT6 in the NAWM which was predominantly expressed in oligodendrocytes (Fig. 1). Moreover, co-localization of STAT6 and members of its signaling pathway (JAK1, IL-4R and IL13R) in oligodendrocytes suggests that oligodendrocytes might actively participate in the immune regulation of the CNS.

Parallel to this study, we also investigated the functional role of the Myelin and Lymphocyte protein (MAL), a membrane protein involved in the formation, transport and maintenance of glycosphingolipid microdomains, the so-called “rafts”. We have shown previously that lack of MAL resulted in structural as well molecular alterations resulting in disruption of axon-glia interaction at the node of Ranvier in the CNS (Schaeren-Wiemers et al., 2004). These results demonstrated a critical role for MAL in the maintenance of CNS paranodes, likely by controlling the trafficking and/or sorting of Neurofascin 155, myelin associated glycoprotein (MAG) and other membrane components in oligodendrocytes. Consequently, we have investigated the functional role of MAL during myelination of the peripheral nervous system (PNS). Our results show that the lack of MAL leads to enhanced myelination, whereas in contrast, the myelination in MAL-overexpressing mice was retarded. These results suggest a critical functional role of MAL in axon-glia interaction during the process of myelin initiation and sheath formation.

We further investigated the functional role of the two MAG isoforms, which are cell adhesion molecules that play an important role in axon-glia interaction during myelination. We have generated a transgenic mouse line that specifically expresses GFP-tagged S-MAG correctly regulated and targeted into the myelin sheath allowing the specific discrimination of L- and S-MAG at the subcellular level (Erb et al., 2006). Our study revealed a differential expression pattern and spatial distribution of L-and S-MAG during development as well as in the adult CNS and PNS. In peripheral nerves, where S-MAG is the sole isoform, we observed S-MAG concentrated in different ring-like structures such as periaxonal and abaxonal rings, and discs spanning through the compact myelin sheath perpendicular to the axon. Our analysis provides new insight in the subcellular distribution and function of the two isoforms in myelin formation and maintenance. Further, we are able to directly investigate the dynamic of myelination by monitoring in vitro myelinating cultures by time-lapse video microscopy (Fig. 2).

Fig. 1: Immunofluorescence localization of STAT6 in Multiple Sclerosis normal appearing white matter. STAT6 was mostly colocalized together with the oligodendrocytes marker OLIG2 (arrows).





▲ Fig. 3: 3D-reconstruction of nodal structure in skin nerve of anti-MAG patient: Anti-MAG IgM deposits (blue) are found around nerve fibers (neurofilament in green), Caspr (red) marks the paranode of a myelinated axon.

◄ Fig. 2: In vitro myelinating Schwann cell/ dorsal root ganglion neuron co-cultures from GFP-S-MAG expressing mice. (A) GFP-autofluorescence showing localization of MAG in different myelin sheath compartments. (B) Immunofluorescent colocalization of GFP-MAG (green) with Caspr in the paranodes (red) and Krox20 in the Schwann cell nucleus (SC, red). Axons are stained for neurofilament (blue).

Connection to Clinical Practice

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University Hospital Basel

Molecular investigations of inflammatory neuropathies

Our focus is the investigation of molecular mechanisms of inflammatory neuropathies. In a recently published paper we assessed the presence of IgM deposits on skin myelinated nerve fibers in the anti-MAG neuropathy, a chronic demyelinating condition of the peripheral nervous system (Lombardi et al., 2005). Skin biopsies were performed in patients with anti-MAG neuropathy and in patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). We found IgM deposits on dermal myelinated fibers in all anti-MAG neuropathy patients (Fig. 3), with a greater prevalence at the distal site of the extremities. CIDP patients did not show any IgM deposits. Anti-MAG neuropathy and CIPD patients showed a decrease in epidermal nerve fiber density reflecting an associated axonal loss. In another study we defined a new marker of inflammation in vasculitis. Allograft inflammatory factor-1 (AIF-1) is a cytokine which plays a major role in the immune response and proliferative vasculopathy that occur during chronic allograft rejection. We characterized the cellular expression pattern of AIF-1 in nerve biopsies from patients with vasculitic neuropathy (VAS). We performed immunohistochemistry in human nerve biopsies from VAS and CIDP patients. In CIDP and VAS nerve tissues, AIF-1 expression is elevated when compared to control nerves. AIF-1 is significantly increased in the arterial vessel walls of VAS compared to CIDP cases. Vascular smooth muscle cells in VAS nerves express AIF-1 at a higher level compared to CIDP. These data indicate that AIF-1 plays a role in the pathomechanismen of inflammatory nerve disease and may participate in vascular smooth muscle cell proliferation.

Selected Publications

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Glioblastoma

Notch2

Chromosome 1p deletion

Protein kinase inhibitors

Pro-apoptotic drug synergism

Radiopeptide therapy

Neuro-Oncology

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Elisabeth Taylor (technician)

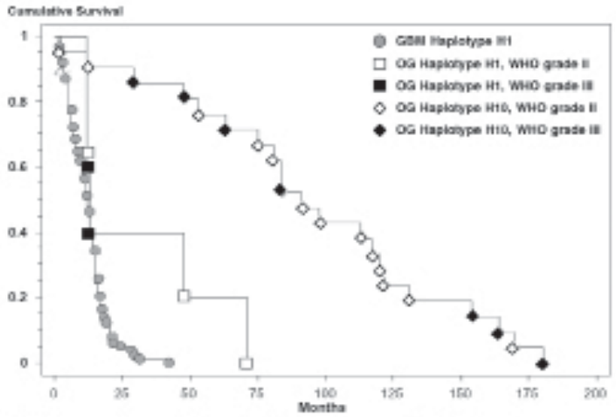
Identification of glioma Notch2 pathway and development of combinatorial therapeutic interference

Loss of NOTCH2 positively predicts survival in subgroups of human glial brain tumors. The structural complexity of chromosome 1p centromeric region has been an obstacle for fine mapping of tumor suppressor genes in this area. Loss of heterozygosity (LOH) on chromosome 1p is associated with the longer survival of oligodendroglioma (OD) patients. To test the clinical relevance of 1p loss in glioblastoma (GBM) patients and identifiy the underlying tumor suppressor locus, we constructed a somatic deletion map on chromosome 1p in 26 OD and 118 GBM. Deletion hotspots at 4 microsatellite markers located at 1p36.3, 1p36.1, 1p22 and 1p11 defined 10 distinct haplotypes that were related to patient survival. We found that loss of 1p centromeric marker D1S2696 within NOTCH2 intron 12 was associated with favorable prognosis in OD (P=0.0007) as well as in GBM (P=0.0175), while 19q loss, concomitant with 1p LOH in OD, had no influence on GBM survival (P=0.918). Assessment of the intrachromosomal ratio between NOTCH2 and its 1q21 pericentric duplication N2N (N2/N2N-test) allowed delineation of a consistent centromeric breakpoint in OD that is also contained in a minimally lost area in GBM. OD and GBM showed distinct deletion patterns that converged to the NOTCH2 gene in both glioma subtypes. Moreover, the N2/N2N-test disclosed homozygous deletions of NOTCH2 in primary OD. The N2/N2N test distinguished OD from GBM with a specificity of 100% and a sensitivity of 97%. Combined assessment of NOTCH2 genetic markers D1S2696 and N2/N2N predicted 24-month survival with an accuracy (0.925) that is equivalent to histological classification combined with the D1S2696 status (0.954) and higher than current genetic evaluation by 1p/19q LOH (0.762). Our data propose NOTCH2 as a powerful new molecular test to detect prognostically favorable gliomas.

Combination of sublethal concentrations of an EGFR inhibitor and a microtubule stabilizer induces apoptosis of glioblastoma cells. The oncogenic EGFR pathway triggers downstream PI3K/RAS-mediated signaling cascades. Complete blockade of EGFR activation does not result in apoptosis in human GBM cells, suggesting additional cross-talk between downstream pathways. Based on these observations, we investigated combination therapies using protein kinase inhibitors (PKI) against EGFR, PDGFR and mTOR assessing GBM cell survival. Clinically relevant doses of AEE788, Gleevec (Imatinib) and RAD001 (everolimus) alone or in combinations did not induce GBM cell apoptosis. In contrast, simultaneous inactivation of the EGFR downstream targets MEK and PI3K by U0126 and wortmannin triggered rapid tumor cell death. Blocking EGFR with AEE788 in combination with sublethal concentrations of the microtubule stabilizer patupilone also induced apoptosis and reduced cell proliferation in GBM cells, accompanied by reduced AKT and ERK activity. These data underline the critical role of the PI3K/AKT and the RAS/RAF/MEK/ERK signaling cascades in the cell-intrinsic survival program of sensitive GBM cell lines. We conclude that drug combinations, which down-regulate both ERK and PKB/AKT activity, may prove effective in overcoming cell resistance in a subgroup of GBM.

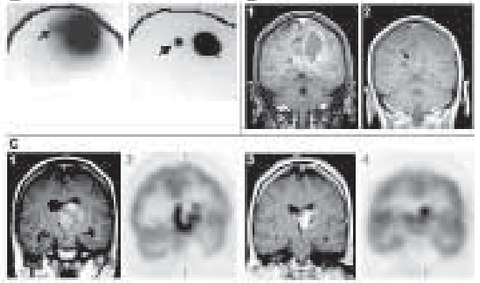
Histone deacetylase inhibition and blockade of the glycolytic pathway synergistically induce cance cell death. Since tumorigenesis is considered a multi-step process of accumulating mutations affecting distinct signaling pathways, combinations of compounds that inhibit non-overlapping pathways are being explored to improve treatment of gliomas. Histone deacetylase (HDAC) inhibitors (HDIs) have proven anti-tumor activity by blocking cell proliferation, promoting differentiation and inducing tumor cell apoptosis. In this report, we show that the HDIs trichostatin A (TSA), sodium butyrate (NaB) and low nanomolar doses of LAQ824 combined with the glycolysis inhibitor 2-deoxy-D-glucose (2-DG) induce strong apoptosis in cancer cell lines of brain, breast and cervix in a p53-independent manner. HDIs upregulate p21, which is blocked by concomitant administration of 2-DG. We propose to simultaneously block histone deacetylation and glycolysis as a novel therapeutic strategy for several major cancers.

Supporting Information

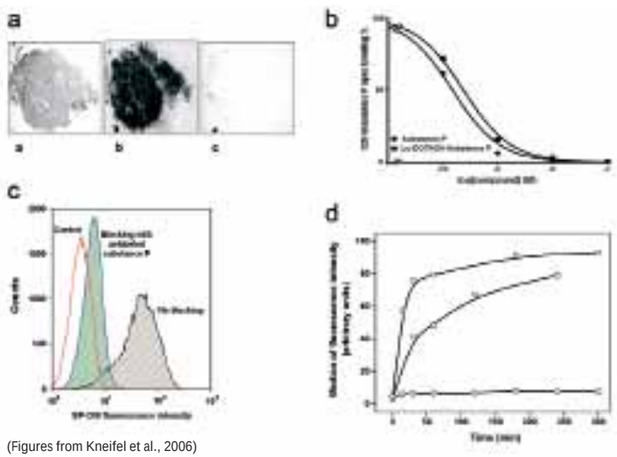


Kaplan-Meier cumulative survival curve of OD Haplotype H10 compared to OD Haplotype H1 and GBM Haplotype H1.

(Details see Boulay et al., 2007)



(Figures from Kneifel et al., 2006)



(Figures from Kneifel et al., 2006)

Connection to Clinical Practice

Local Targeting of Malignant Gliomas by the Diffusible Peptidic Vector DOTA-Substance P

Malignant glial brain tumors consistently overexpress neurokinin-type 1 receptors. The complex geometry of rapidly proliferating high-grade gliomas requires a diffusible system targeting tumor-associated surface structures to saturate the tumor, including its margins. We developed a new targeting vector by conjugating the chelator 1,4,7,10-tetraazacyclododecane-1-glutaric acid-4,7,10-triacetic acid to Arg1 of substance P, generating a radiopharmaceutical with a molecular weight of 1,806 Da and an IC50 of 0.88 F 0.34 nmol/L. Cell biological studies were done with glioblastoma cell lines. Neurokinin type-1 receptor (NK1R) autoradiography was done with 58 tumor biopsies. For labeling, 90Y was mostly used. To reduce the “cross-fire effect” in critically located tumors, 177Lu and 213Bi were used instead. In a pilot study, we assessed feasibility, biodistribution, and early and long-term toxicity following i.t. injection of radiolabeled 1,4,7,10-tetraazacyclododecane-1-glutaric acid-4,7,10-triacetic acid substance P in 14 glioblastoma and six glioma patients of WHO grades 2 to 3. Autoradiography disclosed overexpression of NK1R in 55 of 58 gliomas of WHO grades 2 to 4. Internalization of the peptidic vector was found to be specific. Clinically, the radiopharmeutical was distributed according to tumor geometry. Only transient toxicity was seen as symptomatic radiogenic edema in one patient (observation period, 7-66 months). Disease stabilization and/or improved neurologic status was observed in 13 of 20 patients. Secondary resection disclosed widespread radiation necrosis with improved demarcation.

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- Boulay JL, Miserez AR, Zweifel C, Sivasankaran B, Kana V, Ghaffari A, Luyken C, Sabel M, Zerrouqi A,WasnerM,van Meir E,Tolnay M, Reifenberger G, Merlo A. (2007). Loss of NOTCH2 Positively Predicts Survival in Subgroups of Human Glial Brain Tumors. PLoS ONE 2(6): e576.
- Failly M, Korur S, Egler V, Boulay JL, Lino MM, Imber R, Merlo A. (2007). Combination of sublethal concentrations of epidermal growth factor receptor inhibitor and microtubule stabilizer induces apoptosis of glioblastoma cells. Mol Cancer Ther 6, 773-781.
- Kneifel S, Cordier D, Good S, Ionescu MCS, Ghaffari A, Hofer S, Kretzschmar M, Tolnay M, Apostolidis C, Waser B, Arnold M, Mueller-Brand J, Maecke HR, Reubi JC, Merlo A. (2006). Local Targeting of Malignant Gliomas by the Diffusible Peptidic Vector 1,4,7,10-Tetraazacyclo-dodecane-1-Glutaric Acid-4,7,10-Triacetic Acid-Substance P. Clin Cancer Res 12, 3843-3850.
- Beutler D, Avoledo P, Reubi JC, Maecke HR,Mueller-Brand J,MerloA, Kuehne Th. (2005). Three-Year Recurrence-Free Survival in a Patient with Recurrent Medulloblastoma After Resection, High-Dose Chemotherapy and, Intrathecal Yttrium-90-Labeled DOTA0-D-Phe1-Tyr3-Octreotide Radiopeptide Brachytherapy. High-dose chemotherapy and targeted radiotherapy for relapsing medulloblastoma. Cancer 103, 869-73.
- Kneifel S, Bernhardt P, Uusijärvi H, Good S, Plasswilm L, Buitrago-Téllez C, Mueller-Brand J, Maecke H, Merlo A. (2007). Individual Voxelwise Dosimetry of Targeted 90Y-labelled Substance P Radiotherapy for Malignant Gliomas. Eur J Nucl Med Mol Imaging, 34, 1388-1395.

Spinal cord injury
17beta-estradiol
Neuroprotection
Astrocyte
Functional recovery

Neurosurgery

PD Dr. med. Oliver Hausmann
Department of Biomedicine
University Hospital Basel

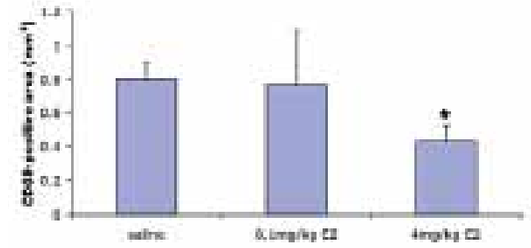
Group Members
Dr. Marie-Françoise Ritz
Yann Curin (PhD student)

Neuroprotective effects of 17β-estradiol in spinal cord injury

Spinal cord injury (SCI) occurs mostly in young people as a result of traffic or sports-related accidents and leads to severe neurological deficits such as paraplegia and quadriplegia.
Secondary complications associated with initial injury, such as urinary tract infection, cardiac and respiratory dysfunctions are the leading causes of long-term-morbidity or even mortality after acute spinal cord injury. The study of SCI pathophysiology immediately highlights the concept of a two-step process involving primary and secondary mechanisms. After the initial mechanical deformation of the spinal cord, a cascade of biochemical and cellular processes initiates further cellular damage and cell death, known as the secondary injury. The secondary injury mechanisms include vascular changes (including ischemia, vasospasms, haemorrhages and thrombosis), ionic disturbances, neurotransmitter (glutamate) accumulation, generation of free radicals (NO), edema, depletion of energy substrates, and activation of a variety of proteases including caspases, phospholipases, endonucleases and metalloproteinases. This active and progressive spread of damage results from a process that begins within minutes and continues for weeks after the initial injury. It constitutes the chronic phase of SCI, in which limited functional recovery occurs. Unfortunately, this secondary segmental neuronal loss is responsible for an often deleterious secondary functional worsening. Inflammation is one key-player that may exacerbate the spreading of the initial lesion. After experimental SCI, transcripts of pro-inflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor alpha (TNFα) are up-regulated within the first few hours in the injured environment.
Considering the multifactorial nature of the secondary spinal cord injury, drugs directed against single target may be ineffective.
Estrogen has been shown to possess neuroprotective activities and to modulate brain neurotransmitter transmission. Studies using in vivo and in vitro models of neurodegenerative disorders such as Alzheimer and Parkinson's diseases suggest that estrogens provide neuroprotection of the central nervous system (CNS) cells. 17β-estradiol exerts multiple neuroprotective effects through both receptor-dependent and -independent mechanisms. These effects include lipid antioxidant activity and attenuation of reactive oxygen species either by direct radical scavenging action or through preservation of endogenous antioxidants. Another important potential mechanism of neuroprotection of 17β-estradiol is its ability to induce and support the expression of the anti-apoptotic factor Bcl-2 and to decrease apoptotic cellular death. 17β-estradiol treatment increases Bcl-2 expression in neuronal cell cultures. It has also been reported that 17β-estradiol exerts protective effects against excitotoxic ischemic damage by reducing NMDA-induced calcium influx through the stimulation of MAP-kinase signal transduction pathways.
Moreover, we have shown that acute treatment with 17β-estradiol reduces excitatory amino acids release induced by transient cerebral ischemia. We evaluated the effects of an immediate treatment with a single physiological dose (0.1 mg/kg) and a supra-physiological dose (4 mg/kg) of 17β-estradiol on the functional locomotor outcome over 4 weeks following spinal cord compression in male rats. In parallel, release of pro-inflammatory cytokines, activation of astrocytes, and the development of the lesion were evaluated during this period.
Our study demonstrates the stimulating effect of 17β-estradiol on the release of the inflammatory cytokines IL-1α, IL-1β and IL-6, the acceleration of astrogliosis in the vicinity of the injury site, limiting inflammatory cells diffusion (Fig. 1) and leading to an improved functional outcome during the critical acute phase of secondary damage following SCI.

We are now investigating the molecular pathways involved in these stimulations, and we will test the efficacy of treatments at later time points, that are more clinically relevant as well as of repeated treatments. Moreover, the effect of this treatment on revascularization of the injured spinal cord and regeneration of axons will be analyzed.

1A



1B

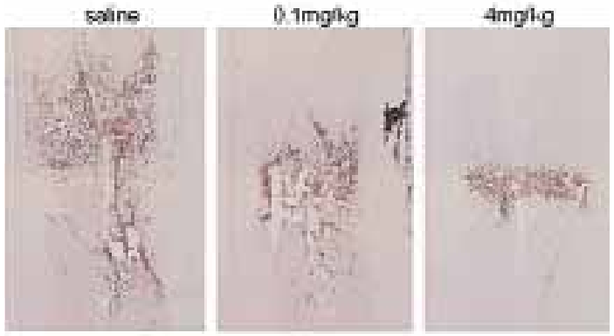


Fig. 1: Relative amount of CD68-positive cells 1 week following SCI in saline and 17β-estradiol-treated rats (n=3 for each condition), expressed as the percentage of CD68-positive immunoreactivity in the region of interest (A) and representative pictures of horizontal sections of injured spinal cords from saline and 17β-estradiol-treated rats stained with antibodies against CD68 (B). Treatment with estradiol significantly reduced the number and spreading of inflammatory cells in the parenchyma surrounding the lesion. Magnification 25 x.

Selected Publications

– Hausmann, O. N., Fouad, K., Wallimann, T., and Schwab, M. E. (2002). Protective effects of oral creatine supplementation on spinal cord injury in rats. Spinal Cord 40, 449-456.
– Hausmann, O. N., Hu, W. H., Keren-Raifman, T., Witherow, D. S., Wang, Q., Levay, K., Frydel, B., V, Z. S., and J, R. B. (2002). Spinal cord injury induces expression of RGS7 in microglia/macrophages in rats. Eur J Neurosci 15, 602-612.
– Hausmann, O. N. (2003). Post-traumatic inflammation following spinal cord injury. Spinal Cord 41, 369-378.
– Ritz, M.-F., Schmidt, P., and Mendelowitsch, A. (2002). 17b-estradiol effect on the extracellular concentration of amino acids in the glutamate excitotoxicity model in the rat. Neurochem Res 27, 1669-1675.

DBM Focal Area
Cell Plasticity and Tissue Repair

Focal Area Coordinators

Prof. Dr. R. Zeller
Department of Biomedicine
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Division of Hematology
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Cell plasticity and tissue repair constitutes one of the four main focuses within the life science strategy of the University of Basel and the Department of Biomedicine. Research efforts by many groups world-wide during the last decade have established that stem cells of both adult and embryonic origin can be induced to differentiate into the various cell-types that form many of the tissues and organs in the human body. The department is active in various aspects of this fascinating research field with relevance to both basic, mechanistic and clinically applied, translational research.

An increasing number of research groups in the Department of Biomedicine are devoted to studying specific aspects of stem cell biology. Groups active in basic research try to identify and isolate stem cells and to understand how stem cells are maintained in their normal niches within the embryo and/or body. For example, several groups are studying how stem cells of the blood are maintained in the bone marrow, differentiate into the various different cell-types of the haematopoietic system and how their differentiation potential is altered in malignant states that result in stem cell-based cancers (e.g. leukaemia or lymphomas). Due to the close interactions of clinical with basic researchers, this research aims to bridge the gap between fundamental and translational research. For example, attempts to grow and differentiate mesenchymal stem cells, which are isolated from human bone marrow, into different cell- and tissue-types in vitro are rather advanced and may e.g. lead to clinically relevant cartilage and bone replacement therapies in the not too distant future. Donor derived haematopoietic stem cells are followed after clinical stem cell transplantation in their new host with respect to their potential to differentiate into haematopoietic cells and their potential to trans-differentiate into cells of other embryonic tissue types. Information gained from these experiments is essential for future, clinically applied tissue engineering.

In spite of these impressive advances, it is important to gain a much better understanding of how stem cells interact with their niche to either maintain their multi-potency or give rise to daughter cells that upon leaving the niche undergo controlled transient amplification in concert with cell-type specification and differentiation. The challenge is to define culture conditions that allow one to maintain stem cells in culture and induce their specification and differentiation into functional tissues in an efficient and controlled manner. As organs and tissues are composed of different, well organised and functionally interacting cell-types, it is important to understand the functions of embryonic signaling centres in the process of tissue patterning und cell-type specification by combining tissue engineering attempts with knowledge gained from analysing cell-type, tissue specification and organogenesis during normal embryonic development. Recent studies by others have begun to reveal the mechanism by which adult cells (e.g. skin cells) can be re-programmed to revert to stem cells, thereby providing an novel source of defined multi-potent progenitor cells for cell-differentiation and tissue engineering studies. The research groups in the department will also have to incorporate the use of such cells, which can be easily obtained from patients, into their experimental strategy. This fits with the strategy of the department to promote collaborative efforts between basic research groups and clinicians to close the gap between the lab bench and patient's bed as much and as fast as possible.

Moreover, the department has realised the need to broaden its collaborative network. Groups at the Biozentrum, the FMI, the University Hospital and research institutions in the industry have come together and founded the Basel Stem Cell Network and many take part in the Swiss Stem Cell Network. The concept paper describing the Basel Stem Cell Network has been approved both by the Faculty of Medicine and the Faculty of Natural Sciences. The goal is to create a Competence Centrum in Stem Cell Research a part of the Life Science Strategy of the University.

Members of the Department of Biomedicine have been key to establishing these two grass-root organisations that have already proven successful in generating the critical mass and awareness for stem cell research, which is in turn essential to generate the necessary extra-mural funding. Further appointments within the areas of basic stem cell research and applied tissue engineering are required to strengthen the ongoing basic and translational research efforts in this rapidly emerging and highly competitive research field.

Myocyte biology
Cytokines
Protein metabolism
Differentiation
Cardiac remodeling
Heart failure

CardioBiology

Prof. Dr. Marijke Brink
Department of Biomedicine
Institute of Physiology
University of Basel
and University Hospital Basel

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Dr. med. Thomas Dieterle
Dr. Frédérique Dubouloz
Dietlinde John (technician)
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Dr. Silvia Meili-Butz
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Bettina Stokar (student)
Dr. Vivian Suarez Domenech*

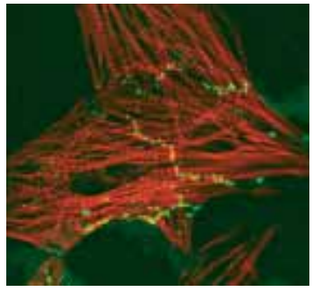
* left during report period

Molecular Mechanisms of Heart Disease

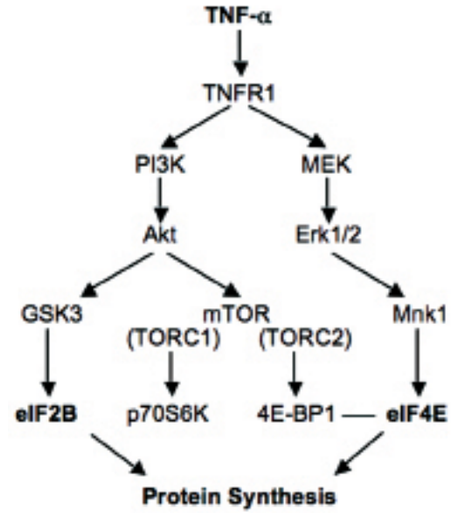
Central in the activities of our laboratory is the molecular understanding of the heart in health and disease. Of the three projects currently ongoing, the first aims to define signaling nodes and pathways by which combinations of hormones and cytokines such as insulin, the insulin-like growth factors (IGF), angiotensin II and tumor necrosis factor (TNF)-α change the expression pattern, the activity and the turnover of cardiac and skeleletal muscle proteins during differentiation and de- or re-differentiation. The second project assesses the function and regulation of the recently discovered urocortins in hypertension and heart failure. In our third project the identification of genetic alterations in patient samples contributes importantly to the understanding and diagnosis of familial cardiovascular disease and frequently directly leads to improved therapy (see also "relation to clinics").
A common theme in our experimental projects is the cardiac remodeling process. Cardiac remodeling refers to the structural and functional adaptations that occur for example in hypertension or after myocardial infarction. The remodeling process in the long-term becomes maladaptive and gradually leads to cardiac chamber dilation and dysfunction, with heart failure as a result, a condition in which the heart is not able anymore to deliver sufficient output for tissue perfusion. The process is thought to be associated with a loss of cardiomyocytes via apoptosis and insufficient cardiac regenerative capacity.

Cardiomyocyte generation and hypertrophy as a result of altered protein degradation? In this project, we are acquiring a mechanistic molecular understanding of the cardiomyocyte, which has to effectively interact, contract and respond to a multitude of stimuli, including hormones and nutrients, in its environment of the healthy or diseased heart. We are currently analyzing the role of the highly selective ubiquitin-proteasome pathway (UPP) in the turnover of myocyte-specific contractile and regulatory proteins. Our analysis of hypertrophic and failing rat hearts revealed distinct changes in muscle-specific E3 ligases which, due to their rate-limiting and highly selective features, are most critical components of the UPP. In differentiated myocytes, we are testing if selected E3 ligases modulate the response to stimuli such as insulin, the insulin-like growth factors, angiotensin II, and TNF-α. Similarly, in myocyte precursor cells, we investigate if the UPP and muscle-specific E3 ligases modulate expression of proteins that drive the differentiation process.
Regarding TNF-α, which in the setting of several severe disease states including heart failure was long considered solely as a catabolic factor, we recently established that it also has a beneficial function in muscle. We demonstrated that TNF-α promotes hypertrophic growth in C_2C_{12} and primary rat myotubes. Moreover, with selective inhibitors we provided evidence that TNF-α increases protein synthesis by enhancing protein translation via the TNF-R1, and that two signaling cascades are implicated: (1) MEK/Erk1/2 and its target eIF4E and (2) PI-3K/Akt and its downstream effectors GSK3, p70S6K and 4E-BP1 (Fig. 1 and Plaisance 2007). Our preliminary data also suggest involvement of mammalian target of rapamycin complex 2, and studies on this topic are currently ongoing with neonatal and adult cardiomyocytes as well as transgenic mice.

Regulation and function of urocortin 2 in hypertensive heart disease. Recent data suggest that the urocortins, novel corticotropin-releasing factor (CRF)-related peptides, modulate cardiovascular function and responses to stress. Our aim in this project is to build a thorough understanding of the mechanisms that regulate Ucn 2 and its receptor in the cardiovascular system, to establish how the peptide exerts its potent cardiovascular physiological actions, and to evaluate its potential for the treatment of hypertension and heart failure in animal models. In recent experiments with salt-sensitive Dahl rats, we established that Ucn 2 gene expression is increased in hypertrophic hearts and that Ucn 2 administration has significant beneficial effects on blood pressure and cardiac function without having effects on Ucn receptor expression. Ucn 2 had direct cardioprotective effects when applied to isolated failing hearts in the Langendorff setup. Using luciferase-linked Ucn 2 promoter expression in cultured cells we found support for the involvement of oxygen and HIF-1 in the transcriptional regulation of the Ucn 2 gene. A thorough knowledge of these effects and mechanisms forms an important basis for a potential future use of Ucn 2 in the therapy of hypertension, cardiac hypertrophy and congestive heart failure.



◄ Fig. 1: Cardiomyocytes isolated from neonatal rat hearts. Staining with rhodamine-phalloidin shows the cross-striated pattern of F-actin in the sarcomeres, and immunolabeling of connexin43 shows intercellular contacts as bright dots (image by Isabelle Plaisance).



► Fig. 2: In myotubes, TNF-α induces multiple pathways that enhance protein translation.

Connection to Clinical Practice

Prof. Dr. Peter Buser
Division of Cardiology
University Hospital Basel

Towards improved prevention and therapy of heart failure

Heart failure is the main epidemic of the next decades. Improved survival of acute coronary disease, valvular and congenital heart disease and changing demographics are the main contributors. In the Division of Cardiology a range of clinical research projects and trials is ongoing (http://www.cardiobasel.ch) and like in our basic science projects, the common focus of these investigations is aimed to improve prevention and therapy of heart failure. Here the projects that run both in the clinical setting and in our basic science lab are described.
The most common genetic cardiac disorders are familial hypertrophic cardiomyopathy and dilated cardiomyopathy, which are structural heart diseases, and the long QT syndrome, the Brugada syndrome, the congenital conduction defect and the congenital sick sinus syndrome, which are diseases of the cardiac ion channels. In our CardioGenetics program, headed by PD Dr. Dagmar I. Keller, patients are based on the clinical diagnosis selected for genetic testing, subsequently performed using PCR with primers to cover all exons and important intronic sites of known disease-causing genes, followed by DHPLC analysis, and confirmation of the genetic variants by sequencing. The genetic test will confirm a clinically determined diagnosis, predict risk and prognosis in a clinically affected patient, and importantly, it provides options for therapy not only in the patient, but also in clinically unaffected relatives who carry the disease-causing mutation. Whenever novel mutations are discovered in ion channel genes, these are assessed for their biophysical characteristics (Keller 2006).
Clinical investigations in the setting of the urocortin project of Dr. Thomas Dieterle include the analysis of plasma samples of heart disease patients, while aiming to assess its potential as diagnostic marker for heart failure patients. We have also measured tissue and plasma IGFs, their binding proteins and the IGF receptors in relation to plasma levels of inflammatory cytokines.

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Angiogenesis
Myoblasts
Mesenchymal stem cells
Gene therapy
Cell therapy
Ischemia

Cell and Gene Therapy

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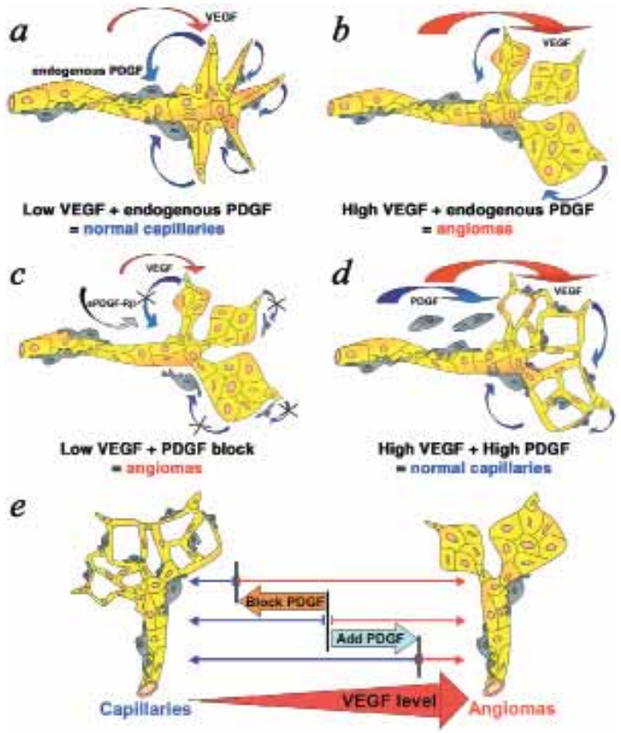
Dr. med. Thomas Wolff
Dr. med. Philipp Fueglistaler
Dr. med. Heidi Misteli

Cell and gene therapy for controlled angiogenesis in regenerative medicine

Therapeutic angiogenesis aims at restoring blood flow to ischemic tissues by the formation of new vessels. Our research focuses on understanding the basic principles governing the growth of blood vessels and translating this knowledge into therapies for peripheral and myocardial ischemic diseases. We use precursor cells genetically engineered to express controlled levels and combinations of angiogenic factors, in order to provide both vascular growth and tissue regeneration, combining the specific advantages of cell and gene therapy.
Vascular endothelial growth factor (VEGF) is the most potent and specific known angiogenic factor. However, its effects can be deleterious if uncontrolled, leading to increased leakiness of blood vessels and the induction of vascular tumors (angiomas).We are developing novel methods to deliver the VEGF gene alone or in combination with maturation factors, in order to increase safety and expand its therapeutic window in vivo. We are further applying these methods to multipotent mesenchymal progenitors to achieve rapid vascularization of tissue engineered constructs for bone and cardiac regeneration, in collaboration with groups in Switzerland and the USA.

1) Controlled microenvironmental expression of VEGF
We have recently shown that the therapeutic window of VEGF delivery does not depend on the total dose administered, but rather on the microenvironmental levels of expression (Ozawa et al, J Clin Invest 2004). In fact, since VEGF remains tightly localized in tissue around the cells producing it, different growth factor concentrations do not average each other, even between neighboring muscle fibers. Therefore, a few "hotspots" of high expression are sufficient to cause hemangioma growth even if the total VEGF dose is rather low. This finding helps to explain the apparent difficulty to achieve a manageable therapeutic window in clinical trials of VEGF gene therapy. In fact, currently employed gene therapy methods, such as direct injection of constitutive adenoviral and plasmid vectors, only allow control on the total dose (titer) of gene delivered, but not the distribution of microenvironmental levels in vivo. Therefore, in order to avoid even rare "hotspots" of expression, the total dose must be kept low and efficacy is wasted (Banfi et al, Curr Atheroscl Rep 2005).
In order to safely deliver therapeutically efficacious levels of the VEGF gene, it is desirable to rapidly purify cells expressing the required VEGF level from a randomly transduced autologous population. We have recently developed a Fluorescence Activated Cell Sorter (FACS)-based technology to predict the level of VEGF expression in single live cells and to purify populations homogeneously expressing specific levels, yielding only normal and stable vessel growth, while completely avoiding angioma growth.

2) Co-delivery of VEGF and maturation factors
VEGF can induce normal capillaries at low levels and angiomas at high levels and the transition between normal and aberrant angiogenesis does not occur gradually, bur rather as an all-or-none response across a threshold VEGF dose. However, we found that such threshold is not an intrinsic property of VEGF dose, but depends on the balance between angiogenic stimulation induced by VEGF and vascular maturation mediated by PDGF-BB signaling and pericyte recruitment. (Fig. 1, Banfi et al, manuscript submitted).
Current projects are aimed at understanding the mechanism by which PDGF-BB modulates VEGF-induced angiogenesis and determine the dose-dependent effects of their coexpression. Furthermore, VEGF and PDGF-BB coexpression leads to homogeneous normal angiogenesis despite heterogeneous expression levels. Therefore, we hypothesize that PDGF-BB coexpression can overcome the requirement for control on the microenvironmental level distribution of VEGF and make adenoviral gene therapy approaches, which are unsuitable to VEGF alone, safe and efficacious.
The results of these projects should provide fundamental knowledge necessary for the design of future clinical trials in patients suffering from chronic myocardial ischemia and perypheral artery disease.



Selected Publications

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ABC-transporter
Drug-drug interaction
Pharmacokinetics
P-glycoprotein
Blood-brain barrier
Multidrug resistance

Clinical Pharmacology

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* left during report period

Impact of ABC transporters on drug transport, drug-drug interactions, multidrug resistance and their role in disease processes

Drug disposition is a major determinant for the therapeutic efficacy or toxic effects of drugs. It is therefore important how a drug is absorbed, distributed and excreted in the body. Small and mildly to moderately lipophilic drugs are often passively absorbed, whereas large or polar/charged molecules need transport mechanisms to pass biological membranes in order to enter the body or the site of action. The existence of drug transporting proteins in intestine, liver, kidney and the blood-brain barrier is a major factor influencing drug absorption and disposition. Among the transport proteins, those belonging to the ATP-binding cassette (ABC) superfamily, the so-called ABC-transporters, are of special importance.
Members of the ABC transporter family are proteins like P-glycoprotein (ABCB1), breast cancer resistance protein (ABCG2) and several other multidrug resistance related proteins (ABCC1-5) are a main topic in our research group. They were first detected overexpressed in human cancer, where they mediate drug resistance to cancer therapy. In general, they affect drug transport at the level of intestinal drug absorption, in limiting the distribution of drugs towards different tissues such as CNS (blood-brain barrier) and contribute to active elimination of drugs in liver and kidney. A major source for drug-drug interactions is based on the competition of drugs for the same transporter-binding site. Furthermore, gene induction of transports by several drugs such as rifampicin, phenytoin, nelfinavir and St. John's worth represents a fundamental source for changes in the pharmacokinetics of drugs.
Our research group is interested in the impact of ABC transporter for absorption, distribution and elimination of drugs. In this context the generation of expedient in vitro models to study ABC-transporter mediated mechanisms of drug transport is of interest. During the last two years, we established and characterized in vitro models that constitute important barriers for intestinal drug absorption, renal elimination and blood-brain barrier transport.
A model for renal excretion of drugs based on primary kidney proximal endothelial cells was established in our laboratory and characterized with respect on impact of transporters on renal drug elimination. Furthermore we published also data on the role of GLP1 receptor in this system. Besides the gastric peptide GLP1, several other gastric peptides such as ghrelin and CCK where investigated in different clinical studies.

One special area of our research is the function of the blood brain barrier. Herein we characterized an immortalized human brain capillary endothelial cell line with respect to ABC transporter function and barrier properties. Further research in this field is currently ongoing.

The main focus during the last two years aimed towards the role of transport proteins in the intestine. Here we performed a detailed analysis of ABC transporter expression along the intestinal tract in human biopsies, tested the effect of anti-inflammatory drugs such as budesonide on transporter expression and investigated the effect of surfactants on P-glycoprotein, leading to changes in intestinal talinolol absorption. Furthermore we could show, that the antibiotic flucloxacillin induced P-glycoprotein expression and cytochrome P450 (CYP) 3A4 in the intestine. Natural green tea extract has also been shown to affect intestinal CYP P450. Of special interest in our research, is the influence of diseases on functional expression of intestinal transportes. Here we could show, that the ileal sodium dependent bile acid transporter as well as breast cancer resistance protein is decreased in the duodenum of patients with obstructive cholestasis. In a current project, we investigated the expression patterns of several ABC transporters in biopsies of patients with ulcerative colitis. We could show that this patients exhibit a decreased expression of certain transporters in inflamed but not in uninflamed sites of the mucosa which could contribute to an enhanced accumulation of toxic compound and changes of drug pharmacokinetics.

Connection to Clinical Practice

Impact of ABC transporters in drug disposition and inflammatory diseases

Drug transporters such as P-glycoprotein, breast cancer resistance protein and multidrug resistance-associated proteins in the intestinal wall restrict oral bioavailability of drugs. Knowledge about the expression patterns of these transporters is therefore useful to develop new targeting strategies for enteral drug delivery. We investigated systematically site-specific expression of P-glycoprotein, breast cancer resistance protein and MRP1-5 along the gastrointestinal tract in human biopsies and could show localisation specific differences in expression patterns.
Furthermore, we could show in samples of patients with ulcerative colitis and inflammatory colitis that the expression of transporters is changed during acute inflammation returning back to normal levels after remission of the disease. Pathophysiologically, a decreased expression of certain efflux pumps might increase the accumulation of food-derived carcinogens and toxins and thereby trigger inflammatory processes. In addition, it may influence the pharmacokinetics of various drugs. We could also show by in vitro and in vivo evaluations that ABC transporters are able to influence the absorption, distribution and elimination of drugs and to modulate therapeutic effects. They constitute therefore a clinically important source of severe drug-drug interactions. Knowledge of the impact of transporters can help to explain clinically observed drug-drug interactions and might help to improve safety of individual drug therapy in the future.

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Mitochondria
Apoptosis
Necrosis
Toxicity
Carnitine
Liver

Clinical Pharmacology

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Effects on energy metabolism as a cause of drug toxicity

Our group mainly focuses on the toxicity of drugs on the liver, skeletal muscle and/or bone marrow. Special emphasis is placed on the mechanisms of cell death, in particular apoptosis and necrosis. In recent projects, we have investigated the toxicity of amiodarone and benzbromarone on hepatocytes and the toxicity of statins on skeletal muscle. Current projects include the toxicity of clopidogrel and ticlopidine on hepatocytes and on myelocytes, the molecular mechanisms of toxicity of statins on skeletal muscle and other cells and the cellular toxicity of amiodarone and amiodarone derivatives on hepatocytes and pneumocytes in relation to hERG channel inhibition.
We have shown previously that mitochondrial diseases can be risk factors for the manifestation of toxicity associated with idiosyncratic toxins. In order to investigate this hypothesis in more detail, we studied the toxicity of valproate in an animal model with carnitine deficiency. Since carnitine deficiency is associated with impaired mitochondrial β-oxidation, it could also be regarded as a mitochondrial disease. As expected, valproate turned out to be more hepatotoxic in carnitine deficient as compared to wild type mice, supporting our hypothesis. Interestingly, although the skeletal muscle carnitine content was also decreased in carnitine deficient mice, valproate showed no skeletal muscle toxicity.
Statin-associated toxicity towards skeletal muscle is another problem we studied in detail. We could demonstrate previously that statins are mitochondrial toxins. In future studies, we plan to perform in vivo experiments in mice with a defect in β-oxidation (e.g. mice with carnitine deficiency or mice with a knock-out of an enzyme involved in β-oxidation) to test the hypothesis that underlying mitochondrial diseases are risk factors for statin-associated rhabdomyolysis.
In order to be able tom screen drugs for idiosyncratic toxicity, such risk factors have to be known and to be introduced into suitable in vitro systems. Defects in β-oxidation or other mitochondrial functions can be introduced in suitable cells, which could then serve as screening tool for toxicity studies. Such experiments are currently going on.

Energy metabolism

Further fields of interest of our research group are mitochondrial function and metabolism, in particular concerning β-oxidation and carnitine. Carnitine is a small molecule needed for transport of long-chain fatty acids into the mitochondrial matrix. We have developed a sensitive LC/MS-method for the determination of carnitine and acylcarnitines. Current projects include the investigation of carnitine homeostasis in vegetarians, the regulation of the expression of the carnitine carrier OCTN2 and investigations of the interactions of drugs and other compounds with OCTN2, which can potentially lead to secondary carnitine deficiency.
We have finished a study in healthy vegetarians where we assessed carnitine homeostasis before and after treatment with carnitine (2g a day for 3 months). As expected, skeletal muscle carnitine stores were decreased in vegetarians due to reduced dietary intake of carnitine and carnitine precursors. After treatment with carnitine, there was no difference in the skeletal muscle carnitine content between control subjects and vegetarians, suggesting accumulation of carnitine in skeletal muscle of vegetarians during treatment with carnitine. Vegetarians had a higher skeletal muscle expression of OCTN2, supporting these findings.
We have prepared cells with stable overexpression of OCTN2 which we can use for carnitine transport assays. Using these cells, we could study the interactions of many drugs with OCTN2 and interactions of carnitine reabsorption with acylcarnitines. We could for instance demonstrate that acylcarnitines are competitive inhibitors for renal carnitine reabsorption and that acylcarnitines can also be transported by OCTN2.
In further projects, we plan to study the regulation of OCTN2 expression in skeletal muscle and in kidney. Furthermore, we are currently developing mice with organ specific over-expression of OCTN2 in order to get more insight in cellular functions of carnitine.

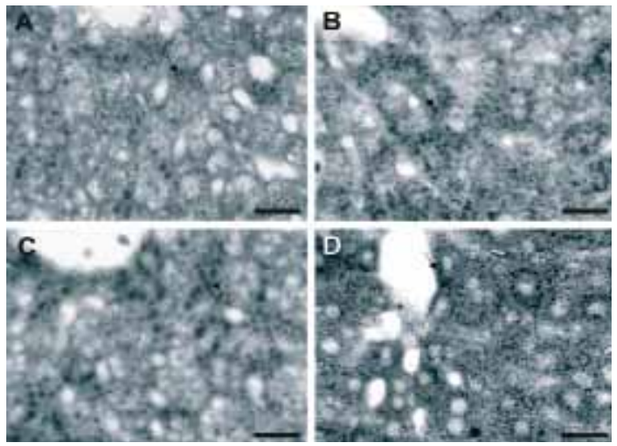

Fig. 1: Hepatic accumulation of fat in vehicle-treated wild type (A), valproate-treated wild type (B), vehicle-treated jvs+/- mice (C) and valproate-treated jvs+/- mice (D). Jvs mice have systemic carnitine deficiency due to a mutation in OCTN2, the renal carnitine transporter. Vehicle-treated wild type livers contain only few hepatocytes with Sudan B stainable material (small intracellular dark droplets, see arrows) (A). Valproate treatment of wild type mice for two weeks (B) or heterozygousity for OCTN2 (vehicle-treated jvs+/- mice) (C) are both associated with a slight increase in microvesicular fat. VPA-treated jvs+/- mice show the highest accumulation of microvesicular fat, mainly in the pericentral region of liver lobules (D). Sudan black B staining, the micron bars represent 20 μm.

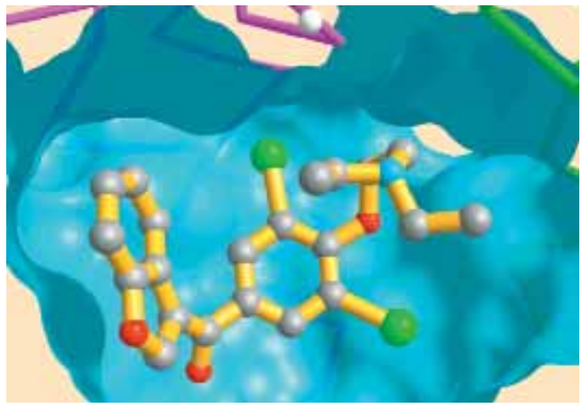

Fig. 2: Docking of amiodarone into the vestibule of a hERG model. hERG channels are potassium channels responsible for the repolarization of depolarized cardiomyocytes. Two of the four chains of the hERG homo-tetramer are shown in C-alfa representation (green, magenta). The Connolly surface was produced with a probe radius of 1.4Å. A white ball represents a potassium ion in the filter region. Amiodarone fits exactly into the vestibule of the channel and impairs potassium conductivity.

Connection to Clinical Practice

Preventing of idiosyncratic drug toxicity

Many of our problems studied in basic research origin from clinical questions. Idiosyncratic drug toxicity is a potentially serious problem for affected patients. It is therefore important to have accurate test systems for this type of toxicity as screening tools before drugs enter the market and to accurately diagnose this type of toxicity in patients.
The first steps in achieving these aims are to obtain more information about the mechanism of action of this type of toxicity and to find out risk factors for its appearance. Many of our projects described in the preceding section focus on these areas. We have for instance identified the importance of mitochondrial damage for the toxicity of amiodarone, benzbromarone, valproate and also statins. Furthermore, at least for valproate, we could show that impaired β-oxidation is a risk factor for hepatotoxicity. Once mechanisms and risk factors have been identified, future steps include the formation of easy to use and accurate tools for drug screening and for diagnosing patients. This type of research can therefore potentially bring a large improvement for drug safety.

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Skin lesion
Hereditary cancer
Genetics

Dermatology

new group since July 2006

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Hereditary cancer and skin lesions

The new research lab of dermatology has started with work since July 2007 and grows up continuously.
In our first study, we examine a large number of patients carrying genetic predisposition for specific cancer syndromes. Many patients show skin lesions characteristically for the single cancer syndrome. These lesions are more frequent in patients with special cancer than in the general population. Unfortunately, in the majority of cases the cause of these skin lesions is unknown and no connection is known between mutations site and type of skin lesion. Carriers of interfamilial identical mutations exhibit different phenotype relating to skin lesions, even in families with the same mutation in the same predisposition gene the occurrence of such skin lesions varies between individuals. Modifier genes or other reasons for the development of skin manifestations are currently still enigmatic and the correlation to the cancer predisposition remains unclear, mainly of lack on studies. Knowledge of modifier genes would essentially contribute to a better presymptomatic treatment of patients. Until now it's impossible to make a prognosis for developing these lesions or not and for their recurrence after excision, and to do a prediction to the patient.
Our project aims at understanding skin lesions developing in some patients with special kind of hereditary cancer. Such knowledge makes it possible to give a better consultation and make an individual risk evaluation for developing different skin lesions. The skin lesions we will analyse are benign tumors such as fibromas, epidermal cysts, and lipomas. First, we have to analyse a broad range of different lesions (kind of lesion like epidermal cysts, fibroma, lipoma etc., further affected organs, histology of neoformation) as it occurs in indexpatient and his family getting the necessary data. Second, the skin lesions of patients will be analysed for gene expression, somatic mutations, and chromosomal rearrangements, compared with a control group. Because of the methodical comparison of patients carrying the same mutation affiliated to the same family or different and controls of the common population we are able to look for unknown effects (genetically or environmentally) affecting the manifestation of skin lesions, subsequently realising the principle why some patients with predisposition for cancer are affected by skin lesions and others are unaffected.
To identify associations between the skin lesion and the mutations responsible for cancer will help to appreciate the skin lesion, resulting in an improved therapy and follow-up strategies, and give a better consultation to the patients.

Selected Publications

– Lugassy, J., Itin, P., Ishida-Yamamoto, A., Holland, K., Huson, S., Geiger, D., Hennies, H.C., Indelman, M., Bercovich, D., Uitto, J., et al. (2006). Naegeli-Franceschetti-Jadassohn syndrome and dermatopathia pigmentosa reticularis: two allelic ectodermal dysplasias caused by dominant mutations in KRT14. Am. J. Hum. Genet. 79, 724-730.
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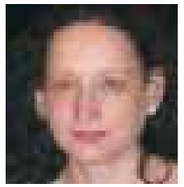
Embryogenesis
Kidney
Limb
Mouse genetics
Signaling
Signal antagonism
Transcriptional regulation

Developmental Genetics



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Genetic and Functional Analysis of Cell-Cell Signaling during Vertebrate Organogenesis

During development of a vertebrate embryo, the formation of functional organs is controlled by cell-cell signaling interactions (= cell communication). In particular, these interactions define the cells that will participate in formation of different organs by controlling cell proliferation, determination of their identities and differentiation.

We are interested to understand how particular embryonic cells that have the capacity to "organize" the development of organs such as kidneys and limbs (= arms and legs) communicate with the up to several 100'000 other cells required to make the tissues. We are using mouse molecular genetics in combination with organ rudiment cultures and cell-biochemical analysis to analyse the rather complex signaling feedback loops that control orderly progression of kidney and limb development. Our current research aims at understanding how such signaling feedback loops are established and generate stable cell-cell communication networks. Interestingly, we were able to show that the initiation of e.g. kidney development requires inhibition of the BMP4 signal a specific antagonist called Gremlin1. In fact, its is becoming increasingly clear that vertebrate embryonic cells produce many antagonists, which indicates that cells try to tune down/turn off signals once they have received them. Therefore, we now study mostly how the balanced interplay of signals and antagonists controls normal development. We also aim to gain insight into how a small number of embryonic cells are selected to form an organizer and turn on the production of potent signals, which instruct embryonic cells with respect to their proliferation and differentiation potential. The understanding of how this organizer potential is suppressed is as important as understanding how an organiser forms as deregulated organizer signaling results in severe congenital malformations. For our studies, we take advantage of the excellent molecular genetic approaches in the mouse, which is the best model to analyse embryonic development of mammals. In addition, we analyse organ development in vitro, which allows us to combine genetics with experimental manipulation. Such systematic genetic and cell-biochemical analysis of organ development in mouse embryos will enable us to uncover the molecular networks that orchestrate the formation of complex and functional tissues during embryonic development.

Along with other groups, we have recently discovered that the expression of key regulatory molecules such as the Sonic hedgehog (SHH) morphogen and the BMP antagonist Gremlin1 are controlled by very far away cis-regulatory elements. Their respective transcription units are embedded into large chromosomal landscapes, which control the complex and dynamic regulation of their expression during embryogenesis. These chromosomal landscapes encompass in general several structurally and functionally unrelated genes, whose expression is co-regulated e.g. during limb bud development by a so-called global control region (GCR). At present, it is unclear how these GCRs function and to what extent classical enhancer/repressor elements and epigenetic modifications may play a role in GCR-mediated gene regulation. We are currently in progress to molecularly and genetically dissect the GCR regulating Gremlin1 expression and identify the essential transacting factors interacting with the 800 kb upstream regulatory region required for Shh expression in limb buds. Mutations in the Shh regulatory region cause congenital limb malformations in humans, which underscores the importance of tight transcriptional regulation of this key regulatory signal during development. Aberrant expression of these key regulatory signals can induce tumorigenesis. Therefore, we will also use our genetically altered mouse strains for the study of particular types of tumour models. We hope to make important contributions to the understanding of the molecular alterations underlying the initiation and progression of neoplastic cell transformation.

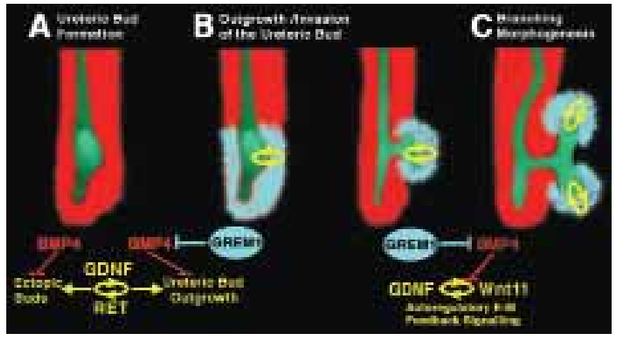

Fig. 1: Local Reduction of BMP4 Activity by the BMP Antagonist GREMLIN1 induces Metanephric Kidney Development
(Michos et al., 2007, Development 134, 2397)

Reduction of BMP4 activity by GREMLIN1 in the mesenchyme around the ureteric bud is essential to enable ureteric epithelial outgrowth, GDNF/RET and Wnt11-mediated e-m feedback signaling and branching morphogenesis.
(A) The ureteric bud forms in the caudal-most part of the Wolffian duct under the influence of GDNF/RET signaling. During this inductive period, Bmp4 is expressed by the mesenchyme enveloping the Wolffian duct. High levels of mesenchymal BMP4 activity (red) inhibit the formation of ectopic epithelial buds and epithelial branching.
(B) Expression of the BMP antagonist Grem1 is up-regulated in the mesenchyme around the mascent ureteric bud, thereby locally reducing BMP4 signal transduction. This reduction of BMP4 activity by GREM1 (light blue) enables initiation of ureteric bud outgrowth and ist invasion into the metanephric mesenchyme.
(C) GREM1 is required to maintain and propagate expression of Wnt11 in the ureteric epithelial tip(s) and Gdnf in the mesenchyme via e-m feedback signaling (yellow), which permits progression of kidney organogenesis.

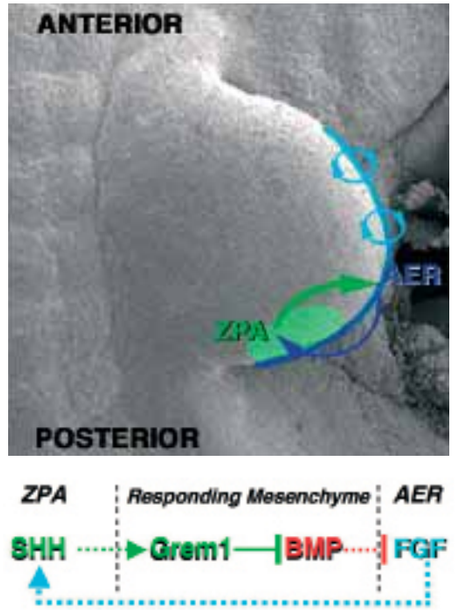

Fig. 2: The Epithelial-Mesenchymal (E-M) Feedback Signaling Interactions that Control Limb Bud Morphogenesis and Digit Patterning
(From Zeller and Zuniga, 2007. Curr. Op. Genet. Dev., 17, 428-434.

The zone of polarizing activity (ZPA, green) and the apical ectodermal ridge (AER, blue communicate by reciprocal feedback sigaling interactions (arrows). SHH signaling by the ZPA up-regulates Grem1 expression the posterior mesenchyme. The extra-cellular GREMLIN1 protein antagonizes BMP ligands, which in turn relieves repression of Ffg expression in the AER. FGF signaling by the AER is required to maintain and propagate Shh expression. The resulting positive signaling feedback loop between the mesenchyme and the AER propagates outgrowth and limb development. Dotted lines represent genetic interactions, while the solid line represents the antagonistic GREM1-BMP interaction.

Selected Publications

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Obesity
Adipocyte
Mitochondrial proteomics
Mitochondrial targeting
Melanoma
Receptor-mediated tumor targeting

Endocrinology

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* left during report period

Human obesity and targeting of mitochondria in fat cells

Impaired function of mitochondria has been associated with metabolic diseases, in particular (morbid) obesity and type 2 diabetes. These diseases, although prevalent in later life, have become an increasing problem also at young age. The molecular details causing dysfunction of mitochondria in patients are poorly understood. Our research project is a collaborative study between the Laboratory of Endocrinology of the DBM, the St.Claraspital Basel (Dr. R. Peterli, Prof. T. Peters), the pediatric and surgical clinics of the UKBB (PD Dr. U. Zumsteg, Prof. J. Mayr), and the MRC Dunn Human Nutrition Unit in Cambridge U.K. (Prof. Sir J.E. Walker, Dr. I. Fearnley), which focuses on a comparative analysis of mitochondrial activity in human visceral and subcutaneous adipose tissues of patients with different degrees of obesity (from normal to morbidly obese). The first part of the project includes (1) functional studies with isolated mitochondria using a range of enzyme and membrane potential assays; (2) determination of mitochondrial copy numbers per cell; (3) mitochondrial proteome analysis by 2D separations and gel electrophoresis as well as by LC-MS-MS mass spectrometry; (4) expression analysis using gene expression profiling, combined with proteome analysis, and (5) analysis of ATP synthase and other key proteins of the respiratory chain. To date, we have analyzed about 100 obese and control patients and found obesity- or diabetes-related changes in the function of the entire respiratory chain or individual complexes of the electron transfer chain. We have also demonstrated that mitochondrial proteome analysis using anion-exchange based chromatography followed by 1D SDS-PAGE is feasible and very robust. The mitochondrial proteome is simplified by fractionation, allowing the identification of differences in protein patterns that can be identified by mass spectrometry.
The second part of the project, a collaboration with Prof. P.W. Schiller, IRCM, Montréal, focuses on mitochondrial targeting in human adipose tissue and isolated adipocytes using short cell-penetrating antioxidant peptides. These peptides have become a promising approach to potentially counteract cellular oxidative injury implicated in many diseases such as diabetes, atherosclerosis, arthritis and other inflammatory as well as neurodegenerative disorders, aging and many more. As opposed to various other antioxidants, the "Szeto-Schiller (SS-)" peptides accumulate up to a 1000-fold at the inner mitochondrial membrane, i.e. near to the site of free radical generation, the cause of oxidative injury. At present, we investigate effects of antioxidant peptides on human adipocytes and adipose tissues as well as undifferentiated and differentiated mouse 3T3 cells as (pre-)adipocyte models. The goal is to identify ways or mechanisms of increased fat mobilization and energy expenditure. Although the mitocount in adipocytes is much lower than, e.g., in muscle or liver, any new insight gained on how to increase the number and activity of mitochondria in fat tissue is relevant for the design of future treatment strategies. Experiments with mouse 3T3 cells have demonstrated that antioxidant SS-peptides markedly elevate the mitocount in differentiated adipocytes. We are in the process of clarifying the combined effect of hormonal stimulation of adipocytes and action of antioxidant peptides on an attempted controlled reduction of fat tissue. Another aim relates to the establishment of a method for routine adipocyte cultures based on surgical fat samples. Although various attempts have been reported in the literature to cultivate human pre- and adipocytes, longer-term cultures could not be established, except with transformation.

Analysis and direct modulation of mitochondrial activity in human fat tissues or cultivated adipocytes is a novel approach in the understanding of human adipocyte regulation. The (expected) identification of key proteins of the mitochondrial proteome which show altered expression and/or function in the obese state may serve as disease markers (outcome, severity) and/or as potential targets for future therapeutic intervention.

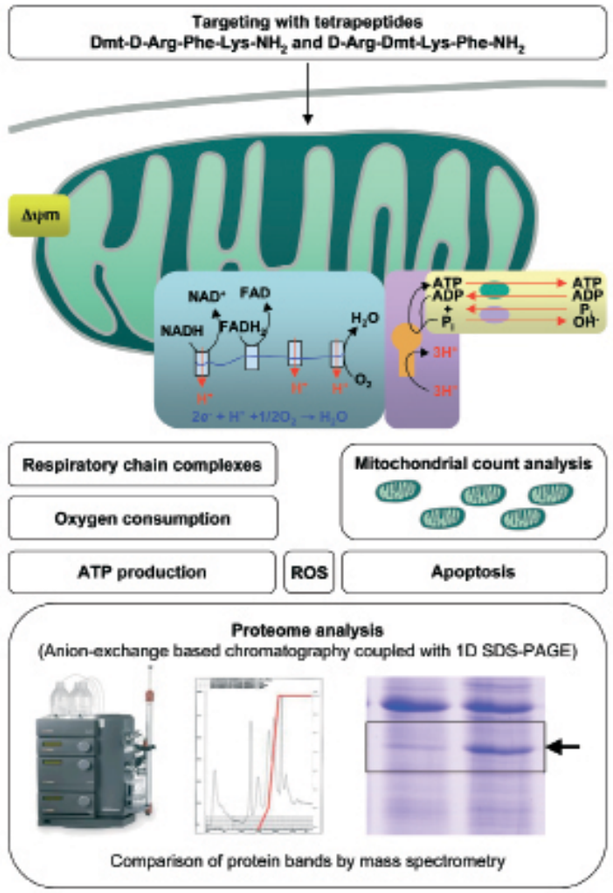

Fig. 1: Targeting and analysis of mitochondria in human adipocytes.
Activity of respiratory chain complexes, oxygen consumption, rate of ATP production, amounts of reactive oxygen species (ROS) and the number of mitochondria per cell are measured to describe the mitochondrial function. Furthermore changes of the membrane potential ($\Delta\Psi_m$) and the tolerance against induction of mitochondria-mediated apoptosis are investigated. In addition pretreatment of fat cells using cell-permeable antioxidant tetrapeptides Dmt-D-Arg-Phe-Lys-NH_2 and D-Arg-Dmt-Lys-Phe-NH_2 is applied to examine possible protective effects against ROS-induced damage of mitochondria. To investigate the mitochondrial proteome, extracted proteins are first divided into several fractions by anion-exchange chromatography and then separated by SDS-PAGE. Subsequently bands of interest are identified by mass spectrometry.

Connection to Clinical Practice

Receptor-mediated targeting of melanoma metastases

The concept of receptor-mediated targeting of melanoma metastases using synthetic analogues of α-melanocyte-stimulating hormone (MSH) labelled with the radiometals ^{111}In, ^{68}Ga or ^{90}Y was presented in the Annual Report 2000-2004 of the DKBW. This clinically related project was further developed in the past three years, with a major focus on increasing the ratio between tumor uptake of the radiopeptides and non-specific uptake by the kidneys, a significant problem in the clinic occurring with all types of radiopeptides. To this end, glycosylated and other types of MSH peptides were designed, synthesized and tested in vitro and in vivo with melanoma-bearing mice. It turned out that in the animal model an MSH analogue containing a galactose residue yielded the highest tumor-to-kindey ratio of all MSH radiopeptides published to date. The suitability of this peptide for human melanoma targeting will be assessed in a forthcoming study.

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Hematopoiesis, Myeloproliferative disorders, Kinase inhibitors, Transgenic mice, Familial predisposition, Genomic rearrangements, Hematopoietic stem cells, Transplantation, Human leukemia, flt3 ligand, Natural killer cells, Immunotherapy

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The pathogenesis of hematopoietic stem cell disorders (R. Skoda)

Myeloproliferative disorders (MPD) are a group of blood diseases characterized by aberrant proliferation of precursors of the myeloid, erythroid and megakaryocytic lineages. They represent clonal stem cell disorders with a tendency towards leukemic transformation. Currently, no curative therapy is available. MPDs comprise 3 entities: polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). The goal of our studies is to better understand the molecular events that cause and influence the progression of MPD.

JAK2 mutations in myeloproliferative disorders

In 2005, our group and three other laboratories described a recurrent mutation in exon 14 of the Janus kinase 2 (JAK2) gene that substitutes a valine to phenylalanine at position 617 (JAK2-V617F). This mutation leads to constitutive activation of the Jak2 kinase and is found in the vast majority of MPD patients, in particular PV. Recently, activating mutations in exon 12 of JAK2 have been described in patients with PV that are negative for JAK2-V617F. Despite this progress, many questions remain unsolved including how a single JAK2 mutation causes three different MPD phenotypes, what other genes might be involved and what determines the progression to acute leukemia. We are examining these questions by combining three approaches: molecular studies in patients with sporadic MPD, genetic analysis of familial MPD and transgenic mouse models that mimic the human disease.

Analysis of clonal progression in MPD

In a subset of patients with sporadic MPD we found evidence for mutations in as yet unknown genes, some of which may precede the acquisition of JAK2-V617F. In some MPD patients only a small percentage of blood cells carries the JAK2-V617F mutation, while surprisingly, the remaining cells are clonal. Deletions on chromosome 20q (del20q) can represent such a clone, suggesting that an additional oncogenic event may be located on chromosome 20q. In other patients we found co-existence of the JAK2-V617F mutation with a mutation in JAK2 exon 12. Futhermore, when patients with JAK2-V617F positive MPD transform into acute leukemia, the leukemic blasts are frequently negative for JAK2-V617F. These results are compatible with the hypothesis that a clonal pre-JAK2 event is present in a subset of stem cells in these patients. This pre-JAK2 mutation predisposes these stem cells to acquire JAK2-V617F and in rare cases also to acquire other mutations and progress to acute leukemia (Fig. 1).

Familial predisposition for MPD

Familial syndromes resembling MPD can be grouped into two classes:

1. Inherited disorders with high penetrance and polyclonal hematopoiesis.
2. Hereditary predisposition to true MPD, with low penetrance, clonal hematopoiesis and occurrence of somatic mutations, e.g. in JAK2-V617F.

We identified mutations in the thrombopoietin (THPO) gene as the cause for an inherited form of thrombocythemia in several families with a “class 1” phenotype. In another family we found a previously described mutation in the gene for the thrombopoietin receptor (MPL). However, in the majority of families neither THPO nor MPL is mutated. The search for these disease genes is ongoing. Families with “class 2” phenotype are more common than generally assumed. These germ line mutations increase the likelihood of acquiring a somatic JAK2-V617F mutation. We are using genetic methods to map the locus for these pre-disposing mutations.

Mouse models for MPD

Recently, we generated JAK2-V617F transgenic mice that express a human JAK2-V617F gene. This conditional construct can be activated by Cre-recombinase. Depending on the mode of Cre-mediated activation, these mice developed a phenotype resembling ET with strongly elevated platelet counts or a PV-like phenotype with increased hemoglobin, thrombocytosis and neutrophilia. We found that the ratio of mutant to wild type JAK2 correlated with the phenotypic manifestation. These results suggest that the relative activity of the mutant JAK2 may be a major determinant of the ET versus PV phenotype. Furthermore, this mouse model will be valuable for preclinical testing of JAK2 inhibitors.

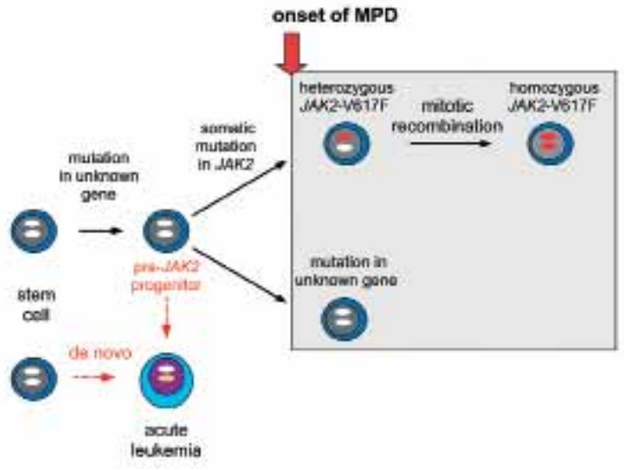

Fig. 1: Model of the clonal progression in MPD. The onset of MPD is preceded by a somatic mutation (sporadic MPD) or germ line mutation (familial MPD) in an as yet unknown gene(s). The onset of MPD coincides with the acquisition of a mutation in JAK2 or other genes (gray box). Acute leukemia can arise de novo or from the MPD clone. Leukemic transformation of a “pre-JAK2” progenitor or stem cell could explain the absence of JAK2-V617F in leukemic blasts from MPD patients previously positive for JAK2-V617F.

The control of blood cell development and pathogenesis of hematopoietic stem cell disorders (A. Wodnar-Filipowicz)

The first aim of our studies is to understand the mechanisms, which regulate the recovery of bone marrow function after hematopoietic stem cell transplantation (HSCT). We demonstrated in patients with leukemia undergoing HSCT that chemotherapy-induced myeloablation is associated with a profound overexpression of flt3 ligand (FL), a hematopoietic cytokine interacting with tyrosine kinase flt3 receptor expressed by HSC and early progenitor cells. To assess the importance of FL in the regeneration of bone marrow function, mouse models of HSCT have been established. We found that following myelosuppression, FL expression is up-regulated in stromal fibroblasts in the bone marrow and in peri-vascular fibroblasts in the thymus, and results in enhanced recovery of hemat-

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- Theocharides, A., Boissinot, M., Girodon, F., Garand, R., Teo, S. S., Lippert, E., Talmant, P., Tichelli, A., Hermouet, S., and Skoda, R. C. (2007). Leukemic blasts in transformed JAK2-V617F-positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation. Blood 110, 375-379.
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- Nowbakht, P., Ionescu, M-C., Rohner, A., Kalberer, C.P., Rossy, E., Mori, L., Cosman, D., De Libero, G. and Wodnar-Filipowicz, A. (2005). Ligands for natural killer cell-activating receptors are expressed upon the maturation of normal myelomonocytic cells but at low levels in acute myeloid leukemias. Blood 105, 3615-3622.
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opoietic and immune systems. This indicates that FL belongs to the cytokine network which defines the function of stem/progenitor cell niches. We are now extending these studies to the regulation of leukemic stem cells (LSC) niches. LSC arise from mutations occurring at the stem/progenitor cell level and are responsible for the generation and persistence of human leukemia. Using the mouse models of myelosuppression and transplantation of leukemic blasts, we are studying the role of FL in controlling the LSC localization and movement within the bone marrow microenvironment.

The potential of natural killer (NK cells) for immunotherapy against leukemia

The second aim of our studies addresses the potential of natural killer (NK cells) for immunotherapy against human leukemia. To understand the molecular interactions between NK cells and malignant cells, we focus on the role of the activating NK receptor NKG2D and its ligands (NKG2D-L) in recognition and elimination of leukemic targets. We demonstrated that leukemic blasts in patients with acute myeloid leukemia (AML) display a defective expression of NKG2D-L and a compromised immunogenicity. To reverse this tumor escape mechanism from the immune recognition, we used histone deacetylase (HDAC) inhibitors and demonstrated an up-regulation of NKG2D-L cell surface levels, conferring an increased sensitivity of AML blasts to NK cell lysis. Further studies aim at elucidating the molecular mechanisms regulating expression of NKG2D-L by post-transcriptional regulation involving mRNA silencing/degradation by microRNAs. To assess the cytotoxic properties of NK cells against AML blasts in vivo, we established a model of human leukemia in immunodeficient NOD/SCID mice. The in vivo function of HDAC inhibitors and their synergy with adoptively transfered NK cells in reducing the tumor load is studied in mice that had previously been transplanted with AML cells. These approaches provide the basis for the ongoing clinical study which uses NK cells, acivated and expanded ex vivo, as cellular immunotherapy to enhance the immune response in patients with AML and prevent disease relapse after HSCT.

Connection to Clinical Practice

Improved diagnostics of MPD and new therapeutic aproaches: From bench to bedside (A. Tichelli and R. Skoda)

The primary challenge in the diagnostic approach to MPD is to distinguish between reactive changes (i.e. elevated blood counts secondary to other diseases) and true MPD (i.e. primary disease of the bone marrow cells). In a second step, the definitive category of the MPD, i.e. polycythemia vera (PV), essential thrombocythemia (ET) or primary myelofibrosis (PMF), has to be established. Until recently, MPD was a diagnosis of exclusion and sometimes long-term follow-up was needed to definitively distinguish MPD from reactive alterations. The discovery of the JAK2-V617F mutation has completely changed the diagnostic approach to patients with a suspected MPD (Fig. 2). Since JAK2-V617F is absent in reactive thrombocytosis, erythrocytosis or leukocytosis, the presence of a JAK2 mutation can be used to exclude such reactive changes. JAK2-V617F can be found in about 95% of patients with PV and in approximately 50-60% of PMF and ET and also in other chronic myeloid neoplasms, such as refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T). Therefore, mutation screening for JAK2-V617F cannot distinguish between different forms of MPD and blood counts, erythropoietin levels and additional parameters (bone marrow threphine and cytology, cytogenetic analysis) and search for less frequent mutations (JAK2 exon 12, MPL, thrombopoietin, Epo-receptor and others) have to be taken into consideration. In the near future, classification and diagnosis of myeloid neoplasm will be mainly based on disease-specific genetic markers. Furthermore, inhibitors of JAK2 are being developed and some of them are already undergoing clinical trials. There is hope that these JAK2-inhibitors will prove to be effective for treating patients with MPD.

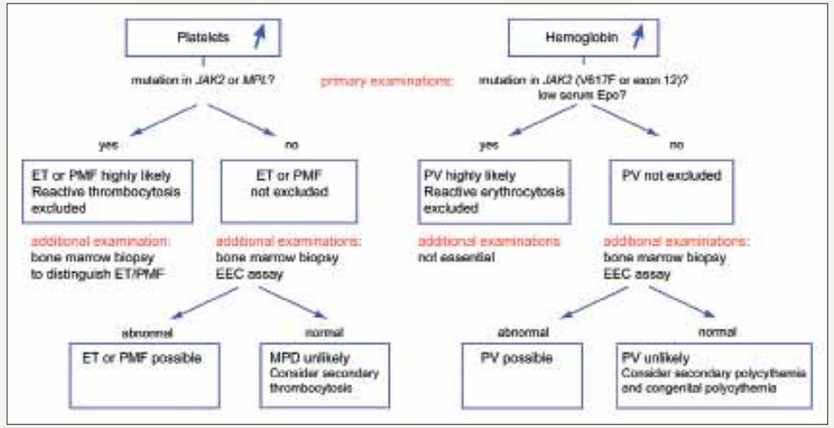

Fig. 2:
Diagnostic workup in patients with suspected myeloproliferative disease. PV, polycythemia vera;p ET, essential thrombocythemia; PMF, primary myelofibrosis; EEC, endogenous erythroid colonies.

Immunotherapy of AML with NK cells: From bench to bedside (A. Gratwohl and A. Wodnar-Filipowicz)

This study aims at advancing the immunotherapeutic NK cell trials by developing clinically-suitable approaches to increase leukemia recognition by NK cells from haploidentical donors (Fig. 3). The project combines and advances the existing expertise in NK cell research and clinical application at the Laboratory of Experimental Hematology and the Stem Cell Transplant Team at the Clinic of Hematology in Basel. The aims of the project are:(i) The infrastructure for large scale NK cell expansion is being set up and the most efficient and secure procedures to obtain a highly purified NK cell product for clinical use are being determined. (ii) Feasibility, safety and efficacy of the expanded NK cells will be evaluated in the clinical settings with patients undergoing HSCT from haploidentical donors.

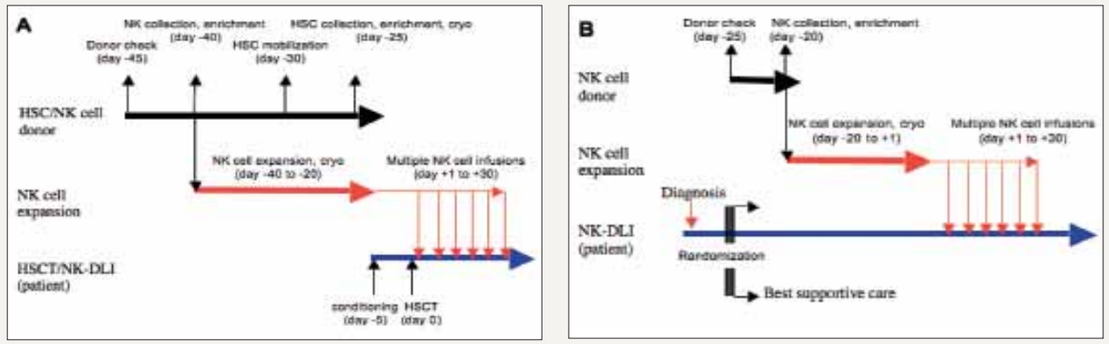

Fig. 3: Scheme of NK cell infusions (NK-DLI) offer HSCT (A) and in non-transplanted elderly patients (B).

Appetite regulation
Gastrointestinal signals

Gastro-enterology

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Gastrointestinal signals in regulating food intake

The investigation of human eating behaviour, especially the regulation of appetite and satiety, has become a very active field of research with potential for the development of a specific therapy for obesity. The information available about the biochemical processes that control hunger and satiety is still insufficient. There is evidence that pre-absorptive factors are important cofactors in this regulatory control system. Specific nutrient intake is associated with the secretion of a number of gastrointestinal hormones, including peptide YY (PYY), glucagon-like peptide-1 (GLP-1), GIP, neurotensin and cholecystokinin (CCK). These peptides interact with appetite centres in the brain and the brainstem in order to induce satiety. Recent studies have shown that the administration of PYY or GLP-1 reduces energy intake in healthy subjects and in obese persons. This research has stimulated interest in these hormones as targets for the development of anti-obesity therapies. The present research focuses on the physiology, mechanism of action and interactions of the gut hormones GLP-1 and PYY as satiety hormones to prepare the path for potential therapeutic application.

Connection to Clinical Practice

Gastric sensorimotor functions and hormone profile in normal weight and obese people

Obesity is an increasing global epidemic. In the United States, 65% of the population is considered obese or overweight. Obesity is associated with diabetes type 2, coronary heart disease, gall bladder disease, increased incidence in some cancers, respiratory complications, osteoarthritis, and increased mortality. With increase in the prevalence of obesity, it is in the public interest to better understand the pathogenesis of obesity. Food intake is modulated by the sensation of hunger and satiation. The gastrointestinal tract is a key element in this complex system. The upper digestive tract, especially the stomach and the proximal small intestine, trigger signals which induce satiation in response to calorie ingestion or volume consumed. The role of gastric emptying and gastric sensory functions have been studied in normal weight subjects and in obese persons, but contradictory results have been provided for obese persons. Earlier studies reported that obese subjects have accelerated gastric emptying rates of solid particles implying that accelerated emptying could lead to shorter periods of satiety or fullness, leading to a shorter period time to the next meal. Other studies have provided contradictory evidence with normal or even delayed gastric emptying. The discrepancies between different studies can be explained by different factors: inadequate methodology to measure gastric emptying, insufficient number of subjects. Currently, the influence of gastric motor functions and the role of satiety hormones in the process of satiation and in relation to body mass is still unclear.

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Assisted reproduction
Granulosa
Stem cell
Ovary
Oestradiol
Embryo

Gynecological Endocrinology

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Interplay between hormonal regulation and oocyte-signaling in ovarian follicular growth and atresia

During a woman´s reproductive life the ovary is characterized by the cyclical growth of single dominant follicles followed by ovulation and luteal body formation. This process is highly selective and involves a constant remodelling of the ovarian tissue, proliferation and apoptosis. Ovarian function consists of a complex interplay between various endocrine signaling pathways, regulatory functions of the maturing oocyte and a network of intra- and extracellular protein-protein interactions. Using our ovary-enriched gene expression databases, we constantly search for new factors active in ovarian physiology and have been able to identify and characterize several new players, such as hBOK, Bcl2-L10, Bcl2-L12, ADAMTS16 and EULIR. The three first genes are involved in apoptosis, the fourth is regulated by FSH and encodes a protease in the antrum of preovulatory follicles, whereas EULIR is active as a inhibin binding protein-cofactor. For detailled characterization of these agents the entire armamentarium of modern molecular biology is being used, including flow cytometry and the production of knockout mice (e.g. for EULIR). Until recently, the granulosa cells, being at the center of the regulation of follicular growth and oocyte maturation, could not be cultured over prolonged periods of time. We therefore initially developed and characterized one of the first extant immortalized granulosa cell lines (COV434), with which we studied the interaction between granulosa and the oocyte. Recently, our research group managed to culture luteinizing granulosa cells, collected from preovulatory follicles and previously considered to be doomed for apoptosis after two weeks in culture, over prolonged periods of time. Furthermore we discovered putative ovarian follicular stem cells that exhibited all the characteristics typical of stem cells of the mesodermal lineage. By culturing them in a collagen scaffold it has been possible to conserve the functionality of these cells including the expression of the FSH receptor and aromatase, which opens many possibilities for further research.

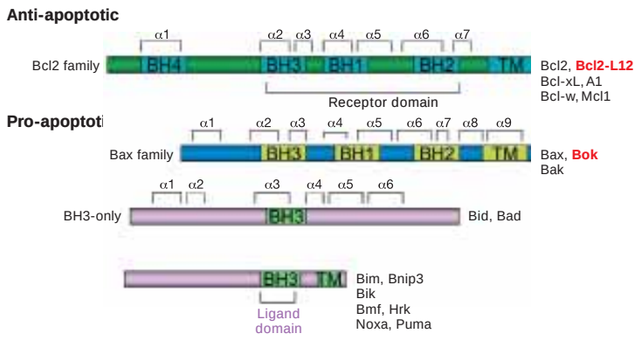

Fig. 2: Overview of the structural-functional relationship of various members of the Bcl2 family, involved in apoptosis
The proteins marked in red have been a major target in our research. Based on functional studies and the retention of BH e.g. "Bcl-2 homology") domains, the Bcl-2 family are divided into three subgroups:
1. The Bcl-2 subgroup including all anti-apoptotic proteins, such as Bcl-2, Bcl-xL, A1/Bfl-1 and Mcl-1.
2. The Bax subgroup consisting of multi-BH domain pro-apoptosis members, such as Bax, Bak, Bok and Bcl-rambo.
3. The third subgroup containing BH3-only proteins, such Bid and Bim.

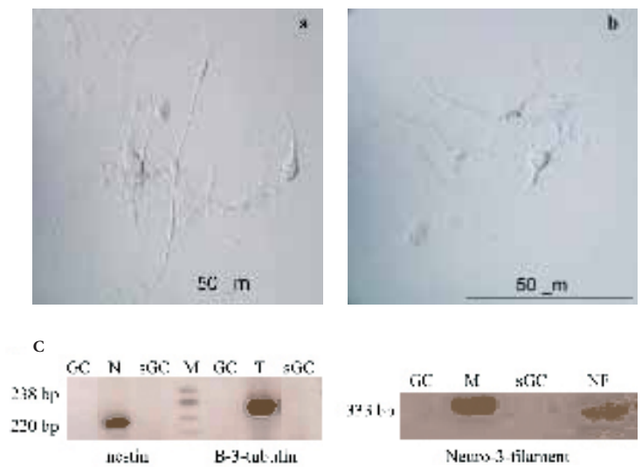

Fig. 1: Neuro-induction of granulosa cells collected from preovulatory follicles and cultured over prolonged time periods in medium supplemented with leukaemia-inhibiting factor
Neurogenic differentiation of GCs, selected after prolonged culture in medium supplemented with LIF (a, b) and RT-PCR results (c) showing expression of the neuronal markers, nestin (N), B-3-tubulin (T) and neuro-3-filament (NF).
GC: GCs cultured in control medium supplemented with LIF, sGC: sorted GCs.

	Total no. of pups	%
+/+	54	88.5
+/-	7	11.5
-/-	0	0

Fig. 3: Production of a EULIR knockout mouse
We identified a protein, which is associated with the putative receptor of inhibin B, InhBP. We consider the novel protein, characterized in our lab, as an inherent part of the InhBP/p120 complex and termed it EULIR, an E3 ubiquitin ligase E2. Whereas the InhBP/p120-knockout mouse was proved to be normally fertile (Bernard et al., 2003), the EULIR +/- knockout mouse produced in our research unit is subfertile, as shown in the table.

Connection to Clinical Practice

Transcriptional response of luteinizing granulosa cells to follicle-stimulating hormone

In ovarian physiology the granulosa cells, situated in the antrum of the growing follicles, play an eminent part in the cyclical growth of the follicles, in containing the oocyte and guiding its maturation and in the production of the dominant female sexual hormones, the estrogens. The function of the granulosa cells largely depends on the presence of the FSH-receptor. There are huge differences in the density of the FSH receptor during the various phases of the menstrual cycle, between individual follicles and between individual women. Among other factors, different genetic polymorphisms have been identified, which co-determine granulosa cell function. Polymorphisms of the FSH receptor and of the alpha-estrogenreceptor have been identified as being significant. The effect of each of these polymorphisms on the gene expression profiles of the FSH-receptor in both infertile and fertile women is currently being studied systematically. For that purpose, sorting of granulosa cells out of the follicular antrum was set up and culture conditions were optimised. It is intended to establish prospectively a well defined cell bank with granulosa cells collected from patients treated with assisted reproduction. Knowing the final outcome of the treatment and the characteristics of the patients, the genotype of granulosa cells function and ovarian receptivity to the endocrine treatment can be established prospectively. In addition to that, it is now possible to culture granulosa cells from mature follicles, previously thought to be terminally differentiated, over prolonged time periods both in the differentiated and in the undifferentiated status. Those findings have clearly demonstrated that ovarian follicles contain, as other tissue types with both rapid proliferating cells and with cells exerting specialized functions, stem cells. Those ovarian follicular stem cells may well play a role in the pathogenesis of ovarian cancer and may be part of the coelomic metaplasia theory of ovarian endometriosis.

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- Gao, S., Fu, W., Dürrenberger, M., De Geyter, Ch., Zhang, H. (2005) Membrane translocation and oligomerization of hBok are triggered in response to apoptotic stimuli and Bnip3. Cellular and Molecular Life Sciences 62: 1015-1024.
- Gao, S., De Geyter, Ch., Kossowska, K., Zhang, H. (2007) FSH stimulates the expression of the ADAMTS-16 protease in mature human ovarian follicles. Molecular Human Reproduction 13: 465-471.
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Liver
Signaling
Viral Hepatitis
Hepatocellular Carcinoma
Interferon

Hepatology

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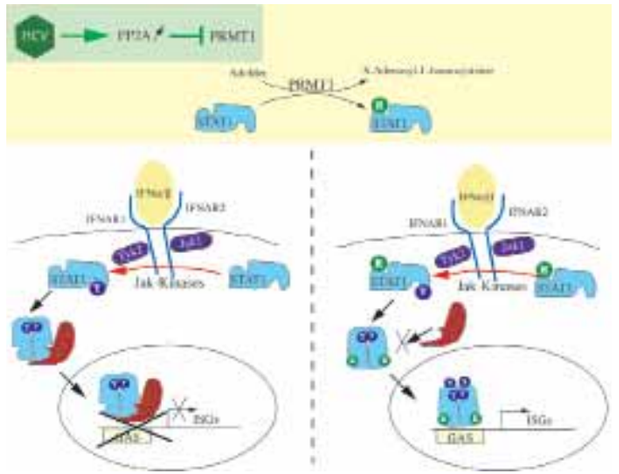
Interferon signaling in chronic viral hepatitis

Chronic hepatitis C (CHC) affects more than 180 Million people worldwide and is one of the most frequent causes of liver disease. CHC can lead to cirrhosis and hepatocellular carcinoma. Indeed, end stage CHC has become to most frequent cause for liver transplantation in Western countries. All current therapies of CHC rely on the antiviral effects of type I interferons (IFN). About half of the patients can be cured with a combination therapy consisting of pegylated IFN alpha and ribavirin. The reason for treatments failure in the other patients is not well understood. The analysis of the interaction between hepatitis C virus (HCV) and the host liver cell is one of the main focuses of our laboratory, especially in regard to viral interference with IFN signal transduction from the cell surface to the nucleus.The Jak-STAT pathway is the most important signal transduction pathway for IFNa. STATs (signal transducers and activators of transcription) are activated at the IFN receptor by members of the Jak kinase family through phosphorylation of a single tyrosine residue. Phosphorylated STATs form dimers, translocate into the nucleus, and activate IFN target genes through binding to specific response elements in their promoters. Important negative regulators of IFNa signaling through the Jak-STAT pathway are member of the suppressor of cytokine signaling (SOCS) family, and protein inhibitor of activated STAT1 (PIAS1). PIAS1 binds to tyrosine phosphorylated (activated) STAT1 and inhibits the binding of active STAT1 dimers to response elements in interferon stimulated genes (ISGs). The binding of STAT1 to PIAS1 is regulated by arginine methylation of STAT1, a process that is catalyzed by protein arginine methyl transferase 1 (PRMT1). Our analysis of IFNa signaling in cells expressing HCV proteins, in liver extracts from transgenic mice expressing HCV proteins in hepatocytes and in extracts from liver biopsies of patients with chronic hepatitis C consistently showed normal expression levels of the signaling components important for IFN signaling (Jak1, Tyk2, STAT1, STAT2, IRF9, SOCS1, SOCS3, PIAS1), and an intact and normal tyrosine phosphorylation of STAT1 and STAT2 (Christen et al., 2007a; Duong et al., 2004). However, electrophoretic mobility shift assays with nuclear extracts from HCV protein expressing cells or from liver biopsies of patients with chronic hepatitis C disclosed an impaired binding of STATs to their DNA response elements (Christen et al., 2007a; Duong et al., 2004). Further analysis of HCV interference with IFN signaling revealed a novel molecular mechanism of viral interference with the IFN system. HCV induces the over-expression of protein phosphatase 2A (PP2A), an important serine/threonine phosphatase involved in a wide range of cellular processes. This over-expression of PP2A in cells inhibits IFNa signaling at the level of DNA binding of STATs (Duong et al., 2004), because PP2A inhibits PRMT1 (Duong et al., 2005), and as a consequence, induces a hypomethylation of STAT1 (see figure). Surprisingly, PRMT1 is also involved in the arginine methylation of an important viral protein, the non-structural protein NS3 (Duong et al., 2005).In recent work we investigated how HCV infection could lead to PP2A over-expression. We found that the expression of HCV proteins in cells activates an endoplasmatic reticulum (ER) stress response. During this ER stress response, Ca2+ leaks from the ER into the cytoplasma, where it activates calcium/calmodulin-dependent protein kinase. The kinase phosphorylates and activates the transcription factor CREB. Phosphorylated CREB binds to CRE elements in the promoter of PP2Ac and stimulates the transcription of the gene (Christen et al., 2007b).
The consequences of HCV induced PP2A over-expression are summarized in the figure, with the right panel showing normal signaling with methylated STAT1, and the left panel showing impaired signaling with unmethylated STAT1. As depicted in the top panel of the figure, the methyl group donor for STAT1 methylation by PRMT1 is S-adenosylmethionine (AdoMet or SAMe), a compound that has been used to treat alcoholic liver disease and is available in many countries as a non-prescription drug. Consequently, we hypothesized that AdoMet could be used to correct the defects in IFNa signaling induced by HCV. Indeed, pre-treatment of HCV protein expressing cells and of cells with HCV replicons with AdoMet corrected IFNa signaling and potentiated the inhibitory effects of IFNa on HCV replicons (Duong et al., 2006). An ongoing clinical study with previous non-responders to IFN-ribavirin combination therapy will clarify if the addition of AdoMet (and betaine) to a standard therapy with pegIFNa and ribavirin could improve the sustained response rate in these difficult to treat patients (ClinicalTrials.gov Identifier: NCT00310336).



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– Duong, F.H., Christen, V., Berke, J.M., Penna, S.H., Moradpour, D., and Heim, M.H. (2005). Upregulation of Protein Phosphatase 2Ac by Hepatitis C Virus Modulates NS3 Helicase Activity through Inhibition of Protein Arginine Methyltransferase 1. J Virol 79, 15342-15350.
– Duong, F.H., Christen, V., Filipowicz, M., and Heim, M.H. (2006). S-adenosylmethionine and betaine correct hepatitis C virus induced inhibition of interferon signaling in vitro. Hepatology 43, 796-806.
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Aminoglycosides
Apoptosis
Hair cells
Hearing loss

Inner Ear Research

new group since December 2007

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Molecular mechanisms involved in hair cell survival and death

Hearing loss can be caused by damage to either external, middle or inner ear. Today, hearing loss caused by diseases of the external and the middle ear can be treated well, while disorders affecting the inner ear cannot. Often, only prosthetic devices offer some help. For mild to moderate hearing loss conventional hearing aids are used, while for profound hearing loss cochlear implantation is the standard of care today.
Loss or damage of hair cells and/or neuronal cells, which are the sensorineural elements of the inner ear, results in a so-called sensorineural hearing loss. The neurons of the spiral ganglion are progressively lost over a period of months and years, presumably as a result of lack of trophic support. However, the hair cells are the most vulnerable elements in the cochlea, and damage to them is the most common cause of sensorineural hearing loss. When the hair cells are lost from the adult organ of Corti, spiral ganglion dendrites retract and are possibly lost. Total loss of hair cells can result in degeneration of most cochlear neurons.
Hair cell damage can result from a variety of causes, including genetic disorders, infectious diseases, overexposure to intense sound and certain drugs. In the last few years, progress has been made in understanding hair cell damage. Our group has discovered key steps in the molecular pathways involved in hair cell damage and death after aminoglycoside exposure. Our and other groups have demonstrated a crucial role of mitogen-activated protein kinase signaling in aminoglycoside-induced ototoxicity. Mitogen-activated protein kinases are important mediators of signal transduction from the cell surface to the nucleus. C-Jun N-terminal kinases, members of the mitogen-activated protein kinase family, are strongly activated in cell culture conditions by stress inducing stimuli, including ultraviolet light, heat shock and tumor necrosis factor; therefore they are also referred to as stress-activated protein kinases. In hair cells aminoglycoside treatment was shown to activate the c-Jun N-terminal kinase signaling pathway. Activation of Jun N-terminal kinase leads to phosphorylation and thereby activation of transcription factors such as the AP-1 complex and consequently to altered gene expression. We were able to demonstrate that aminoglycoside treatment of explants of organ of Corti results in increased AP-1 binding activity. The main component of these AP-1 complexes is the c-Fos protein. Moreover, we showed that the AP-1 induction is transient and occurs exclusively in hair cells of rat organ of Corti explants (Albinger et al., 2006).
One concept of apoptosis is that cells are thought to exist in a finely tuned balance between survival and cell death. There are pathways that signal cell survival, whereas other pathways promote cell death. Under physiologic conditions, cell survival pathways are active and keep the cells alive, while cell death promoting pathways are inactive. Cell stress disrupts this balance, and if the stress is severe, apoptosis promoting pathways predominate and dell death occurs. Recently, survival signalling pathways have been described in the inner ear. We were able to demonstrate that in immature hair cells NF-kappaB is constitutively active and keeps the cells alive: inhibition of NF-kappaB therefore results in rapid hair cell loss (Nagy et al., 2005). Using DNA microarray technology, immune fluorescence microscopy and a biochemical assay we were able to link NF-kappaB-dependent hair cell death to phosphatidylinositol 3-kinase signalling (Nagy et al., 2007).
We want to continue our studies and define the stress pathways and survival pathways that operate in auditory hair cells.

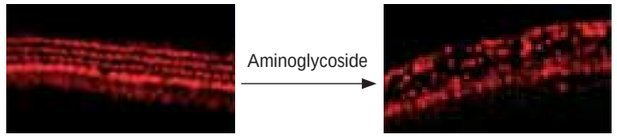

Fig. 1: Aminoglycoside exposure results in hair cell degeneration

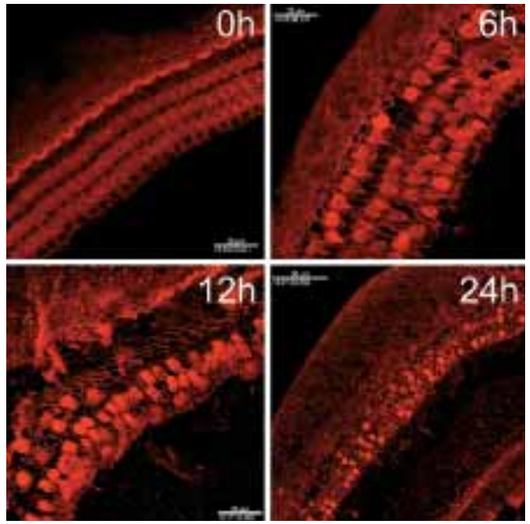

Fig. 2: NF-kappaB inhibition results in rapid hair cell degeneration

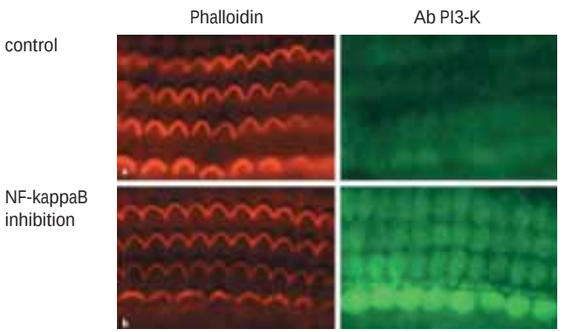

Fig. 3: NF-kappaB inhibition up-regulates phosphatidylinositol 3-kinase in hair cells

Connection to Clinical Practice

Electrophysiological measures of central auditory processing deficits in presbycusis

Hearing loss is a common consequence of the aging process, characterized by a bilateral decrease in hearing sensitivity, predominantly in the high-frequency range. Presbycusis is frequently associated with speech understanding difficulties, particularly in adverse listening conditions, such as background noise or reverberant environments.
Peripheral hearing loss alone cannot account for the speech understanding problems in elderly persons. Central auditory processing deficits may also contribute to these problems. The central auditory system is thought to be necessary for analysis of specific signal attributes, such as frequency, intensity, and duration, and for the processing of more complex stimuli, such as extracting signals from a competing background of noise.
We investigated age-related changes in central auditoy processing using psychoacoustic tasks and auditory event-related potentials (ERPs), an electrophysiological measure that may provide objective information about the level and localization of the processing deficits. ERPs have an excellent temporal resolution information on the sequence of cerebral events that underlie hearing and auditory processing. They comprise potentials that can be elicited regardless of the subject's attention to the stimuli and are labeled as "passively evoked" or "exogenous" (P1, N1, P2), representing the sensory-perceptual stages of processing, and potentials that require the subject's attention to the stimuli, labeled as "cognitive" or "endogenous" (N2b, P3a, P3b or P300). To elicit ERPs, small frequency contrasts were presented in an oddball paradigm under unattended and attended conditions.
Behavioral frequency discrimination was not affected by age, but deteriorated significantly with hearing loss. In contrast, aging, both in elderly subjects with relatively normal hearing and with hearing loss, was associated with pronounced changes of the later ERP components compared to those of the young subjects, reflecting a decrease in inhibitory control of irrelevant stimuli, a decreased sensitivity of automatic preattentive stimulus discrimination, and a more effortful and delayed stimulus evaluation (Bertoli et al., 2005; Bertoli and Probst, 2005). These results support current concepts of presbycusis suggesting a combination of peripheral, central-auditory and cognitive factors underlying the hearing difficulties of elderly persons.

Selected Publications

- Albinger, A., Hegyi, I., Nagy, I., Bodmer, M., Schmid, S., and Bodmer, D. (2006). Neuroscience 137(3), 971-80.
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- Bertoli, S., and Probst, R. (2005). Lack of standard N2 in elderly participants indicates inhibitory processing deficit. Neuroreport 16, 1933-1937.

Calcitonin Peptides
Inflammation
Insulin resistance
Stem cells
Hormokines

Metabolism

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Regulation, Function and Plasticity of Calcitonin Peptides and other Hormokines during inflammation and infection

The calcitonin (CT) family of peptides includes procalcitonin (ProCT), CT gene-related peptide (CGRP) I, CGRP II, amylin and adrenomedullin (ADM). CT peptides are suspected inflammatory mediators in several diseases, including severe bacterial infections and sepsis (ProCT), migraine (CGRPs), cancer (ADM), diabetes (amylin, ADM) and rheumatoid arthritis (CGRP, ADM). However, knowledge on expression regulation and molecular mechanisms is scarce.
The present work represents a continuation of our own previous research on cytokine-induced non-endocrine calcitonin-1 gene expression and ProCT production. The inflammation-mediated expression and possible functions of CT-peptides were investigated in several human adipose tissue models including biopsies, mature explanted adipocytes, preadipocyte- and mesenchymal stem cell (MSC)-derived adipocytes. With the exception of amylin, production of all CT-peptides was demonstrated in interleukin-1β (IL-1β)- and endotoxin-exposed adipocytes. Interferon-γ (IFN) blocked IL-1β-induced ProCT and CGRPs production but augmented ADM. ProCT and CGRPs were inducible in differentiated adipocytes exclusively. In contrast, ADM expression occurred also in undifferentiated MSC and numerous cell lines. CGRP and ADM exerted specific positive feedback regulation of their own expression and dose-dependently (10-10 and 10-6 M) enhanced lipolysis.
In summary, CT gene expression is not restricted to specialized neuro-endocrine cells during inflammatory conditions. CT peptides exert paracrine/autocrine metabolic and positive feedback regulatory effects in human mature adipocytes, but not in undifferentiated precursor cells. Since viral infections are known to release IFN, the inhibiting effect of IFN on inflammatory ProCT production might explain the clinical observation of a blunted inflammation-mediated increase in serum ProCT concentrations observed in viral as compared to bacterial infections.

Specifically, the following subprojects are ongoing:
– Lentiviral vectors to efficiently transduce human mesenchymal stem cell- and preadipocyte-derived mature adipocytes
– Regulation of expression and effects of inflammation-mediated calcitonin gene products in human adipocytes
– Hyperglycemia-induced inflammatory gene expression in human adipocytes

Role of toll-like receptor 3 and 4 agonists polyIC and LPS in adipocytes (collaboration with VETSUISSE)
– Antimicrobial effects of calcitonin peptides (Collaboration with Landmann R., Trampuz A.)
– To analyse various additional culture conditions in order to enrich the stem cell population with the greatest developmental capacity (Zulewski H.).
– Role of AMP-dependent protein kinase (AMPK) in inflammation-associated insulin resistance in human adipocytes (Christ-Crain M.)

Connection to Clinical Practice

Hormokines in respiratory tract infections – Diagnostic guides to antibiotic prescription, prognost

The term “hormokine” encompasses the cytokine like behaviour of hormones during inflammation and infections. The concept is based on our finding of an ubiquitous expression of calcitonin peptides during sepsis. All these peptides are increased to variable extents during inflammation and infection. Most prominently, circulating procalcitonin (PCT) levels increase several-thousand fold during sepsis. Using a sensitive assay, a PCT-based therapeutic strategy can safely and markedly reduce antibiotic usage in those respiratory tract infections that are mostly viral, and in viral meningitis. Adrenomedullin, another member of the calcitonin peptide superfamily, was shown to complement and improve the current prognostic assessment in lower respiratory tract infections. Other peptides share features of hormokines, e.g., natriuretic peptide and copeptin. Hormokines are not only biomarkers of infection. Hormokines are also pivotal inflammatory mediators. Like all mediators, their role during systemic infections is basically beneficial, possibly to combat invading microbes. Yet, with increasing levels they can become harmful for their host. Multiple mechanisms of action were proposed. In several animal models the modulation and neutralization of hormokines during infection was shown to improve survival and thus might open new treatment options for severe infections, especially of the respiratory tract.

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Editorial: Wunderink R.G. A CAP on Antibiotic Duration. Am J Resp Crit Care Med 2006; 174: 3-5
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Bone architecture
Topographical variation
Endplate mineralisation
Strength
Cervical vertebra
Adaptation

Musculoskeletal Research

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Regional variations in microarchitecture, bone density, bone strength and endplate mineralization within the cervical vertebrae

Recent studies point to the fact, that cancellous bone density and structure present substantial variability inside the vertebral body. By identifying the weakest and strongest regions inside the vertebral body and in the endplates we may be able to focus clinical assessment on the region that will, theoretically show the earliest failure.
Our aim in several studies was therefore a precise analysis of the microstructure at different, well defined locations within cervical vertebrae by means of microcomputed tomography and an assessment of the distribution of mineralization and bone strength within the endplates.
The material for micro-CT examination consisted of 8 cervical vertebrae (C4, 4 male, 4 female, age range 38-62 years). At 24 different well-defined locations parameters like BV/TV, BS/BV, trabecular number (TbN), trabecular thickness (TbTh), trabecular space (TbSp), structure model index (SMI) and the degree of anisotropy (DA) were determined. These data were statistically analyzed using a linear regression analysis (paired and unpaired Student´s t-test).
The mineralization patterns were displayed in 80 endplates (C3-C7) of the same spines by means of CT-osteoabsorptiometry (CT-OAM). Then a summary image of the maximum localization was generated in the form of a point cluster from the coordinates of all endplates investigated. Statistical analysis of these data was performed with the Chi-square test and the level of significance was $p < 0.05$.
Finally indentation testing at several well-defined points within the endplates were performed by means of a material-testing machine to get strength values.

1. Substantial site-dependent differences in bone density (BV/TV) and bone architecture were observed. The posterior areas presented a generally higher density than die anterior areas (Fig. 1)
2. Significant differences are also apparent between cranial and caudal portions (Fig.2): the caudal BV/TV being larger. Furthermore, the caudal and dorsal parts show a higher connectivity density and tend to exhibit a plate-like structure, whereas the cranial and anterior parts tend to exhibit a rod-like structure (SMI). The trabeculae are more numerous in the caudal and dorsal areas, thicker, and the space between the trabeculae is smaller.
3. The distribution of subchondral mineralization also revealed considerable topographic differences within each endplate. The zones of greatest density, in both the inferior and superior endplates, are localized over wide areas of the posterolateral surface.
4. The distribution of strength within an endplate showed as well considerable topographic differences within an endplate. Maximal values occurred in both the inferior and superior endplates in the posterolateral regions.
5. Comparison of strength and mineralization values revealed a high correlation which was statistically significant (r2 between 0,74 und 0,97, $p < 0.059$).

A precise topographic differentiation in the cervical vertebral body reveals significant differences between the cranial and caudal portions and between the anterior and posterior regions. The structurally “strongest” area is found in the posterior caudal region. Moreover there exists a high correlation with the distribution of endplate mineralisation and endplate thickness (literature) and material properties such as strength and stiffness in the vertebral endplates.

The strongest part within a vertebra is caudal posterior, the weakest the cranial anterior area
These results can serve as a basis for improved disc prosthesis design and the anchorage point for various fusion techniques. The advantage of the CT-OAM method used for the assessment of endplate mineralization is that it can also be used in patients clinically to provide information on individual mineralization distribution in an individual and can be used as a basis for surgical planning

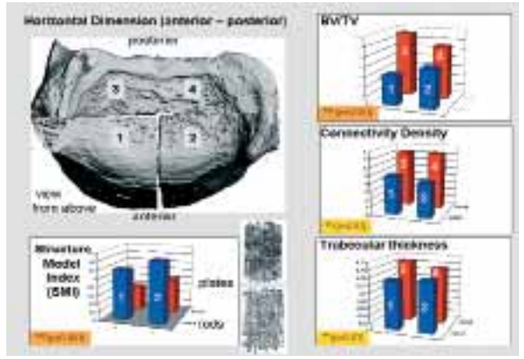

Fig. 1: Mikro-CT results in the horizontal dimension

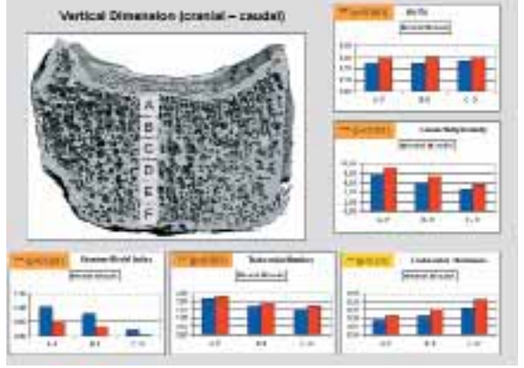

Fig. 2: Mikro-CT results in the vertikal dimension

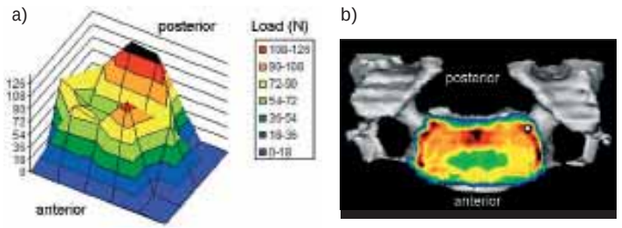

Fig. 3: a) Strength distribution within the superior cervical endplate. b) Distribution of mineralisation within the superior cervical endplate (black and red are zones of highest density followed by yellow, green and blue)

Selected Publications

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Myocardial Remodeling
Reactive Oxygen Species
Cardiomyocytes
Cardiac Progenitor Cells
Hematopoietic Growth Factors
β1-integrin

Myocardial Research

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Berit Rosc (PhD student)
Stéphanie Häuselmann (Master student)
Vera Lorenz (technician)

What Harms and What Heals the Heart: Impact of Oxygen Radicals and Role of Hematopoietic Growth Factors and Cardiac Progenitor Cells

Heart failure is a major complication of various cardiac diseases and a leading cause of death and hospitalization. Myocardial remodeling plays a key role in heart failure and it encompasses myocyte growth (hypertrophy), alterations in cytoarchitecture, gene expression and calcium handling, changes in extracellular matrix and cell death. Reactive oxygen species (ROS) are important mediators of this remodeling. They arise from molecular oxygen (O_2) through action of intracellular enzymes (e.g. NADPH oxidase, xanthine oxidase) or the mitochondria. A number of antioxidant systems are in charge to protect the cell from excessive ROS and maintain a physiological redox-balance (Fig. 1). While excessive ROS can lead to direct cellular injury through oxidation of DNA, lipids and proteins, they likewise participate in cell signaling through activation of redox-sensitive signaling cascades that can initiate both protective (adaptive) or damaging (maladaptive) cellular events.

Understanding the mechanisms of ROS interactions: β_1-integrin as possible target

Although antioxidant treatment ameliorates adverse remodeling in animal studies, clinical studies in humans have yielded disappointing results. In order to develop more effective treatment strategies, we need to improve our understanding of the sources and targets of myocardial ROS and of the mechanisms whereby ROS interact with these targets. ROS can induce oxidative modifications of proteins and thus alter their structure and/or function. In Boston, in the laboratory of W.S. Colucci, we previously found oxidatively modified (nitrotyrosinylated) protein in mice hearts after ascending aortic constriction, a condition going along with increased oxidative/nitrosative stress (Kuster et al, Circulation 111, 420-427, 2005). Further work suggests that post-translational oxidative modification of free reactive thiols on the small G-protein Ras increases Ras activity and thus promotes ROS-dependent hypertrophic signaling in cardiomyocytes (Kuster et al, Circulation 111, 1192-1198, 2005). We are currently focusing on β_1-integrin as possible target of ROS. Integrins are transmembrane receptors that participate in the regulation of cell growth, proliferation and death. β_1-integrin mediates hypertrophy and protects cardiomyocytes against apoptosis. In this project we hypothesize that ROS participate in the control of β_1-integrin activity by regulating amount (transcriptional regulation) and activity of β_1-integrin (post-translational regulation). In turn, β_1-integrin itself may exert its cell-protective effects by modifying ROS-signaling. The results of these studies will further our understanding of how ROS interact with integrins to orchestrate cellular and extracellular events in myocardial remodeling and may help to identify novel targets of antioxidant treatment to prevent myocardial failure.

Cardiac progenitor cells: important determinants of myocardial cell homeostasis

The heart has long been thought of as a terminally differentiated organ, incapable to compensate for the loss of functional cardiac cells or to replenish its cell pool. Recently, however, multipotent cardiac progenitor cells (CPCs) were identified that have the capacity to differentiate into all cardiac cell lineages including functional cardiomyocytes (Fig. 2). Over a lifespan, this resident CPC population maintains myocardial cell homeostasis. Functional impairment of the CPCs has detrimental effects on the integrity of the myocardium and may lead to heart failure. The function of CPCs is largely determined by the surrounding environment. In this so-called "niche", a variety of cytokines and growth factors regulate survival as well as proliferation and differentiation capacities of the CPCs. Analogous to bone marrow derived progenitor cells, CPCs exhibit receptor systems that are responsive to hematopoietic growth factors. Only little is known, however, on the role of these receptor systems in the regulation of CPCs and cardiac cell homeostasis.

Erythropoietin and flt3 ligand as "cardiopoietic" factors

In Boston, in the laboratory of R. Liao, we have previously characterized CPCs and compared them to bone-marrow derived progenitor cells (Pfister et al, Circ Res 97, 52-61, 2005). We also showed that ischemic myocardium is a potent stimulus for the proliferation and migration of CPCs (Pfister et al, Circ Res 97, 1090-1092, 2005). Furthermore, erythropoietin improves post-myocardial infarction remodeling and this effect seems associated with increased mobilization of endothelial progenitor cells (Prunier et al, 2007). In our current projects, we are using primary cell cultures from rodent hearts and transgenic mouse models to study the roles of the hematopoietic growth factors erythropoietin and flt3 ligand in the regulation of CPCs and in myocardial remodeling in vitro and in vivo. Flt3 ligand is a hematopoietic growth factor that promotes survival and proliferation of bone-marrow derived stem cells. Flt3 ligand is enriched in the ischemic myocardium suggesting a role in the regulation of CPCs. In our ongoing project we seek to determine how erythropoietin and flt3 ligand affect the function of CPCs and regulate cardiac cell survival, and to elucidate the underlying molecular mechanisms. The results of these studies will further our understanding of how the heart maintains and restores its structural and functional integrity and help to identify novel "cardiopoietic" factors that could be used for therapeutic stimulation of CPCs to prevent and treat heart failure.

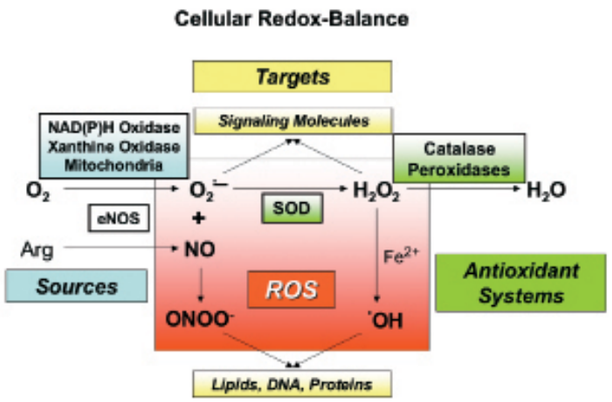

Fig. 1: Perturbation of the cellular redox-balance leads to impaired cell signaling or direct cellular injury going along with cell death, disease and premature aging. Therapeutic strategies to counterbalance oxidative stress may include enhancement of antioxidant capacities, inhibition of sources of ROS or protection of ROS targets.

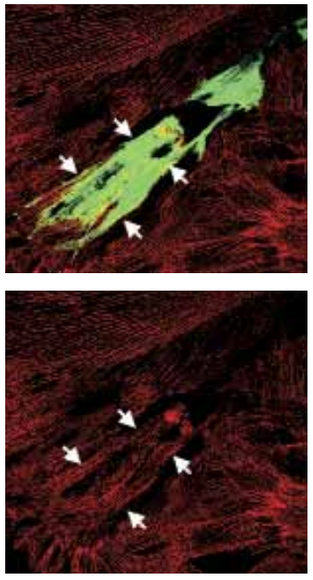

Fig. 2: Cardiomyogenic Differentiation of Cardiac Progenitor Cells
Differentiated cardiac progenitor cell (green, upper panel) that is structurally indistinguishable from the surrounding cardiomyocytes (lower panel).

Selected Publications

- Kuster*, G.M., Kotlyar*, E., Rude, M.K., Siwik, D.A., Liao, R., Colucci, W.S., Sam, F. (*equal contribution). (2005). Mineralocorticoid receptor inhibition ameliorates the transition to myocardial failure and decreases oxidative stress and inflammation in mice with chronic pressure overload. Circulation 111, 420-427.
- Kuster, G.M., Pimentel, D.R., Adachi, T., Ido, Y., Brenner, D.A., Cohen, R.A., Liao, R., Siwik, D.A., Colucci, W.S. (2005). α-Adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated via thioredoxin-1-sensitive oxidative modification of thiols on Ras. Circulation 111, 1192-1198.
- Pfister, O., Mouquet, F., Jain, M., Summer, R., Helmes, M., Fine, A., Colucci, W.S., Liao, R. (2005). CD31- but not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. Circ Res 97, 52-61.
- Pfister*, O., Mouquet*, F., Jain, M., Oikonomopoulos, A., Ngoy, S., Summer, R., Fine, A., Liao, R. (*equal contribution). (2005). Restoration of cardiac progenitor cells following myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. Circ Res 97, 1090-1092.
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Glaucoma
Ocular blood vessels
Leucocytes
Cell signaling pathway
Oxidative stress
Meningothelial cells

Ocular Pharmacology and Physiology

new group since July 2007

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Dr. Xin Xiarong
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Basic Science in Glaucoma – laboratory analysis

Glaucoma is a potentially blinding neurodegenerative disease, affecting about 70 million people worldwide.This disease affects the entire visual system, particularly the retinal ganglion cells and the optic nerve, giving rise to the term glaucomatous opticus neuropathy (GON). There are a number of risk factors for GON including increased intraocular pressure, vascular dysregulation and systemic hypotension.
The clinical studies carried out in the Eye Clinic of Basel are complemented by research in the laboratory, including physiological and pharmacological studies on isolated ocular vessels, analysis of gene expression in leucocytes of glaucoma patients, analysis of blood plasma and studies on morphology of the optic nerve and its meningeal sheets.

Studies with isolated vessels

We investigated the role of endothelial cells on arteries and veins in terms of regulation as well as the influence of drugs that are used locally on the eye and given systemically. In particular, we investigated the role of postanoids U46619, the prostaglandine F 2alpha, latanoprost free acid and travoprost free acid. We intend to study Endothelin blockers in the near future.

Gene expressions of leucocytes

As a proof of principal, we analyzed the neural thread protein which is upregulated in glaucoma patients. Subsequently we found a number of molecules up- or downregulated, both on the mRNA level as well as on protein level. Some of these might be used as molecular markers in the future. To more efficiently measure gene expression in leucocytes, we developed a new method to quantify mRNA with optical methods, based on molecular beacons. This method will be applied to blood of glaucoma patients in the near future.

Analysis of blood plasma and blood serum

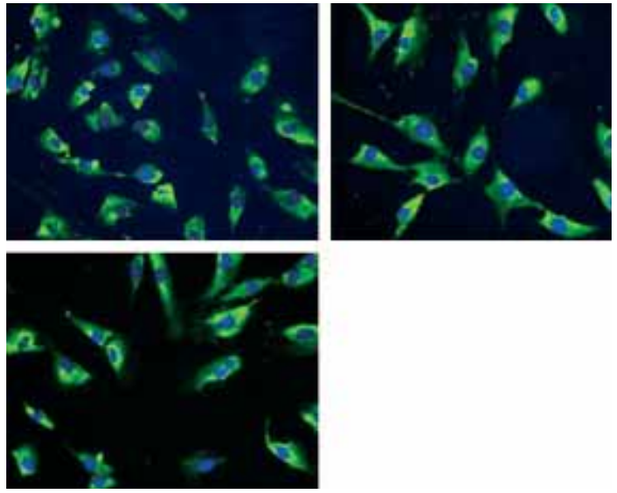
In the blood from patients with glaucoma, citric acid is markedly reduced whereas Endothelin is increased. In the next step we will analyse cytokines and chemokines, obiquitine and molecules involved in the cell signaling pathway.

Quantification of oxidative stress

A number of systemic changes indicate an increased oxidative stress. We found a marked increase of DNA-breaks with the Comet Assay Analysis.

Optic nerve and its meningothelial sheets

We have shown that cells of the arachnoid of the optic nerve of glaucoma patients tend to proliferate and to form meningothelial cell nests. We will now study the biological behaviour of these cells in cell culture.



Immunoflorecense staing of meningothelial cells with keratin sulfate in different concentration (1:50 upper left, 1:100 upper right and 1:200 bottom left)

Selected Publications

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Asthma
COPD
Fibrosis
Intra-cellular signaling
Transcription factors
Differentiation control

Pneumology

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The regulation of mesenchymal cell differentiation in chronic airway diseases

Asthma and COPD are the most frequently diagnosed inflammatory lung diseases with increasing pre-valances. Inflammation is often seen as a condition that is mainly due to an over-reacting immune system with little attendance given to the participation of the tissue forming resident cells. Increasing data, however, supports the view that the tissue forming cells are not a mere reactive cell mass, but participate and may be even trigger inflammatory processes. Therefore we included two diseases which are linked to tissue inflammation, lung fibrosis and mesothelioma. In our studies we also include immune cells or pro-inflammatory mediators that are released by those cells with the focus on the pro- or anti-inflammatory response of the tissue forming cells.
Based on our observation that airway smooth muscle cells of asthma patients proliferate faster compared to cells obtained from healthy controls or COPD patients we investigate their motility response to known pro-inflammatory stimulators. We could show that urokinase functions as a supporter for PDGF-induced migration and that this mechanism involved the link of shp-2 to the urokinase receptor (1). Motility is linked to the contractile properties of cells and specifically for smooth muscle cells extended constrictions is a major pathology during an asthma attack. Assessing their contractile response to several pro-inflammatory stimuli we observed a significantly increased constriction of airway smooth muscle cells of asthma patients compared to cells of healthy controls or COPD patients (2). In two additional studies we provided evidence that asthma exacerbation due to viral infection is due to a modified signaling which is based on a distinct synthesis of, and response to IL-6, compared to non-asthma derived airway smooth muscle cells.
We also provided data showing that the synthesis and composition of the extracellular matrix by tissue forming cells (fibroblasts and smooth muscle cells) depends on the density of the cells and of the cell culture condition. In these studies we defined “inflammation” as the presence of high serum concentrations (5-10%) in the growth medium. In an organ high serum concentrations only occur in the tissue during the early phase of inflammation or injury, and a lower than normal oxgen supply of the tissue is common. Under these conditions we demonstrated that the PDGF-induced extracellular matrix synthesis is modified by low oxygen levels (hypoxia) and is cell type specific in human lung cells (fibroblasts versus smooth muscle cells) (3). Furthermore, we showed that the usually reducing effect of steroids on extracellular matrix remodeling is converted into a stimulating effect in the presence of serum (inflammation) which may act as a shield for the affected cells to reduce the further influx of pro-inflammatory stimuli (4). However, the increased deposition of extracellular matrix around a smooth muscle cell may reduce its relaxation after constriction due to forced water loss of the extracellar matrix and therefore increase airway stifness. The role of specific transcription factors in this response shift to steroids is currently investigated. We postulated earlier that the loss of the specific transcription factor C/EPB-alpha is specific for airway smooth muscle cells of asthma patients and this finding could be further supported (5). In the same study we found evidence that the similar symptoms of asthma and COPD may be due to a deregulation of another member of the C/EBP protein family. We observed that in airway smooth muscle cells of COPD patients the expression of C/EBP-alpha is as in healthy controls, but the expression of C/EBP-beta and C/EBP-delta are deregulated compared to cells of asthma patients or controls. C/EBP-beta is generally up-regulated in COPD cells and C/EBP-delta is induced by serum treatment while in controls cells it is down-regulated. Furthermore, our results clearly showed that the expression of at least these three C/EBP members inhuman cells is regulated by translation rather than by transcription as suggested by rat and mouse models. The precise mechanism of this control mechanism is currently investigate

Connection to Clinical Practice

Cell differentiation: a regulator of chronic airway diseases

The prevalence of chronic airway diseases, including asthma, chronic obrstructive pulmonary disease (COPD, and fibrosis have been rising world wide without any tracable cause. It was assumed that all chronic diseases are due to a de-regulated immune response to environmental factors. However, increasing evidence suggests that a pre-disposition of disease specific tissue forming mesenchymal cells.
Our studies in diseased and healthy human airway cells have shown that these cells release signifcant amounts of pro-inflammatory cytokines after stimulation which in turn activate immune cells. Increased numbers of pre-mature airway smooth muscle cells in asthma (large/medium sized airway) and COPD (small airways) are responsible for airway hyperreactivity and secretion of pro-inflammatory cytokines. Steroids down-regulated pro-inflammatory cytokines, but do not lower proliferation of smooth muscle cells. Furthermore, steroids increase the secretion and desosition of extracellular matrix components in the presence of inflammation, while they reduce the them in none-inflammed tissue. Our data explains why there is no change of airway wall thinkening under steroid and beta2-agonist therapy in asthma and COPD and urges the need to develpe novel therapeutic stragties.
In fibrosis we provide data that epithelial cells provide the major factor that controls fibroblast proliferation and differentiation and suggest that in fibrosis the epithelial cell fibroblast interaction is interrupted. Therefore, therapeutic strategies have to be developed which address this target.

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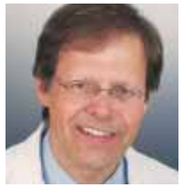
Risk Free Diagnosis
Genetic analysis
Proteomics
Cell Free Nucelic acid in circulation
and Tumor marker's

Prenatal Medicine and Gynecological Oncology



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Women's Health: Non Invasive Prenatal Diagnosis and Tumor Marker's in Gyn. Cancer

The Laboratory of Prenatal Medicine and Gynecological Oncology in Basel, headed by Prof. Wolfgang Holzgreve, MD and Prof. Sinuhe Hahn, PhD, deputy PD Dr. Xiao Yan Zhong and Dr. Olav Lapaire, is a leading research group in the field on non-invasive prenatal diagnosis and earlier diagnosis of gynaecological cancers in Europe. The laboratory is part of the University Women's Hospital Basel, which is a referral centre for high-risk pregnancies, breast cancer and gynecological cancers. The laboratory is well equipped with separate rooms for the preparation of human samples, Magnapure system for automated DNA/RNA extraction, ABI Taqman real-time PCR systems for quantitative PCR analysis, Laser Dissecting Microscope, equipment for FISH analysis, and a Sequenom Mass Array for high throughput DNA and RNA analysis.

In the prenatal area, the Basel lab is a pioneer concerning the use of fetal cells and placenta derived cell-free fetal in maternal circulation for the non-invasive determination of fetal genetic traits. In this context our lab e.g. introduced the use of MACS (magnetic cell sorting) for the enrichment of rare fetal erythroblasts from maternal blood, and participated as the only non-US based group in the NIH funded Nifty study, with Prof. Holzgreve functioning as Principle Investigator. During this study we processed and examined close to 1000 maternal blood samples. Our group also made the novel discovery that preeclampsia, a severe disorder peculiar to human pregnancy, is associated with elevated fetal cell trafficking, and that this disturbance occurs prior to the onset of symptoms. Pioneering studies also addressed the potential use of this material to study abnormal placentation, such as pregnancies at high altitude in Tibet. The leading role of our lab in the field has permitted us to play a major role in the establishment of the EU funded SAFE Network of Excellence, in which Prof. Hahn is acting scientific director and leader of the work package addressing the use of fetal cells. The Basel lab has also played a leading role in the examination of cell-free fetal DNA and mRNA, performing some of the first large scale studies which indicated the remarkable accuracy of this approach for the detection of certain fetal genetic loci such as that for Rhesus D. Our lab also made the important observation that fetal cell-free DNA fragments can be enriched for on the basis of a smaller size than maternal fragments, and that this can be used to detect otherwise masked fetal loci, such as point mutations involved in β-thalassemia for which a patent was accorded. These studies are now being extended with the use of a new cutting edge Sequenom Mass Array mass spectrometer, for which we transferred a patent to the company.
After 15 years of research now already more than 40'000 cases of non-invasive prenatal diagnoses have been conducted, especially for Rhesus D and Kell factor, but also by sex-chromosome analysis for X-linked, compound heterozygous and paternally inherited autosomal dominant diseases.

In the cancer area, PD Dr. Zhong (leader of oncology section) was previously involved in the project of detection of micrometastatic breast tumour cells in bone morrow and peripheral blood at the University Women's Hospital Heidelberg. Zhong et al developed a highly sensitive method to detect rare human breast cancer cells, which combines an immunomagnetic separation (IMS) using antibody BM2 against MUC-1 with cytokeratin-19 (CK19), and the reverse transcriptase/polymerase chain reaction (RT-PCR) and/or immunocytochemistry.We have extensive experiences using MALDI-TOF Mass ARRAY to detect SNPs in prenatal diagnosis and gynecologic oncology. We are establishing a new quantitative high-throughput MassARRAY system to determine cancer derived cell-free and cellular DNA in circulation. For this the laboratory is funded by two FP6 European Commission projects SAFE and Pregenesys and Krebsliga Beider Basel, OncoSwiss and the SNF.

Connection to Clinical Practice

University Women's Hospital
Non-invasive Prenatal Diagnosis

The possibility of obtaining a reliable prenatal diagnosis from fetal material in the maternal circulation has finally become a clinical reality. The analysis of cell-free fetal DNA from maternal plasma has now resulted in the ability to reliably detect single gene mutations, which are not present in the maternal genome. Good examples were the diagnosis of the Rhesus factor in Rh-negative women, where the non-invasive technique has been applied successfully in more numerous cases, or the detection of the Y chromosome, which has been used for detecting X-linked more than 10'000 diseases and fetal autosomal dominant diseases inherited through the paternal line. We were able to show recently that even the diagnosis of compound heterozygously affected fetuses (e.g. beta-thalassemia), using DNA-size separation and DNA-clamping was possible. The influx of fetal material may be associated with specific pregnancy associated diseases, such as preeclampsia. The influx of apo-necrotic fetal debris has been shown to be toxic to the maternal endothelial system, and that the increase of this influx preceeds the onset of preeclampsia by some weeks because it allows functional placental studies for the first time in a non-invasively. The study of fetal RNA in the maternal circulation is also proving to be useful in the study of pregnancy-related disorders. The analysis of placentally derived cell-free mRNA can be used to detect fetal Down syndrome by quantifying relative expression of chromosome 21 specific gene (PLAC4) alleles to each other by the mass spectrometric analysis of SNP loci. Altogether, an impressive and growing number of conditions can already be detected non-invasively from maternal blood.

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Atherosclerosis
Inflammation
Angiogenesis
Signal transduction
Cadherin
Stress response

Signal Transduction

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Mechanisms of neovascularization in atherosclerosis

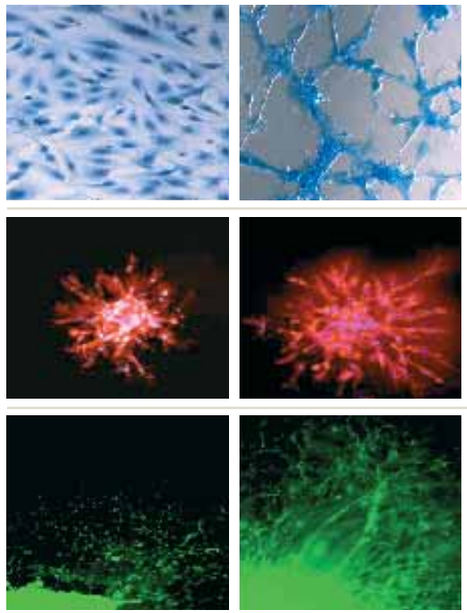
Adhesion molecules

T-cadherin is an atypical GPI-anchored member of the cadherin superfamily of adhesion molecules. We focus on investigating its role in the vasculature. In vivo T-cad expression is up-regulated on vascular smooth muscle (SMC) and endothelial (EC) cells in atherosclerotic lesions and during restenosis, and also on EC in tumour vasculature. These are conditions associated with uncontrolled cell migration and growth and with microenvironmental oxidative stress. In vitro T-cad expression is upregulated on proliferating SMC and EC or EC exposed to oxidative stress, and T-cad redistributes to the leading edge of migrating cells. Further, T-cad overexpression and/or homophilic ligation promotes EC proliferation, motility, survival during oxidative stress and angiogenesis both in vitro (see images) and in vivo. These data support our hypothesis that T-cad possesses novel proangiogenic properties and also suggest that T-cad is both a marker of EC activation/stress and an inducer of an activated EC phenotype. Bi-directional modulation of T-cad expression on EC using gene transfer or silencing offers modalities to either improve outcome of growth factor-dependent proangiogenic therapy or to treat pathological conditions associated with excessive neovascularization.
Signaling mechanisms whereby T-cad mediates all these effects are poorly understood. Induction of the polarized angiogenic phenotype by T-cad requires activation of RhoA/ROCK and Rac. T-cad promoted proliferation and survival involves activation the PI3K/Akt/GSK3β signal transduction axis with downstream targets including mTOR/p70S6kinase, β-catenin/cyclin D and p38MAPK. How cell surface GPI-anchored T-cad transmits signals across the membrane to its intracellular targets is also unclear. We have found that T-cad co-associates as a complex with a number of candidate membrane "molecular/signaling" adaptors, amongst them Integrin-linked kinase (ILK), Integrin β3 and Grp78. ILK and Grp78 are required for T-cad-mediated activation of Akt/GSK3β pathway and survival of EC under conditions of oxidative stress. Grp78, a key mediator of endoplasmic reticulum (ER) stress response, also activates Akt/GSK3β pathway, suggesting cross-talk with T-cad and a possible role for T-cad in adaptive cell response to ER stress. Other recent data show that cultured, activated-EC can release T-cad from their plasma membranes in microparticle-bound and possibly also shedded forms, invoking a potential signaling role for T-cad within the extracellular space and circulation in vivo. These aspects of T-cad biology are under investigation.

Inflammation and angiogenesis in plaque instability

During atherosclerosis small newly formed vessels (vasa vasorum) appear in the plaque area which is characterized by abnormal lipid accumulation and inflammatory cells. Murine models have shown that activation of Natural Killer T (NKT) cells by lipid antigens during inflammation exacerbates atherogenesis and plaque instability. NKT cells react to lipid antigens presented by CD1d molecules which are expressed by foamy cells in plaques. In collaboration with Prof. G. De Libero (Experimental Immunology) and Dr. B. Biedermann (Molecular Nephrology), and using human cell models we investigate the hypothesis that activation of NKT cells may constitute a proangiogenic mechanism in atherosclerosis. Examination of atherosclerotic plaque and normal blood vessel tissues by immuno-based tissue array revealed correlations between CD1d expression and atherosclerotic disease and between the number of $CD1d^+$-cells and vascularity. In vitro data using the EC spheroid model of angiogenesis support that activation of NKT cells creates a milieu which favours angiogenesis. Using 2D monolayer EC cultures we found that induction of angiogenesis occurs via a stimulatory effect on EC motility which is dependent upon IL-8 released by the activated NKT cells. Induction of proangiogenic genes in EC also occurs, suggesting co-operative autocrine angiogenic mechanisms. Immune-cell targeted therapies could reduce atherogenesis and atherothrombosis by limiting neovascularization at sites of inflammation.

Homophilic ligation of T-cadherin induces angiogenesis *in vitro*



Formation of cord-like structures in monolayer endothelial cell culture

In-gel sprout outgrowth from endothelial cell spheroid

In-gel endothelial cell sprout outgrowth from heart tissue

\- + Recombinant T-cadherin protein within substratum

Connection to Clinical Practice

Novel biomarkers of endothelial dysfunction and atherosclerotic disease

Acute coronary syndromes (ACS) are severe and sudden heart conditions caused by myocardial ischemia. Atherosclerosis, which causes harmful build-up of plaque lesions in coronary blood vessels and vessel occlusion, is the predominant underlying disease. In patients with ACS early morbidity and mortality is caused by plaque rupture and ensuing thrombus formation, which can occur very abruptly and without warning. We recently reported on the clinical benefit of searching for, and treating, silent ischemia even if patients lack symptoms of atherosclerosis. Earlier identification and management of patients at risk of ACS needs diagnostic tests which determine coronary artery disease severity or indicate plaque instability or rupture before myocardial damage and necrosis become apparent. To identify a risk profile of rupture-prone plaque we aim to assess atherosclerotic profiles in defined patient groups (i.e. with atherosclerosis, with asymptomatic atherosclerosis, without atherosclerosis) on the basis of vessel physical characteristics (lesions present/not), vessel endothelium function (peripheral arterial tonometry (PAT) signal at the fingertip), plasma biomarkers of endothelial stress/activation/damage (presence/concentrations) and myocardial ischemia (presence/absence); inflammatory status (T-cell profile; collaboration with Prof. G. de Libero, Experimental Immunology). Specific relationships between the various parameters and clinical outcome may be helpful in identifying the relative risk constellation of plaque rupture and mortality. Use of a multi-marker diagnostic paradigm could improve risk stratification of patients with ACS and determination of treatment measures.

Selected Publications

- Bochkov, V. N., Philippova, M., Oskolkova, O., Kadl, A., Furnkranz, A., Karabeg, E., Afonyushkin, T., Gruber, F., Breuss, J., Minchenko, A., et al. (2006). Oxidized phospholipids stimulate angiogenesis via autocrine mechanisms, implicating a novel role for lipid oxidation in the evolution of atherosclerotic lesions. Circ Res 99, 900-908.
- Erne, P., Schoenenberger, A. W., Burckhardt, D., Zuber, M., Kiowski, W., Buser, P. T., Dubach, P., Resink, T. J., and Pfisterer, M. (2007). Effects of percutaneous coronary interventions in silent ischemia after myocardial infarction: the SWISSI II randomized controlled trial. Jama 297, 1985-1991.
- Joshi, M. B., Philippova, M., Ivanov, D., Allenspach, R., Erne, P., and Resink, T. J. (2005). T-cadherin protects endothelial cells from oxidative stress-induced apoptosis. Faseb J 19, 1737-1739.
- Philippova, M., Banfi, A., Ivanov, D., Gianni-Barrera, R., Allenspach, R., Erne, P., and Resink, T. (2006). Atypical GPI-anchored T-cadherin stimulates angiogenesis in vitro and in vivo. Arterioscler Thromb Vasc Biol 26, 2222-2230.
- Philippova, M., Ivanov, D., Allenspach, R., Takuwa, Y., Erne, P., and Resink, T. (2005). RhoA and Rac mediate endothelial cell polarization and detachment induced by T-cadherin. Faseb J 19, 588-590.

Cartilage
Bone
Stem cells
Mechanobiology
Bioreactors
3D cultures

Tissue Engineering

Prof. Dr. Ivan Martin Prof. Dr. Michael Heberer

Department of Biomedicine
Institute for Surgical Research and Hospital Management (ICFS)
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A multidisciplinary team for the engineering of cartilage and bone constructs

The ultimate goal of the research group is to generate grafts based on autologous cells for the treatment of damaged or lost cartilage and bone tissues, as well as of more complex osteochondral lesions. Beyond a potential clinical use as implants, the engineered constructs are also being considered as 3D model systems to investigate fundamental aspects of cell differentiation and tissue development under controlled and defined conditions. The scientific questions addressed are related to (i) the comparison of cell sources (mature, progenitor, stem cells) from a variety of human tissues, (ii) the effect of specific environmental factors and culture conditions (soluble factors, oxygen levels) on cell growth and differentiation, (iii) the interaction of cells with 3D scaffolds in different architectures and compositions (meshes, foams, or nanostructured geometries based on synthetic or natural materials) and (iv) the cell response to controlled regimes of physical stimuli (perfusion, compression) applied to 3D culture models using bioreactor systems. These projects are at the interface between fundamental and applied research and are based on a tight collaboration between biologists, engineers, material scientists and surgeons. Beyond national (SNF, KTI) and industrial support, research is generously funded in the context of european consortia (EU Frameworks VI and VII), which have been instrumental for a strong international networking of the group.

Main recent achievements

1. We have demonstrated that human mesenchymal stromal cells can be selected and expanded by direct perfusion of bone marrow nucleated cells in alternate directions through the pores of ceramic scaffolds. The resulting constructs contained early mesenchymal and hemopoietic progenitors, and generated abundant bone tissue when implanted ectopically in nude mice (Fig. 1). The developed system not only streamlines the generation of osteogenic grafts, but also represents a 3D in vitro model to study interactions between mesenchymal and hematopoietic cells.
2. We have established that 3D perfusion culture of human lipoaspirate-derived cells results in constructs with both osteogenic and vasculogenic capacity when implanted ectopically in nude mice. These cells could represent an ideal source for the engineering of large, pre-endothelialized bone substitutes.
3. In collaboration with the University of Bern, we have identified surface markers to characterize and possibly predict the inter- and intra-individual chondrogenic differentiation capacity of human chondrocyte populations. These markers will be further explored for quality and potency control in cell-based cartilage repair procedures.
4. We have determined that human nasal chondrocytes can respond to physical forces typical of joint loading in a pattern similar to articular chondrocytes. Considering the higher reproducibility of human nasal as compared to articular chondrocytes to engineer cartilage tissues, the study opens new prospectives for transplantation of nasal chondrocytes for articular cartilage repair.
5. An interdisciplinary project combining advanced imaging tools, mathematical modeling and experimental cell culture work has been established to determine operating conditions (e.g., oxygen tension, perfusion flow rate) for the generation of up to 4 mm thick cartilage grafts with a uniform deposition of extracellular matrix.
6. In collaboration with INFORS AG, we have developed a stand-alone bioreactor system for the seeding and culture of cells into 3D scaffolds, based on direct perfusion of cell suspensions or culture medium through the scaffold pores (Fig. 2). The system may be used as a model to investigate cell function and tissue development in a 3D environment under controlled conditions, as well as for the manufacturing of tissues, starting from different types of cells and porous scaffolds
7. In collaboration with the Rheumatology clinic (Prof. A. Tyndall, Dr. C. Bocelli-Tyndall), we have identified and characterized immunomodulatory properties of human chondrocytes and of bone marrow-derived mesenchymal stromal cells from healthy donors and auto-immune disease patients.

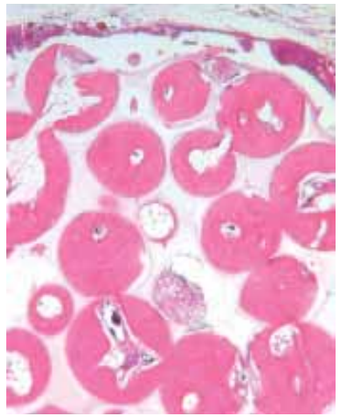

Fig. 1: Engineered bone tissue (dense pink areas) generated by subcutaneous implantation in nude mice of constructs obtained by perfusion of human bone marrow cells through porous ceramic scaffolds

Fig. 2: Stand-alone Tissue-Culture Under Perfusion (T-CUP) bioreactor system (left), allowing uniform seeding and culture of cells within a variety of scaffold sizes and shapes. The right images exemplify the loading of mesenchymal stromal cells into granulate ceramic particles, contained within a basket.

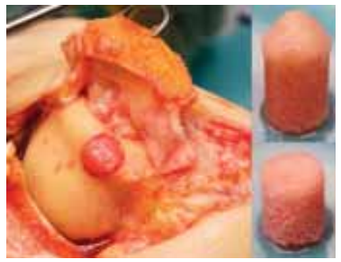

Fig. 3: Osteochondral composite tissues based on adult human cells cultured on biomimetic materials can be engineered in a variety of shapes (see insets on the right) and inserted within confined osteochondral defects, precisely matching the contour of the cartilage surface.

Connection to Clinical Practice

PD Dr. Marcel Jakob
Behandlungszentrum Bewegungsapparat (Operative Medizin)

Musculoskeletal engineered grafts in orthopaedic and reconstructive surgery

The goal of the group is to introduce into specific surgical procedures the use of engineered implants, based on autologous cells and suitable 3D scaffolds. Our vision lies in the standardized production of such grafts within specialized, closed and automated bioreactor systems, where cells are processed and cultured under controlled environmental conditions. Following is a brief description of currently targeted clinical applications:

1. Use of engineered cartilage for reconstruction of the alar septum of the nose following tumour resection, as an alternative to autologous cartilage. A phase I clinical trial has been approved by the internal Ethical Committee and – provided the availability of a GMP-compatible facility to generate the grafts – is expected to start in the near term (PD Dr. J. Farhadi, Dr. M. Haugg)
2. Use of engineered tissues for the treatment of chondral or osteochondral joint defects, with the goal to ensure long-lasting regeneration (Fig. 3). In collaboration with the team of Prof. N. Friederich in Bruderholz, the group is planning a phase I trial for the implantation of autologous cell-based, functional cartilage tissues (Dr. C. Candrian, PD Dr. D.J. Schäfer)
3. Use of engineered osteogenic grafts for bone repair procedures. The group is in the phase of pre-clinical validation of engineered osteogenic tissues to replace autologous bone in spinal fusion surgery and maxillary sinus elevation. An "intraoperative concept" to facilitate clinical use is also under development (PD Dr. C. Jaquiery, Dr. A. Mehrkens, Dr. A.M. Müller, Dr. S. Schären)

Selected Publications

- Candrian, C., Vonwil, D., Barbero, A., Bonacina, E., Miot, S., Farhadi, J., Wirz, D., Dickinson, S., Hollander, A., Jakob, M., Li, Z., Alini, M., Heberer, M., Martin, I. Engineered human nasal cartilage is responsive to physical forces resembling joint loading. Arthritis Rheum 58: 197-208 (2008)
- Scherberich, A., Galli, R., Jaquiery, C., Farhadi, J., Martin, I. (2007). 3D perfusion culture of human adipose tissue-derived endothelial and osteoblastic progenitors generates osteogenic constructs with intrinsic vascularization capacity. Stem Cells 25, 1823-1829
- Marsano, A., Millward-Sadler, S.J., Salter, D.M., Adesiva, A., Hardingham, T., Tognana, E., Kon, E., Chiari-Grisar, C., Nehrer, S., Jakob, M., Martin, I. (2007). Differential cartilaginous tissue formation by human synovial membrane, fat pad, meniscus cells and articular chondrocytes. Osteoarthritis Cartilage 15, 48-58
- Jaquiery, C., Schaeren, S., Farhadi, J., Mainil-Varlet, P., Kunz, C., Zeilhofer, H.F., Heberer, M., Martin, I. (2005). In vitro osteogenic differentiation and in vivo bone-forming capacity of human isogenic jaw periosteal cells and bone marrow stromal cells. Ann Surg 242, 859-867
- Braccini, A., Wendt, D., Jaquiery, C., Jakob, M., Heberer, M., Kenins, L., Filipowicz, A.W., Quarto, R., Martin, I. (2005). Three-dimensional perfusion culture of human bone marrow cells and generation of osteoinductive grafts. Stem Cells 23, 1066-1072

Angiogenesis
Endothelium
Hypoxia
Hypertension
Renin-Angiotensin System
mTOR

Vascular Biology

group left during report period

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Dr. Lourdes Sanchez de Miguel
Dr. Nicole Butz
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Marco Petrimpol (PhD student)
Veronica Munk (PhD student)
Weimin Li (PhD student)
Sonja Jakob (trainee)
Kaija Paris (technician)

Angiogenesis between Hypertension and Hypoxia

Impaired cardiac function during ischemic heart disease or left ventricular hypertrophy may be caused by inadequate blood supply to the myocardium. Restoring blood flow to the heart by controlled induction of angiogenesis, i.e., the formation of new microvessels from existing ones, may improve heart function and relieve heart disease. On the other hand, prohibiting new microvessel formation in cancerous growths has proven to be a new and effective cancer therapy. The Laboratory of Vascular Biology – associated with the Medical Outpatient Department – investigates intracellular signaling in response to stimuli determining pathological aspects of myocardial ischemia, hypertension and cancer (Battegay et al., 2007).

mTORC1 and mTORC2 in the Regulation of Hypoxia-Induced Angiogenesis (Li et al., 2007)

Angiogenesis is a complex process involving multiple steps; Micro-gradients, concentrations and combinations of angiogenic molecules, specific matrix composition and oxygen content of the local microenvironment all influence the development of newly emerging microvessels. Ideally, a common circuit, through which all these signals would act and that controls angiogenesis would be the target of choice.
Mammalian target of rapamycin (mTOR) is a central regulator of cell growth that integrates a multitude of extracellular signals from growth factors, nutrients or stress. We have identified mTOR as a decisive signal relay enzyme in the regulation of angiogenesis in response to hypoxia. Hypoxia is the main angiogenic stimulus that directly monitores insufficient vascular supply to the endothelium. mTOR was recently shown to function in two complexes with distinct functions: mTORC1 and mTORC2. Our data derived from molecular in vitro studies show that mTORC1 and mTORC2 participate in the endothelial response to hypoxia in a reciprocal and timed manner: Whereas mTORC1 is inhibited after longterm hypoxic exposure by translocation into the nucleus (Figure 1), mTORC2 is activated in sustained way and transmits a pro-angiogenic signal via its downstream target PKB. Thus, mTORC2 may be a potent target in ischemia-associated vascular hyperplasia or angiogenesis. Our future research will address these roles of mTORC1 and mTORC2 in vivo, i.e., in VEGF- and tumor-driven angiogenesis in transgenic mouse models.

The Role of the Renin-Angiotensin System in Angiogenesis of the Hypoxic Heart (Munk et al., 2007)

Angiotensin II (ANG) receptor blockers are used for hypertension-induced left ventricular hypertrophy. ANG is a potent vasoconstrictor, however, was also reported to have angiogenic properties. Therefore we assessed whether ANG can increase myocardial vascularization under normoxic and hypoxic conditions. For this purpose we developed a model of angiogenesis of the heart in vitro, where mouse or rat heart pieces are stimulated under controlled conditions in a three dimensional matrix (Humar et al., 2007) (Figure 2). Using this assay we specifically blocked or induced the two ANG receptors AT1 and AT2 in heart pieces from wildtype mice. Further, to corroborate our findings, we examined the angiogenic response in heart tissues derived from mice that lack the AT1 or AT2 receptor and additional associated genes.
Our data show that – under conditions of hypoxia – ANG elicits angiogenesis in mouse hearts by AT2 activation, which promotes Bradykinin-induced B2 receptor signaling leading to nitric oxide synthesis (see also (Munk et al., 2006)) as an obligatory angiogenic effector. Thus, therapeutic blockade of the AT1 receptor in hypertensive patients may potentially be beneficial for improving heart function by AT2/B2 induced neovascularization. Current investigations focus on the role of Bradykinin B1 and B2 receptor activation in angiogenesis of the hypoxic heart in vitro.

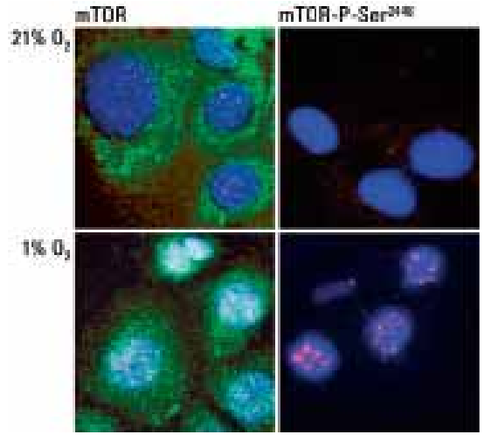
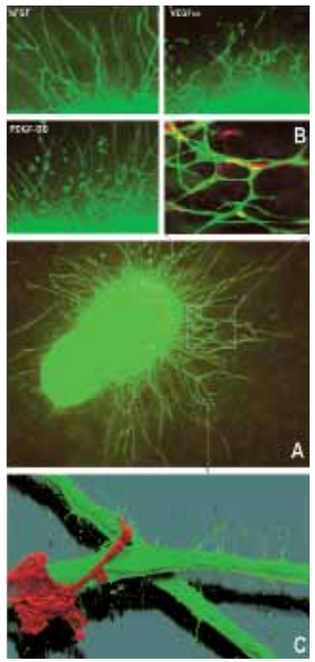


Fig. 1: Hypoxia mediates rapid, dose-dependent, sustained phosphorylation of mTOR Ser 2448 and translocation to the nucleus in rat aortic endothelial cells (RAEC) and thereby deactivates mTORC1. Quiescent primary RAECs were exposed for 6 hours to normoxia (upper panels) and hypoxia (lower panels). Immunestaining of mTOR (green stain), mTOR phospho-Ser2448 (red stain) and nuclear compartment (blue stain) reveals clustering of phosphorylated mTOR (left panels) in the nucleus only under hypoxia.

Fig. 2:
Distinct morphologies of endothelial sprouts are observed after administration of different angiogenic molecules to mouse heart explants: bFGF = basic Fibroblast Growth Factor, PDGF-BB = Platelet-Derived Growth Factor B-dimer and VEGF = Vascular Endothelial Growth Factor. ANG-induced endothelial sprouts resemble endothelial morphology specific to VEGF. (A) Characterization of angiogenic sprouts emerging from hypoxic hearts by lectin G. simplicifolia (green fluorescent) for endothelial cells and (B) magnification including smooth muscle cell specific staining (red). (C) Confocal micrograph of two crossing endothelial tubes. Tiny filopodia reach out and sense the environment for neighboring endothelial tubes.



Connection to Clinical Practice

Medical Outpatient Department & Hypertension Clinic

The Metabolic Syndrome: Prevalence, Clinical Risk Assessment and new Biomarkers

The Metabolic Syndrome (MS) is a constellation of metabolic derangements that includes insulin resistance, hypertension, dyslipidemia, central obesity, type 2 diabetes and accelerated cardiovascular disease. The pathogenesis of the MS is complex and has only been partially elucidated. However, two factors seem to play a major role in the development of the MS: central obesity and insulin resistance.
Proteomics is increasingly used to examine dynamic changes in protein expression providing new insights into cellular processes. Moreover, proteomic analyses have already resulted in the identification of clinically useful biomarkers and can assist in diagnosis and disease staging. Substances contained in body fluids hold an abundance of information, and can be used as a dynamic and concurrent gauge for monitoring the wellbeing of an organism. We hypothesize that proteomic analysis of body fluids such as urine and serum should yield a panel of biomarker peptides useful as additional tools for the diagnosis and monitoring of the MS.
In the clinical part of the project, data on the prevalence of the MS and the risk assessment, as performed by the treating physicians at the Medical Outpatient Department, will be obtained. Furthermore, we will assess if psychiatric co-morbidities, such as depression, are more prevalent in patients with the MS.

Selected Publications

– Battegay, E. J., de Miguel, L. S., Petrimpol, M., and Humar, R. (2007). Effects of anti-hypertensive drugs on vessel rarefaction. Curr Opin Pharmacol 7, 151-157.
– Humar, R., Sanchez de Miguel, L., Kiefer, F. N., and Battegay, E. J. (2007). Formation of New Blood Vessels in the Heart Can be Studied in Cell Cultures. ALTEX Spezial Issue 4.
– Li, W., Petrimpol, M., Molle, K. D., Hall, M. N., Battegay, E. J., and Humar, R. (2007). Hypoxia-induced endothelial proliferation requires both mTORC1 and mTORC2. Circ Res 100, 79-87.
– Munk, V. C., Sanchez de Miguel, L., Humar, R., Kiefer, F. N., Butz, N., and Battegay, E. J. (2006). iNOS is required for in vitro angiogenesis of hypoxic mouse hearts. Seminars in Cardiology 12, 21-26.
– Munk, V. C., Sanchez de Miguel, L., Petrimpol, M., Butz, N., Banfi, A., Eriksson, U., Hein, L., Humar, R., and Battegay, E. J. (2007). Angiotensin II induces angiogenesis in the hypoxic adult mouse heart in vitro through an AT2-B2 receptor pathway. Hypertension 49, 1178-1185.

DBM Focal Area Oncology

Focal Area Coordinators

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Prof. Dr. R. Herrmann
Division of Clinical Oncology
University Hospital Basel

The major goal of this research program is to support and expand research in the field of molecular and clinical oncology in Basel. In particular, we aim at bridging the gaps between basic, translational, and clinical oncology research ongoing within the University of Basel and the non-University research institutes, biotech and pharmaceutical industry in the Basel area. Ultimately, the program should enforce collaborative efforts and common projects between various research groups, research institutes and pharma industry and between different disciplines. An added value is seen in innovative projects that eventually pay off by being transferred to a clinical setting. Apparently, the research program relies heavily on the participating individuals' enthusiasm and initiatives. In the long run, it is hoped that the combined basic, translational and clinical research efforts will form a critical and central part of a comprehensive cancer center in Basel.

The research program is currently led by Prof. Gerhard Christofori, head of the Institute of Biochemistry and Genetics, and Prof. Richard Herrmann, head of Clinical Oncology at the University Hospital. The program focuses on two major areas: first to support basic, translational, and clinical research by either generating additional positions or opportunities for oncology research or by hosting new recruitments, such as SNF Assistant Professors and SCORE fellows within DBM. The second focus is to increase communication between the various researchers, clinicians and pharmaceutical company representatives in Basel and to generate and offer platforms for scientific exchange and technological collaboration. Towards this goal the program has installed a weekly seminar series, the Onco-Seminars, in which members of the various research groups of the DBM present their newest results and discuss common interests. In a more clinical-oriented seminar series, named Onco-Lunch, newest insights into clinical oncology are being discussed. In addition, outstanding international cancer researchers are invited to present lectures within the "DBM Oncology Program Seminars", and an impromptu guest seminar series completes the seminar activities of the research program. Thus far, these communication activities have led to a large number of highly successful collaborations and research networks, notably beyond the borders of institutes and pharmaceutical companies. Accordingly, many of these efforts within the DBM Oncology program have been part of international and national research initiatives that cover innovative approaches to cancer research and treatment, including research on cancer genetics and genetic instability, cancer epigenetics, angiogenesis and metastasis, signal transduction, cancer stem cells, tumor vaccination, and novel therapeutic regimen.

In the past years, the research program Oncology has been strengthened by the recruitment of additional faculty active in oncology research. For example, Prof. Jürg Schwaller has been recruited as Gertrude von Meissner Endowed Professor for Childhood Leukemia and Dr. Olivier Pertz moved as SNF-Assistant Professor from San Diego to the DBM at the end of 2007. On the other hand, PD Dr. Gertraud Orend has recently left the DBM for a position in Strassbourg, and we wish her all the best for the future. The reorganization of the DBM with the existing research laboratories at Hebelstrasse and the Institute of Medical Microbiology at Petersplatz and the new research building at Mattenstrasse has certainly improved the critical mass of research and communication within DBM.

In the years to come, we need to further enforce scientific exchange between basic and patient-oriented research in order to facilitate the identification of clinical problems for the design of appropriate and innovative basic research approaches and, on the other hand, to improve on the rapid translation of basic research results into clinical application.

Inflammation
Cancer
Lipid Signaling
Phosphoinositide 3-kinase
Growth
Cell migration

Cancer- and Immunobiology

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Emilie Collmann (PhD student)
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Markus Buschle (Master student)
Priska Reinhard (technician)

Inflammation and Cancer – Role of Lipid Signaling

Chronic inflammation and cancer share some features, as cells deviate in both disease types from normal growth, proliferation and migration. A prominent signaling pathway, which controls all of these processes, is emerging from the activation of phosphoinositide 3-kinase (PI3K). PI3K produces PtdIns(3,4,5)P_3 at the plasma membrane, where the lipid serves as a docking site for pleckstrin homology domain-containing proteins like protein kinase B (PKB/Akt). PKB thus relays growth signals to the target of rapamycin complex (mTOR). The branching of PI3K activation controls a plethora of events, and is fine tuned by the temporal and spatial activation of four class I PI3K isoforms (designated α through δ, see Wymann and Marone, 2005 for a review).

We have demonstrated previously, that the so-called PI3Kγ isoform plays a major role in the chemokine-mediated recruitment of inflammatory cells to inflamed tissue, and is the main PI3K activated downstream of G protein-coupled receptors (GPCRs). These results – obtained by mouse genetics – could recently be confirmed using PI3Kγ-selective inhibitors in mouse models of rheumatoid arthritis (Camps et al. 2005) and systemic lupus (Barber et al. 2006). Here, disease progression was attenuated by impaired adhesion and migration of neutrophils and T-cells respectively. Genetic and pharmacological targeting of PI3Kγ also alleviated atherosclerosis in low-density lipoprotein (LDL) receptor and apolipoprotein E (ApoE)-deficient mice, which correlated with reduced macrophage and T-cell invasion into atherosclerotic plaques. Additionally, plaques in mice without PI3Kγ were stabilized as judged by collagen content (Fougerat et al. 2008).

Currently, we investigate the role of PI3Ks in mast cell activation downstream of immunoglobulin E (IgE) and GPCRs. We have shown earlier that full-scale mast cell activation requires PI3Kγ signaling in anaphylaxis (Laffargue et al. 2002, Immunity). The clustering of the high affinity IgE receptor (FcεRI) activates protein tyrosine kinases, which trigger the recruitment of so-called class IA PI3Ks (PI3Kα, β, and δ) associated with a regulatory subunit (p85) capable to bind phosphorylated tyrosine residues. This "dogmatic" signaling pathway does not explain the relay from FcεRI to PI3Kγ (the only member of class I B PI3Ks), as PI3Kγ is associated with an adaptor that interacts with Gβγ subunits of trimeric G proteins. In the course of our studies, we have uncovered "non-canonical" activation modes of PI3Kγ, and have collected evidence that PI3Kγ can produce different pools of plasma membrane-localized PtdIns(3,4,5)P3. Of these pools, only one is capable to support mast cell degranulation. This observation provides novel opportunities to target PI3Kγ therapeutically, and provides additional levels of regulation of PI3K output signals.

PI3K seems also to play a prominent role in the progression of cancer and metastasis. Multiple tumors have lost the counter-player of PI3Ks, the lipid phosphatase PTEN, which degrades PtdIns(3,4,5)P_3. Other tumors display up-regulated growth factor receptor signaling (e.g. Erb2, c-kit), effector molecules (Ras, Bcr-Abl) or mutated PI3K (PIK3CA). Melanoma show a frequent loss of PTEN, or harbor mutated Ras driving PI3K activation. In collaboration with Novartis, we have concluded a proof-of-principle study using pan-PI3K inhibitors with drug-like properties, which attenuate the growth of primary and metastatic melanoma. In the course of the study it became clear that the inhibitors also target mTOR complexes. In vitro and in vivo, the compounds efficiently blocked growth and proliferation, and additionally prevented angiogenesis in vivo (Fig. 1). A dual hit of the PI3K/PKB/mTOR pathway seems thus to be therapeutically beneficial, but does not yet clarify the role of individual PI3K isoforms in cancer progression. We presently explore PI3Ks in tumor autonomous processes, and investigate the effect of the immune system and inflammation on tumor progression and dissemination.

To better investigate lipid signal in general (see Wymann and Schneiter, 2008 for a review), we collaborate with the Dept. of Chemistry and Biotech companies to generate novel chemical tools to modulate and monitor protein/lipid interactions (Fig. 2). Up-to-date, our studies have characterized PI3Ks as attractive drug targets in inflammation, allergy, autoimmune disease, cancer and cardiovascular disease.

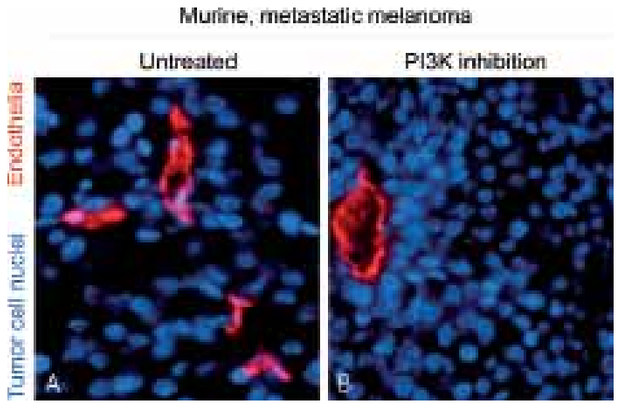

Fig. 1: Treatment of murine, metastatic melanoma with PI3K inhibitors as in B) reduces tumor burden significantly, attenuates tumor cell proliferation and size, and prevents the formation of tumor microvasculature. Preexisting large blood vessels are not affected by the PI3K inhibitors. Blue: nuclei; red: endothelia.

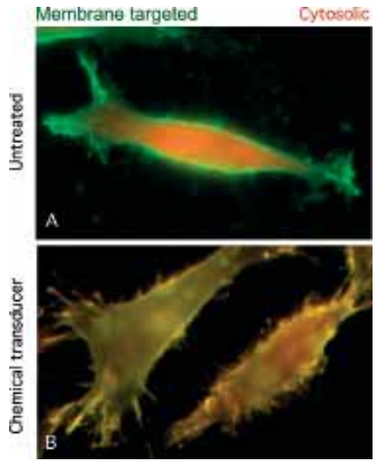

Fig. 2: A cytosolic (red) and a membrane targeted (green) protein fused to domains reacting with a chemical transducer can be provoked to interact with each other. Here the chemical transducer triggers the translocation of the cytosolic protein to the membrane, and the co-localization of the two proteins becomes apparent by the color change to yellow.

Connection to Clinical Practice

The first PI3K inhibitors have recently entered phase I clinical trails in solid cancer. The patent literature reflects the vivid activities of bringing more compounds forward (reviewed in Marone et al. 2008). Rapamycin (Sirolimus) and derivatives, acting downstream of PI3K by targeting the mTOR complex 1 (TORC1), are in clinical use or evaluated for autoimmunity, suppression of transplant rejection, and cancer. A better understanding of drug action and mechanisms causing adverse effects is required.

Selected Publications

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– Camps, M., Ruckle, T., Ji, H., Ardissone, V., Rintelen, F., Shaw, J., Ferrandi, C., Chabert, C., Gillieron, C., Francon, B., Martin, T., Gretener, D., Perrin, D., Leroy, D., Vitte, P. A., Hirsch, E., Wymann, M. P., Cirillo, R., Schwarz, M. K., and Rommel, C. (2005). Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. Nat Med 11, 936-943.
– Ferguson, G. J., Milne, L., Kulkarni, S., Sasaki, T., Walker, S., Andrews, S., Crabbe, T., Finan, P., Jones, G., Jackson, S., Camps, M., Rommel, C., Wymann, M., Hirsch, E., Hawkins, P., and Stephens, L. (2007). PI(3)Kgamma has an important context-dependent role in neutrophil chemokinesis. Nat Cell Biol 9, 86-91.
– Nombela-Arrieta, C., Mempel, T. R., Soriano, S. F., Mazo, I., Wymann, M. P., Hirsch, E., Martinez-A, C., Fukui, Y., von Andrian, U. H., and Stein, J. V. (2007). A central role for DOCK2 during interstitial lymphocyte motility and sphingosine-1-phosphate-mediated egress. J Exp Med 204, 497-510.
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Cell migration
Neuritogenesis
Signaling
Rho GTPase
Systems biology
Live cell imaging

Cell migration and Neuritogenesis

new group since December 2007

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Spatio-temporal Rho GTPase signaling programs in directed cell migration and neuritogenesis

The ability of vertebrate cells to directionally migrate is critical to development, the immune response and wound healing, and its regulation is compromised in pathologies such as metastatic cancer and vascular disease. The capacity of neurons to directionally extend neurites that will subsequently differentiate in axons and dendrites is crucial for the proper wiring of the brain. A detailed understanding of the signaling events that regulate these complex morphogenetic processes is therefore likely to contribute important insights that could be used to target a number of different pathologies. We are interested in the Rho family of small GTPases, which are key molecular switches regulating the cytoskeleton and the cell polarity mechanisms during the two processes mentioned above. Importantly, the activation status of these signaling switches is likely to be highly regulated in space and time at the single cell level, a dimension that is lost when traditional cell biological and biochemical techniques are used. For this purpose, we are developing novel tools to grasp the spatio-temporal dimension of these signaling processes.

Imaging spatio-temporal Rho GTPase signaling

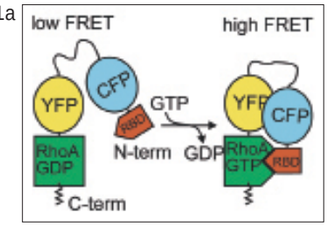
Whereas models for spatio-temporal Rho GTPase signaling have been postulated, direct measurements of the subcellular locations at which Rho GTPases are turned on and off are just emerging. For this purpose, we engineered a genetically-encoded, fluorescence resonance energy transfer-based biosensor that reveals where the small GTPase RhoA is activated in single living cells with high spatial resolution (Fig. 1a). Visualizing the spatio-temporal patterns of RhoA activation during cell migration revealed a much more complex picture of RhoA regulation than previously anticipated. Rather than the classic dogma, in which RhoA is activated at the back of the cell to regulate contractility, RhoA was found to be activated at multiple, discrete subcellular locations with highly defined kinetics. These included the leading edge (specifically during membrane protrusion), the back of the cell (during tail retraction), peripheral membrane ruffles and macropinosomes (Fig. 1b). Thus, rather than RhoA having a unique function controlling cell contractility, each pool of active RhoA is likely to perform a distinct functions by being activated at different subcellular locations. This likely involves interaction of the Rho GTPase with distinct upstream regulators that control its activation status, but also with effectors that transmit downstream signals by binding to the activated GTPase. The challenge is now to map how these different signaling complexes ("signaling modules") assemble and operate in time and space to perform these different functions. This will necessitate a systematic approach using novel methods that reveal this spatio-temporal complexity.

Biochemical analysis of spatio-temporal Rho GTPase signaling

To tackle Rho GTPase spatio-temporal signaling at the biochemical level during neurite outgrowth, we devised a novel assay that enables to biochemically purify in large scale extending neurites from their cell soma (Fig. 2 a,b). This technique can also be used to purify lammellipods from the cell body of migrating cells. This allowed for the first time, a large scale proteomic analysis of the neurite and soma proteomes. Bioinformatic analyses revealed a highly polarized distribution of different proteins in these two cellular domains and allowed a systems biology view of the process with proteins regulating axonal guidance, integrin and actin signaling being highly enriched in the neurite. This dataset was mined for Rho GTPase interacting proteins and revealed a complex regulatory network (Fig. 2c). Functional studies then enabled to define the proteins involved in different spatio-temporal Rho GTPase "signaling modules" regulating neurite extension.

We are now further integrating these imaging (FRET biosensors) and biochemical (lamellipod and neurite purification) techniques to study spatio-temporal Rho GTPase signaling at the systems biology level in different cell migration and neurite outgrowth systems.

1a



1b

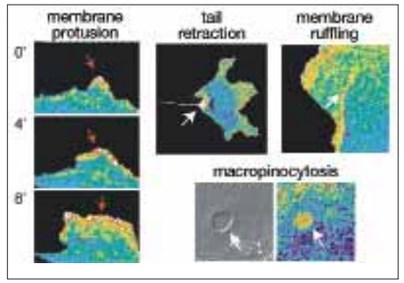
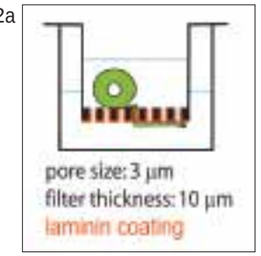
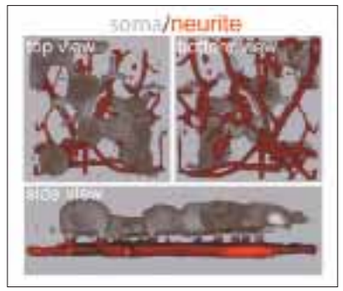


Fig. 1: Imaging RhoA activation in single living cells.
a) Design of RhoA FRET biosensor.
b) Images of RhoA activation in single living cells. Warm/cold colors represent high/low RhoA activation zones.

2a



2b



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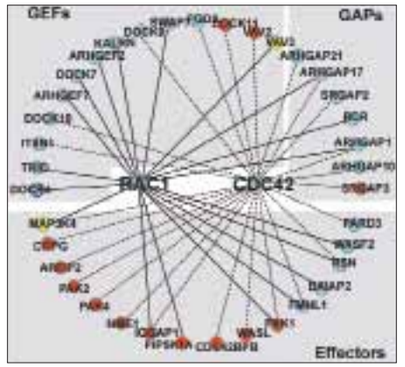

Fig. 2: Neurite purification system.
a) Microporous filter neurite purification assay.
b) 3D reconstruction of neurons on microporous filter.
c) Rac and Cdc42 potential neuron interactome. Proteins are shown as gene names. Red: neurite-enriched proteins, blue: neurite and soma equivalently distributed proteins, yellow: soma-enriched proteins.

Connection to Clinical Practice

Institute for Biochemistry and Genetics

The ability of vertebrate cells to directionally migrate is critical to development, the immune response and wound healing, and its regulation is compromised in pathologies such as metastatic cancer and vascular disease. The ability of neurons to directionally extend neurites that will subsequently differentiate in axons and dendrites is crucial for the proper wiring of the brain. A detailed understanding of the signaling events that regulate these complex morphogenetic processes is therefore likely to contribute important insights that could be used to target a number of different pathologies. We are especially interested in the Rho family of small GTPases, which are key molecular switches regulating the cytoskeletal and adhesion dynamics and the cell polarity mechanisms during the two processes mentioned above. Importantly, these signaling events are highly regulated in space and time, a dimension which is lost when most classic cell biological and biochemical techniques are used. For this purpose, we are developing and using novel tools that enable to grasp the spatio-temporal dimension of these complex processes. (i) One approach takes advantage of novel assays that enable to biochemically separate the leading edge, the "cell front", from the back of polarized migrating cells, or the extending neurite from the soma of neurons. Using state of the art biochemical and proteomics techniques, this enables to study in wide-scale, the subcellular distribution of thousands of proteins, of their activation status and of their post-translational modifications. (ii) A second approach consists in the development fluorescent biosensors that report the activation status of signaling molecules. This then enables to study the changing subcellular locations at which these signaling molecules are activated and inactivated with high temporal and spatial resolution using live cell imaging techniques.

Selected Publications

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Acute leukemia
Molecular genetics
Protein kinases
Small molecule inhibitors
Mouse models

Childhood Leukemia

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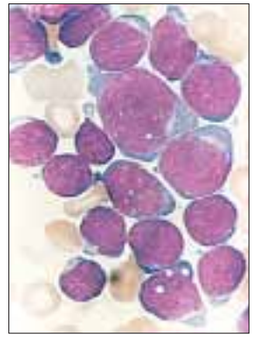
Characterization of molecular genetic alterations in acute leukemia to open new avenues for targeted therapeutics.

Acute leukemia is the most common cancer in childhood. Current strategies allow the curative treatment up to 80% of acute lymphoblastic leukemia (ALL), but only less than half of patients with acute myeloid leukemia, or even less of infants with leukemia. Acute leukemia in adults is characterized by a high percentage of relapse and loss of the patients to the disease in over 50% of the cases. New strategies that target the underlying molecular aberrations are needed. Acute leukemia, like cancer in general, is a disease primarily induced by genetic alterations. Research of the last decade has led to a model of functionally cooperating mutations leading to acute leukemia. Class I mutations, mostly comprising gain of function mutations in protein kinases (such as ABL, PDGFR or FLT3) and related signal transduction mediators, are inducing proliferation and survival without affecting cellular maturation. In contrast, class II mutations, mostly targeting transcriptional regulators of normal haematopoietic cell differentiation (such as CBF, RARα, or MLL) leading to a maturation block at the progenitor level and/or providing aberrant self-renewal capacity. There is strong clinical and experimental evidence that cooperation of two or more class I and class II mutations are necessary for the development of an acute leukemic phenotype. We have recently identified two members (PIM1, PIM2) of the family of PIM serine/threonine kinases as being deregulated in haematopoietic cells transformed by different class I mutations. Interestingly, functional interference by RNA interference (RNAi) or expression of dominant-negative acting mutants significantly impaired proliferation and survival of the cells, suggesting that PIM kinases represent bona fide therapeutic targets (Adam et al., 2006). In collaboration with S. Knapp (University of Oxford) we have used a combined structural and functional approach to identify a group of small molecules that selectively interact and inhibit PIM1. Further detailed characterization of one of those compounds (K00135) demonstrated anti-leukemic activity in vitro not only in cell lines but also in primary cells from patients with acute leukemia, suggesting that small molecule PIM kinase inhibitors may open a new therapeutic avenue for leukemia and maybe other cancers (Pogacic et al., 2007). Currently ongoing experiments using cells from PIM1-/- or PIM2-/- mice should help us to elucidate the role of PIM kinases for the development of a leukemic disorder (in collaboration with J. Duyster, Munich). In another project we have recently identified and molecularly characterized a new chromosomal translocation t(10;11)(q23;p15) from a patient with relapsed AML (in collaboration with C. Mecucci, Perugia). We found that this translocation leads to expression of a fusion of the nucleoporin 98 (NUP98) to the haematopoietically regulated homeobox gene (HHEX). The resulting NUP98/HHEX fusion is a new class II mutation that blocks normal haematopoietic differentiation in vitro, and induces an acute leukemic phenotype in vivo after a long latency. By comparing the gene expression profile induce by NUP98/HHEX with another leukemogenic NUP98-fusion we were able to identify and validate a number of overlapping downstream targets that could represent the first step for the development for new therapeutic strategies for these diseases.

A



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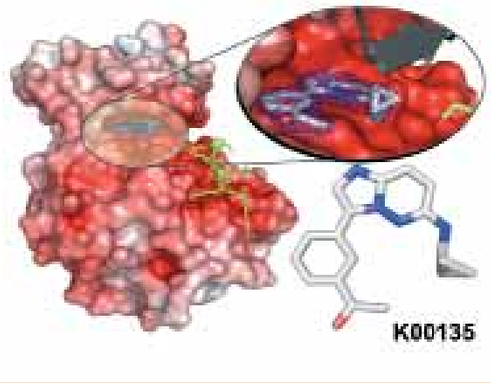

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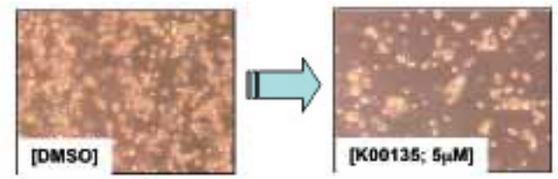

Fig. 1: A) Blood smear from a patient with acute lymphoblastic leukemia
B) The K00135 small molecule inhibitor bound to PIM1
C) Methylcellulose cultures from cells from a patient with acute leukemia untreated (left panel) and treated with K00135 for 48 hours (right panel).

Selected Publications

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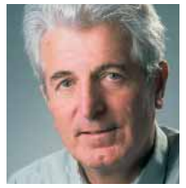
mRNA turnover
Oncogene
mTOR
Embryonal stem cells
Brf1

Experimental Oncology



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Posttranscriptional mechanisms of oncogenesis

Dysregulation of gene expression is the basis of cancer. While gene expression is regulated centrally at the transcriptional level, the role of posttranscriptional regulation – the focus of our research – has gained importance in the last years not only in cell physiology, but also in oncogenesis.

mRNA turnover in oncogenesis: role of the Brf1 protein

AU-rich elements (ARE) are cis elements located at the 3'-terminal ends of many short lived mRNAs. Brf1, a zinc-finger protein binding the ARE and leading to target degradation, has been cloned in our lab (Stoecklin et al., 2002), and its role has been clarified recently (Schmidlin et al., 2004; Benjamin et al., 2006), as summarized in Fig. 1. We found Brf1 to be a target of the akt/PKB kinase, and phosphorylation of Brf1 at two specific serines leads in parallel to a) complex formation with the protein 14-3-3, b) inactivation of Brf1 and c) stabilization and relocalization of Brf1 protein. Our results thus link Brf1 and mRNA turnover of ARE-transcripts with the oncogenic PI3-kinase-PTEN-akt/PKB signaling pathway and argue that perturbations of this oncogenic pathway increase the stability and thus abundance of specific ARE-containing mRNAs.

Studying Brf1 in mouse embryonal stem cells (ES cells)

To investigate whether Brf1 may play a role in cell differentiation, we turned to mouse embryonal stem cells and established a cassette system where a small hairpin RNA (shRNA) of choice can be induced by doxycycline to trigger RNA interference and downregulation of the target gene (Fig. 2A). When Stat3 shRNA was inducibly expressed as a system control, we observed as anticipated vigorous differentiation even in the presence of LIF, the cytokine antagonizing differentiation (Fig. 2B). When Brf1 was targeted, we observed formation of beating bodies, i.e. the generation of cardiomyocytes (Fig. 2C). We postulate that yet to be identified ARE-transcripts lying downstream of Brf1 regulate cardiomyocyte formation. The cassette system described is well suited to study other genes of interest suspected to regulate embryonal development (Wegmuller et al., 2007).

Oncogenic fusion transcripts in human lymphoma

Prof. R. Siebert and his team in Kiel, Germany observed that certain human B-cell lymphomas contain internal deletions of chromosome 14, with one breakpoint in the immunoglobulin gene, and the other in the gene encoding Brf1. In collaboration with Prof. R. Siebert, we characterized fusion transcripts consisting at the 5'-terminal site of sequences from the IgH-region and at the 3'-terminal site of truncated Brf1. Analysis of several transcripts revealed as the most frequent alteration a hybrid transcript where the first exon of Brf1 is lacking, but functional studies of the corresponding protein indicate that the biochemical activity is maintained. The possible role in oncogenesis of the Brf1 alleles found in lymphomas is under investigation.

Recessive TOR pathway mutants and their use for drug discovery

The frameshift mutagen ICR191 allowed generation and isolation of growth factor independent mutants from IL-3 dependent PB-3c cells (Kiser et al., 2006). The mutants were genetically recessive, as indicated by cell fusion experiments. Some mutants contained single nucleotide insertions into the PTEN tumor suppressor gene, generating a truncated protein (Fig. 3). Targeting PTEN or TSC2, another tumor suppressor and upstream regulator of mTOR (Fig. 3) by RNA interference, we could likewise transform cells to IL-3 independence. Interestingly, all mutants and transformants displayed sensitivity to the mTOR-inhibitor rapamycin. As the TOR pathway plays an important role in different cancers the mutant cells are currently being used for high throughput screening (collaboration with Dr. U. Regenass, Actelion), searching for drugs with rapamycin-like activity, or drugs that sensitize tumour cells to rapamycin ("rapamycin sensitizers"). We have subjected our mutants and the wild type precursors to DNA microarray analysis and identified an enzyme involved in glucose metabolism as a robust indicator gene for growth autonomous cell transformation and possibly for rapamycin sensitivity.

Fig. 1

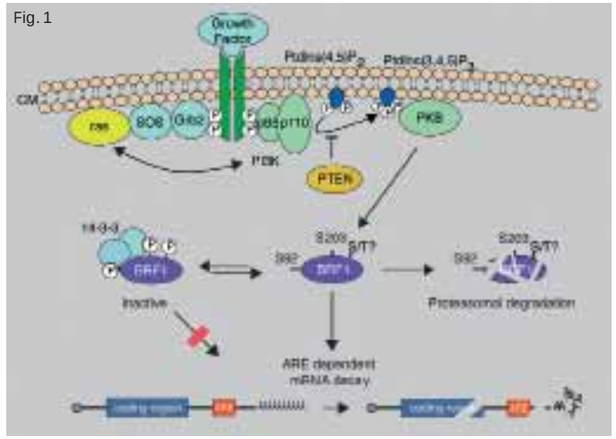

Fig. 2

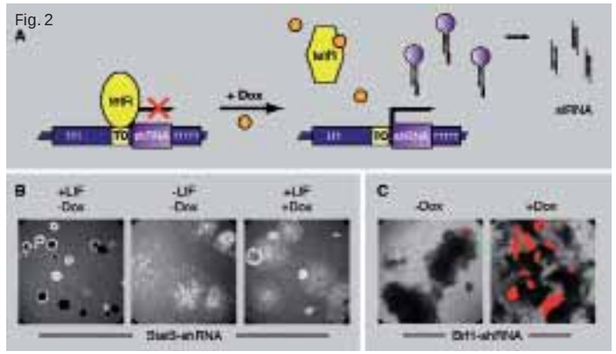

Fig. 3

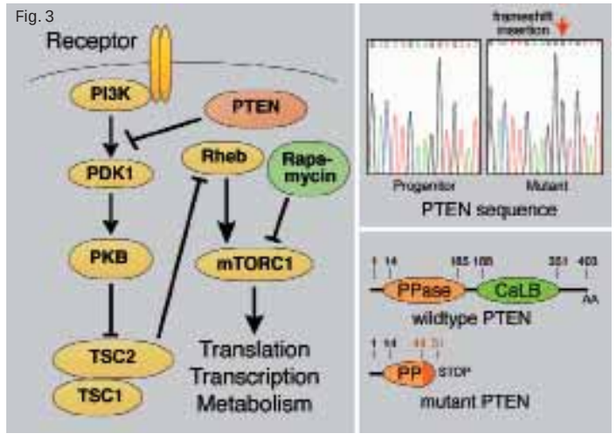

Fig. 1: Receptor signaling leads to activation of the p110 subunit of PI3-kinase, which activates protein kinase B (PKB). Phosphorylation of Brf1 by PKB at serines 92 and 203 abolishes the mRNA decay promoting activity of Brf1 via complex formation with protein 14-3-3 and probably ectopic localization. At the same time, the protein escapes rapid turnover and is probably stored for later use after dephosphorylation.

Fig. 2:
(A) ES cells were engineered to contain inducible shRNA directed against Stat3 or Brf1, which could be induced by doxycyclin and which is processed to siRNA.
(B) Following induction of shRNA targeting Stat3 the morphology of colonies assumes a differentiated phenotype even in presence of LIF, an inhibitor of differentiation (compare right and left panels indicating that the system "works").
(C) Following dowregulation of Brf1, formation of "beating bodies" shown in red, reflecting cardiomyocyte formation, is induced. It is concluded that Brf1 is a negative regulator of transcripts favouring cardiomyocyte formation.

Fig. 3: Left: Shown is a simplified version of how the mTOR kinase, present in mTOR complex 1, is regulated by the oncogene PKB and the tumor suppressors PTEN, TSC1, TCS2. Following frame-shift mutagenesis, we isolated growth factor independent mutants with activated mTOR, sensitive to rapamycin. The mutant shown on the right carries a frameshift leading to truncation in the phosphatase domain (PPase) of PTEN. Mutant lines with mutations in the different components of the pathway shown are currently used for drug discovery and profiling.

Selected Publications

- Benjamin, D., Schmidlin, M., Min, L., Gross, B., and Moroni, C. (2006). BRF1 protein turnover and mRNA decay activity are regulated by protein kinase B at the same phosphorylation sites. Mol Cell Biol 26, 9497-9507.
- Kiser, K. F., Colombi, M., and Moroni, C. (2006). Isolation and characterization of dominant and recessive IL-3-independent hematopoietic transformants. Oncogene 25, 6595-6603.
- Schmidlin, M., Lu, M., Leuenberger, S. A., Stoecklin, G., Mallaun, M., Gross, B., Gherzi, R., Hess, D., Hemmings, B. A., and Moroni, C. (2004). The ARE-dependent mRNA-destabilizing activity of BRF1 is regulated by protein kinase B. Embo J 23, 4760-4769.
- Stoecklin, G., Colombi, M., Raineri, I., Leuenberger, S., Mallaun, M., Schmidlin, M., Gross, B., Lu, M., Kitamura, T., and Moroni, C. (2002). Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover. Embo J 21, 4709-4718.
- Wegmuller, D., Raineri, I., Gross, B., Oakeley, E. J., and Moroni, C. (2007). A cassette system to study embryonic stem cell differentiation by inducible RNA interference. Stem Cells 25, 1178-1185.

Tumor microenvironment
Tenascin-C
Fibronectin
Signaling
Cancer
Proliferation
Migration
Angiogenesis

Extracellular Matrix Adhesion

group left during report period

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Tenascin-C-Induced Signaling in Cancer

Tumorigenesis is largely determined by uncontrolled interactions of cells with their microenvironment. A highly regulated crosstalk with the extracellular matrix (ECM) controls cellular signaling, that restricts cell proliferation and motility. In contrast, a deregulated high expression of the ECM molecule tenascin-C in cancer modulates cell adhesion and may support tumor progression. Cell adhesion to tenascin-C promotes tumor cell proliferation, migration and invasion, presumably by activating oncogenic and blocking tumor suppressive signaling. Together with a strong expression of tenascin-C in most solid tumors, which has a negative prognostic value for some cancers, it is likely that tenascin-C plays an important role in cancer.
We are employing techniques in biochemistry, cell and molecular biology as well as transgenic mouse models of carcinogenesis to address the role of tenascin-C in tumor progression. We showed that tenascin-C competes with syndecan-4 binding to fibronectin, thus preventing cell spreading and stimulating tumor cell proliferation. We identified Wnt and endothelin receptor type A (EDNRA) signaling and tropomyosin (TM1) as candidates, the expression and function of which is regulated by tenascin-C. Tenascin-C caused downregulation of the Wnt inhibitor Dickkopf 1 (DKK1). In consequence, canonical Wnt signaling was initiated leading to stabilisation of β-catenin and induction of its target “Inhibitor of differention-2” (Id2). Using glioma tissue arrays, we observed a link of high tenascin-C and high Id2 expression to the most malignant gliomas, which suggests that a linked tenascin-C and Id2 expression might be suitable as negative prognostic marker in gliomas. In addition to blocking syndecan-4, tenascin-C also stimulated EDNRA expression and corresponding MAP kinase signaling at later time points, which maintains tenascin-C-induced cell rounding. Activation of EDNRA by tenascin-C blocked three molecules critical for cell spreading: focal adhesion kinase (FAK) remained inactive, and the small GTPase RhoA and the actin filament stabilising tumor suppressor-like molecule TM1 were degraded. This occurred in a MEK-dependent manner. In contrast to EDNRA, signaling through EDNRB restored cell spreading in the presence of tenascin-C as well as expression and function of RhoA and TM1, and FAK, respectively. This involved activation of the epidermal growth factor receptor, phospholipase C, c-Jun N-terminal kinase, and the phosphoinositol-3 kinase pathway. Thus, tumorigenesis might be enhanced by tenascin-C involving EDNRA signaling, that is linked to angiogenesis and, to epithelial mesenchymal transition, a hallmark of cancer progression. Inhibition of tenascin-C in combination with blocking both endothelin receptors could present a strategy for sensitization of cancer and endothelial cells toward anoikis.
We recently generated transgenic mice that mimic high tenascin-C expression in cancer tissue. Tenascin-C was ectopically expressed in the beta-cells of the pancreas of RipTag mice, that develop insulinomas due to ectopic expression of the SV40T-antigen. The phenotype of the double transgenic mice is currently under investigation.

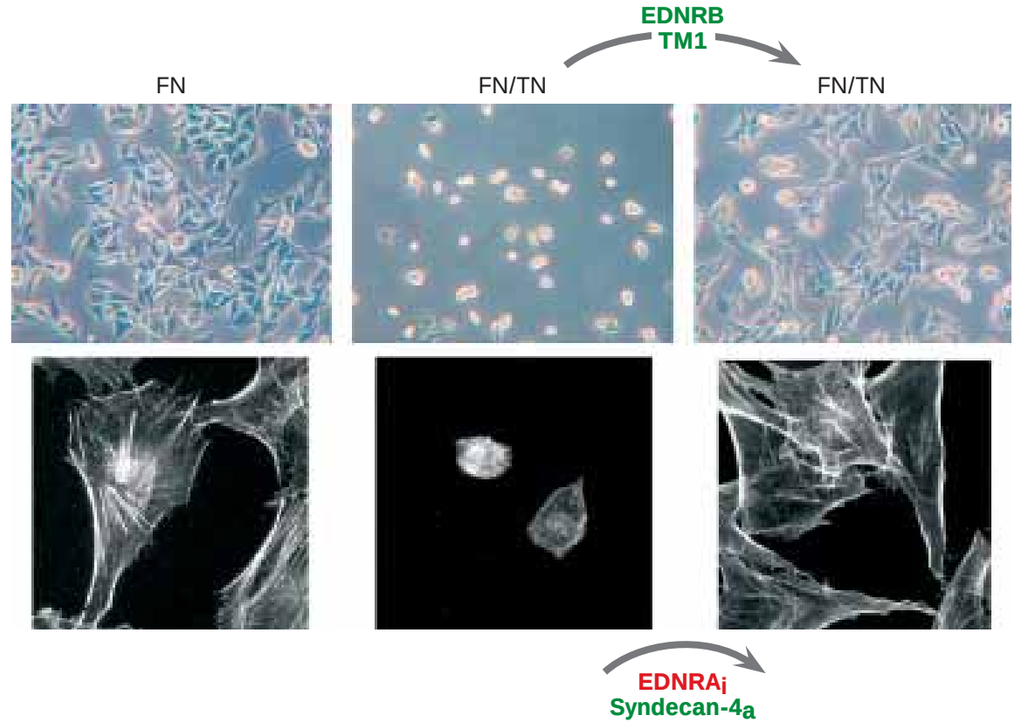

Fig. 1: Modulation of tenascin-C-induced cell rounding: potential use in cancer diagnosis and therapy
Cell rounding is a first response toward tenascin-C, which promotes cell migration and tumor cell proliferation. Glioblastoma cells grown on fibronectin (FN) establish an actin cytoskeleton (lower panel) that is absent when cells are grown on a fibronectin/tenascin-C substratum (FN/TN). Inhibition of the endothelin receptor type A (EDNRA) and activation of endothelin receptor type B (EDNRB) restores cell spreading in the presence of tenascin-C. Activation of syndecan-4 and ectopic expression of tropomyosin-1 (TM1) also prevents cell rounding by tenascin-C.

Selected Publications

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Familial cancer
Hereditary colorectal cancer
Anticipation
Somatic alterations
Presymptomatic genetic testing
Cancer prevention

Human Genetics

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(Epi)genetic alterations in hereditary colorectal cancer

Colorectal cancer (CRC) is a leading cause of morbidity and mortality in the Western world. Most CRCs develop from pre-existing benign polyps and individuals with adenomas are at an increased risk of CRC. Five to ten percent of all CRCs can be attributed to an ascertainable cancer predisposition, in particular familial adenomatous polyposis (FAP) caused by germline mutations in the APC or MUTYH genes and hereditary non-polyposis colorectal cancer (HNPCC) caused by mismatch repair (MMR) gene alterations.
Since 1979 the activities of our research group have focused on the continuous collection, identification and characterisation of families exhibiting hereditary cancer predisposition syndromes with the goal of providing reliable genetic counseling and targeted medical care to affected patients and their relatives. The database actually consists of more than 750 index patients/kindreds referred from all parts of Switzerland and creates the basis for our research projects.

Evidence for genetic anticipation in HNPCC mutation carriers

Thus far, only limited data exist on the occurrence of genetic anticipation in HNPCC, i.e. the earlier age at diagnosis of CRC in successive generations. Performing nonparametric distribution-free statistical analyses, we investigated 55 parent -child pairs diagnosed with CRC and coming from 21 Swiss HNPCC families with characterised MMR germline mutation. Descendants of HNPCC patients (median age at diagnosis 39 years, IQR=12) were found to be diagnosed with CRC significantly earlier than their parents (47 years, IQR=10), with the median of the paired age difference amounting to 8 years (IQR=15; $P < 0.0001$; Fig. 1). Birth cohort effects could be excluded, since the same, statistically significant, age difference was also observed in the oldest offspring birth cohort (birth year <1916; $P=0.01$). Intriguingly, genetic anticipation appeared to be more pronounced when the disease allele was transmitted through the father than through the mother (median age difference 11 vs. 4 years, respectively; both $P < 0.01$).

Characterisation of the "second hit" in tumors from attenuated FAP (AFAP) patients

In a collaborative effort with the the Molecular Population Genetics group of Ian Tomlinson, London, we analysed somatic APC mutations in 235 tumors from 35 patients (16 families) with a variety of AFAP-associated germline mutations (Fig. 2). We observed bi-allelic changes ("third hits") in some polyps with the "third hit" probably initiating tumorigenesis. Most "third hits" left three 20-amino acid repeats (20AARs) on the germline mutant APC allele, with loss of heterozygosity (LOH), or proximal somatic mutation, of the wild-type allele. Not all polyps appeared to need "three hits", however. In addition to effects of different germline mutations, modifier genes may be acting on phenotypic variablity in FAP, perhaps influencing the quantity of functional protein produced by the mutant allele.

Gene conversion is a frequent mechanism of allelic inactivation in HNPCC cancers

Applying a recently developed method, multiplex ligation-dependent probe amplification, to study gene copy number changes we investigated the frequency and nature of loss of heterozygosity as a second, somatic event, in tumors from MLH1/MSH2 germline deletion carriers in 18 cancer specimens from two independent sets of Swiss and Finnish mutation carriers. Surprisingly, somatic mutations identical to the ones in the germline were found to occur frequently in colorectal cancers (6 of 11; 55%) and also present in extracolonic HNPCC-associated tumors (Fig. 3). Chromosome-specific marker analysis implied that loss of the wild-type allele predominantly occurs through locus-restricted recombinational events, i.e., gene conversion, rather than mitotic recombination or deletion of the respective gene locus.
Current projects assess the role of specific (epi)genetic events in key signaling pathways in hereditary CRC which are not only of particular importance for genetic counselling as well as therapeutic and preventive measures in affected patients and their relatives but which may also provide new insights into sporadic carcinogenesis.

Fig. 1

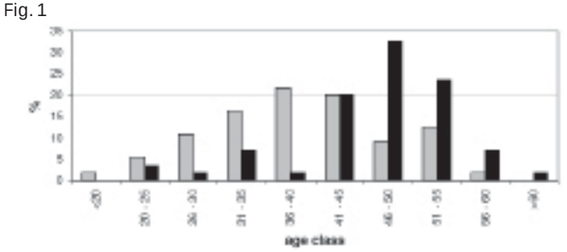


Fig. 2

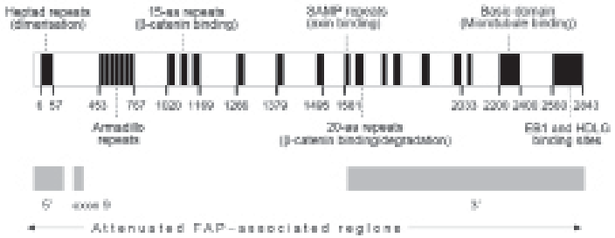
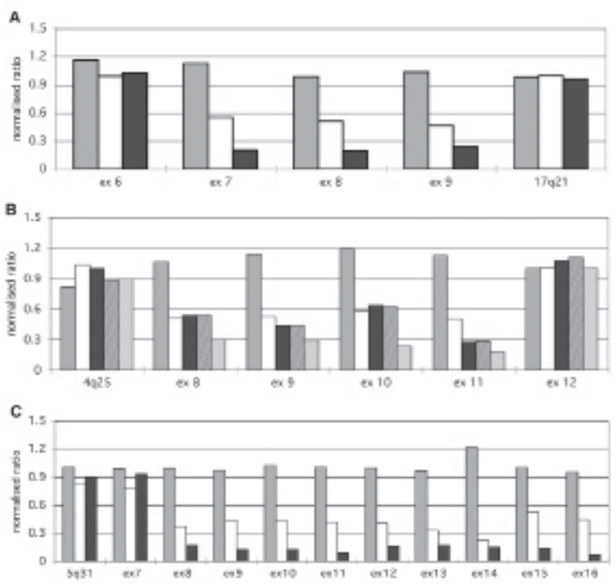


Fig. 3



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DNA microarrays, Tumor
Stroma, Microenvironment
Cell-cell Interaction
Breast cancer
Liposome, Immunoliposomes
Antibody, Drug Delivery
EGFR, VEGFR

Medical Oncology

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Understanding the pathophysiology of breast cancer gene expression profiles by in vitro analysis of the tumour-stroma interaction

Gene expression profiling studies with DNA microarrays have produced a detailed picture of the molecular features involved in human cancer. Over the last few years, gene expression profiles were determined for most common human cancers, allowing the identification of novel molecularly defined disease subtypes with distinct clinical outcome. The major challenge raised by these large amounts of detailed results is now identifying the pathophysiology underlying the specific gene expression patterns. The long-term goal is to identify mechanisms underlying the gene expression profiles of breast cancer. Considering the contributions of the microenvironment for the development of a tumor we will focus our study on the interaction between the tumor cells and their neighboring host cells. That the interaction between tumour cells and neighbouring cells of the host play an essential role in the development cancer has been shown in multiple studies, each of them focusing on a few specific molecules. However, the effects on global gene expression due to heterotypic cell-cell interaction are not yet well characterized. We speculate that the effects of heterotypic interaction, which are expressed by specific gene signatures, are important for cancer development and the course the disease takes.
To elucidate the basic principles in the gene expression effects of heterotypic interaction between breast cancer cells and a panel of stromal cells, we will analyze an in vitro co-culture model system using DNA microarrays and define their in vivo relevance by comparing the data to large publicly available datasets of breast cancer with annotation of clinical parameters.

Development of Immunoliposomes for specific and enhanced transport of anticancer drugs

Site-specific delivery of anti-cancer therapeutics is paramount for both reducing nonspecific toxicities and increasing efficacy of chemotherapeutic agents. Due to their small molecular size and nonspecific mechanisms of action, most conventional chemotherapies result in significant toxicities that limit the effectiveness of treatment and reduce the overall quality of life for cancer patients. Encapsulation of these toxic agents inside lipid-based carrier systems or liposomes, results in passive targeting of liposomes to solid tumors due to a discontinuous microvasculature supporting the tumor and a significantly milder toxicity profile. Recently we have further increased the specificity of delivery by attaching monoclonal antibodies or antibody fragments to the surface of liposomes (= immunoliposomes, antibody-linked nanoparticles) to induce their internalization by target cells. In addition to increasing the efficacy of chemotherapeutic drugs, we have evidence that drug resistance, a major challenge in cancer treatment, might be overcome by such delivery systems. Logical and accessible targets for such approaches using antibody-linked nanoparticles include 1) the epidermal growth factor receptor (EGFR), which is an important molecular contributor to tumorigenesis and a readily accessible cell surface receptor commonly overexpressed in tumor cells and 2) vascular endothelial cell receptors, such as VEGFR and vascular endothelial (VE) - cadherin.

Recently, we demonstrated that by targeting the epidermal growth factor receptor (EGFR) using anti-EGFR immmunoliposomes, the specificity and efficacy of various anticancer drugs was clearly improved. Anti-EGFR immunoliposomes were prepared using Fab‘ fragments derived from the monoclonal antibodies C225 (cetuximab, erbitux™) or EMD72000. In fact, we demonstrated in a series of in vitro and in vivo studies, that immunoliposome drugs were markedly more cytotoxic in target cells than the corresponding free drug or non-targeted liposomal drug. In these experiments, classic liposomal drugs, such as doxorubicin and epirubicin were used; in addition, we also have recently established procedures for the stable liposomal encapsulation of newer anticancer agents, such as vinorelbine and irinotecan. Interestingly, preliminary studies with a cell line featuring multi-drug-resistance showed clearly improved activity of immunoliposomal drugs in comparison to free and non-targeted liposomal drug, indicating the ability to overcome drug resistance mechanisms.
Combining the advantages of (1) promising cytotoxic compounds stably encapsulated into liposomes and (2) the specific targeting function of monoclonal antibodies could result in a potentially ideal delivery system for anticancer agents due to enhancing efficacy and reducing toxicity simultaneously. The final goal is to translate this research into the clinic and to establish a new treatment strategy against cancer.

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(Epi)Genome Stability
DNA Damage
DNA Methylation
DNA Repair
Tumorigenesis
Cancer Therapy

Molecular Genetics

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Maintenance of (Epi)Genome Stability Through DNA Surveillance and Repair

Reactive agents of endogenous and environmental origin pose a constant threat to the integrity of our genomic material, the DNA. DNA damage destabilizes genomes and, thus, increases the risk of cancer (Fig. 1). We explore biological processes that enforce (epi)genetic stability at the level of DNA damage response and repair. Our objective is to provide a thorough understanding of the molecular mechanisms involved and the consequences of their dysfunction for cancer development and therapy.

DNA Base Excision Repair (BER)

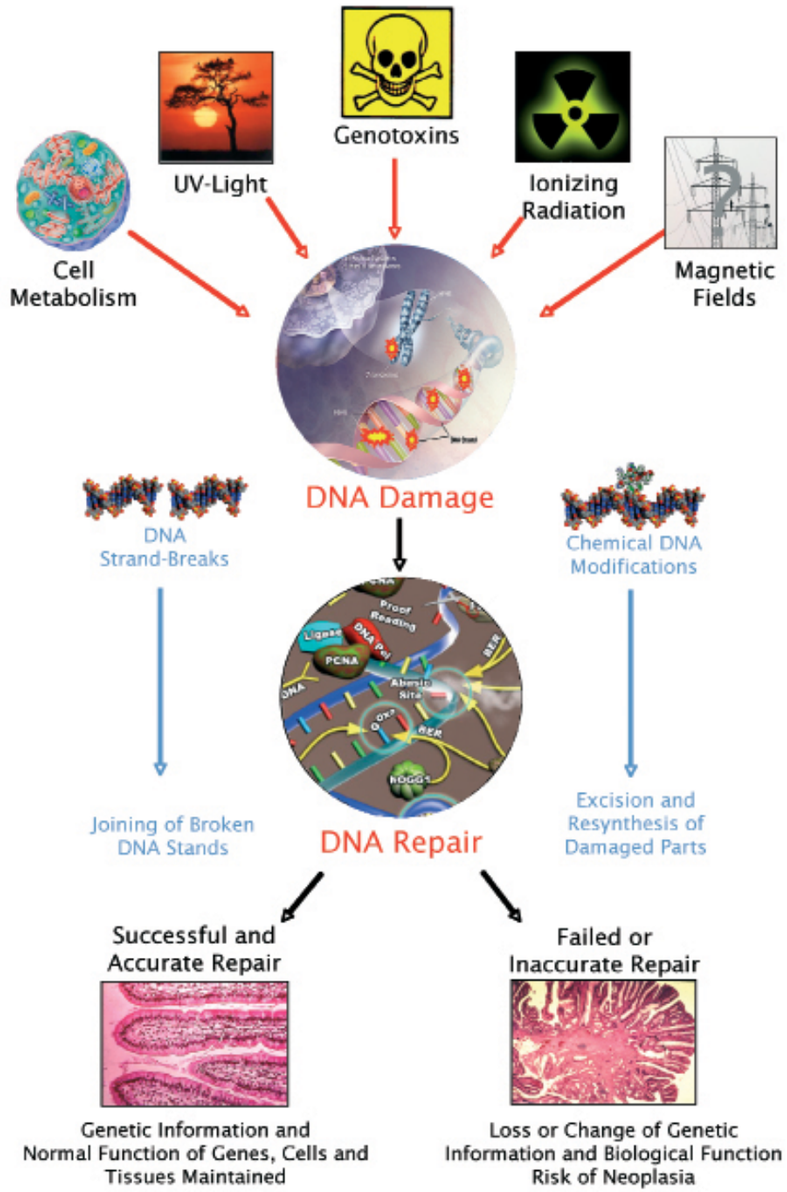
CT changes in the DNA sequence are the prevalent type of mutation found in most human cancers. They occur primarily through deamination of cytosine or 5-methylcytosine, a relatively frequent event that generates U•G and T•G mispairs in the DNA. The faithful restoration of the GáC pair is accomplished by BER. Thymine DNA-Glycosylase (TDG) is capable of hydrolyzing mispaired U or T bases from the DNA backbone and may thus be a key player in this repair process. We are pursuing biochemical and genetic strategies to explore the role of TDG in DNA repair, carcinogenesis, and cell differentiation.In protein interaction studies, we discovered that dynamic SUMO-conjugation is required for full functionality of TDG. We showed that SUMOylation modulates the interaction of TDG with DNA and other repair proteins and thereby established a novel regulatory concept for the coordination of DNA transactions. Work with yeast and mouse genetic models led to two fundamental discoveries, namely that BER contributes critically to the DNA directed effects of chemotherapeutic drug 5-FU, and that the excision of DNA bases by TDG can induce chromosomal instability. The mouse Tdg knockout project revealed further an embryonic essential function for the glycosylase. This unexpected phenotype may relate to a role of TDG in modulating the transition of epigenetic states during cell differentiation.

DNA double strand-break repair (DSBR)

DNA double-strand breaks are the most severe form of DNA damage. They arise through genotoxic insult or as a result of DNA transactions involved in cell proliferation or differentiation. Cells utilize two distinct modes of DSBR; homology directed repair and non-homologous-end-joining (NHEJ). Both are critical for genome stability.Having pioneered work on NHEJ in yeast, we used this model to identify regulatory factors of DSBR. We isolated Nej1 and Ntr1, two proteins that interact with Lif1, an ortholog of human XRCC4. Lif1 also complexes with Dnl4 to constitute the DNA ligase active in NHEJ. While Nej1 turned out to be a regulator of cell-type specific NHEJ, the function of the essential Ntr1 in DSBR remains enigmatic. Protein interaction studies showed that Ntr1 interferes with the formation of an active DNA ligase complex by occupying the DNA ligase binding site of Lif1. Ntr1 also interacts with PinX1, a protein with dual functions in the regulation of telomerase activity and in RNA processing. Like PinX1, Ntr1 localizes to telomeres and nucleoli, suggesting a function in local suppression of NHEJ by sequestering Lif1 into an inactive complexes.

Cancer Epigenetics

Aberrant CpG methylation contributes to tumorigenesis by dysregulating gene expression. Exactly why, how and when changes in DNA methylation arise during carcinogenesis is unknown. We aim to identify physiological conditions promoting DNA hypermethylation and, thereby, to assess the underlying molecular mechanisms.We examined the normal appearing colorectal mucosa of healthy individuals for the presence of cancer-prone methylation changes in the promoters of the hMLH1 and MGMT genes. We detected aberrant methylation in a gene-, age-, and gender-specific manner. Methylation levels were significantly elevated in females, but not in males, and only at the hMLH1 promoter in biopsies from the proximal colon of women above 60. Strikingly, this methylation profile reflected perfectly the epidemiology of sporadic hMLH1 deficient colorectal cancer, presenting preferentially in the proximal colon of females of advanced age. This suggests a causal relationship between hMLH1 hypermethylation in normal mucosa and colorectal carcinogenesis.



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Cancer Immunotherapy
Vaccines
Recombinant vaccinia virus
Melanoma
Lung cancers
Urological cancers

Oncology Surgery

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Tumor immunotherapy: swinging between laboratory and bedside

The characterization of tumour associated antigens (TAA) allowed immunologists to envisage targeting cancer cells with high specificity. We developed a recombinant vaccinia virus (rVV) encoding 3 melanoma associated HLA-A0201 restricted epitopes (Melan-A/Mart-1_{27-35}, $GP100_{280-288}$ and $Tyrosinase_{1-9}$) in the form of minigenes including sequences coding for the adenovirus E3/19K signal peptide, driving resulting fusion products in the endoplasmic reticulum and bypassing antigen processing steps. Genes encoding CD80 and CD86 co-stimulatory molecules were added to this construct (fig.1). This reagent was tested in replication incompetent form in 2 consecutive outpatient phase I/II clinical trials addressing stage IIb to IV melanoma with promising clinical results. Optimal presentation of HLA class I restricted peptides to CD8+ T cells requires "licensing" of antigen presenting cells (APC), provided by CD40 binding by activated CD4+ T cells expressing CD40 ligand. To mimic these mechanisms we constructed a rVV encoding CD154, and we demonstrated its capacity to activate different APC. This reagent enhances peptide specific CTL induction and promotes expression in APC of genes encoding different cytokines, including TNF-α, GM-CSF, IL-12 and IL-15, a soluble factor efficiently supporting generation and survival of memory CD8+ T cells.

Melanoma antigen E (MAGE) family TAAs belong to the so-called cancer/testis (C/T) subclass expressed in tumors of unrelated histologic origin and in a restricted number of healthy tissues. We generated monoclonal antibodies (mAbs) specific for C/T TAA gene products and used them to assess the extent of their expression in clinical samples. In collaboration with the Institute of Pathology providing multi tumor array technology we showed that strong expression of MAGE-A TAA, correlates with poor prognosis in transitional cell carcinoma of the bladder and in non small cell lung cancer (fig.2). These data prompted us to envisage specific vaccination strategies and a rVV encoding different epitopes from C/T TAA has been designed and produced by our group.

During the past three years a project in collaboration with the Tissue Engineering research group focused on generation and characterization of 3D cultures of neoplastic cells. NA8 metastatic melanoma cells routinely cultured in monolayers give rise within 24 hours to the formation of spheroids in culture trays treated with polyHEMA, preventing cell attachment (fig.3). Each spheroid, presenting 400-500 μm diameters, contains from 10'000 to 30'000 cells and, upon prolonged culture (>10days), shows necrotic cores resulting in hollow centers with large, compact cells detectable in the periphery. We addressed oligonucleotide array gene profiling in NA8 cells cultured in standard 2D conditions or in spheroids. Expression of about 11'000 genes was detectable in NA8 cells. 106 genes showed evidence of upregulation and 73 of downregulation with change factors .3 in spheroids as compared to their 2D counterparts. Notably, a significant upregulation of the expression of genes encoding chemokines, including CXCL1, CXCL2, CXCL3, IL-8 and CCL20 was detectable in spheroids, as compared to 2D cultures. Some of these genes are known to be overexpressed in highly aggressive metastatic melanomas. More recently we have addressed the functional activities of HLA-A0201 restricted Melan-A/MART-1_{27-35} specific cytotoxic T lymphocytes (CTL) using, as targets, melanoma cells expressing both antigen and appropriate restriction determinants cultured in different conditions. Culture of HBL melanoma cells expressing Melan-A/Mart-1 TAA and HLA-A0201 on polyHEMA coated plates results in the generation of spheroids. HLA-A0201 restricted Melan-A/Mart-1_{27-35} specific CTL clones produce high amounts of IFN-γ upon 3-24 hours co-incubation with HBL cells cultured in 2D. However, they fail to do so when target cells are cultured in 3D, suggesting altered antigen recognition in these conditions.

Altogether, results obtained by our unit mirror progress and disappointments of the tumor immunology research field whose continuing refinements provide reasoned optimism for the future.

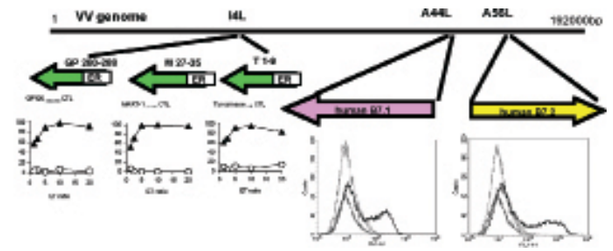

Fig. 1: A schematic representation of the rVV used in the Basel melanoma immunotherapy trials. The indicated HLA-A0201 restricted epitopes are encoded by the penta-mel-rVV in the form of minigenes including an adenovirus derived leader sequence driving the resulting fusion products in the endoplasmic reticulum (ER). CD80 and CD86 (B7.1 and B7.2, respectively) expression was tested on constitutively negative cells infected with psoralene UV inactivated virus. Effective expression of antigenic epitopes was tested by using rVV (triangles) or wild type VV (circles) infected HLA-A0201+ cells as targets of CTL clones recognizing the indicated antigens in standard ^{51}Cr release assays.

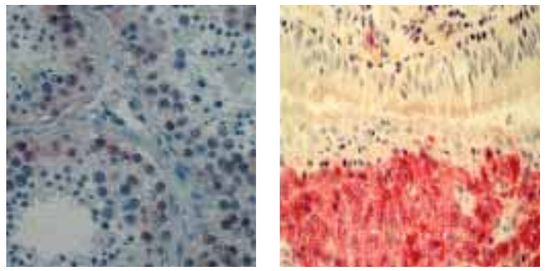

Fig. 2: Expression pattern of cancer/testis tumor associated antigens. Paraffin embedded sections from a testis sample (left panel) and from a lung cancer (right panel) were stained with 57B monoclonal antibody recognizing multiple MAGE proteins. Specific staining is limited to spermatogonia in healthy testis and to tumor cells in the cancer specimen.

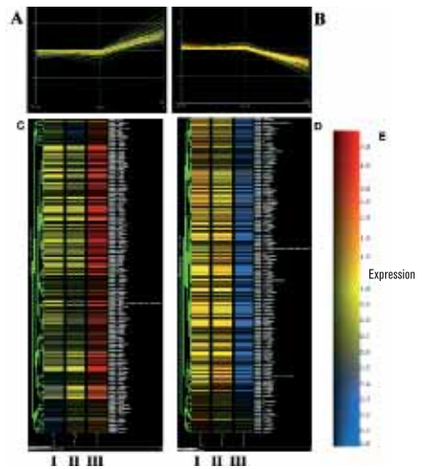

Fig. 3: Modulation of gene expression in NA8 cells cultured in 2D or spheroids. NA8 melanoma cells were cultured in standard monolayers (I), in 2D in the presence of solid phase bound collagen (II) or in spheroids (III). Panels A and B refer to genes significantly up or down-regulated, respectively, in either of these culture conditions. The extent of the up or down-regulation of each gene is presented in panels C and D, respectively, according to the color scale shown in panel E.

Connection to Clinical Practice

Pre-clinical and clinical studies in melanoma, NSCLC and urological cancers.

Our laboratory, being part of a surgical research institute enjoys a close interaction with clinical departments involved in cancer immunotherapy research. In particular, since 1999, we conduct active specific immunotherapy clinical trials in patients with advanced stage melanoma by using rVV (see above) in collaboration with the Division of General Surgery of the Dpt. of Surgery. Additional projects deal with antigen specific immune responses in lung and urological cancers. Regarding lung cancers we mainly focused on MAGE-A TAA whose expression is associated with poor prognosis in non small cells lung carcinomas (NSCLC) (see above). In the past three years we explored responsiveness to MAGE-A TAA in CD8+ tumor infiltrating lymphocytes (TIL) from patients with NSCLC following in vitro stimulation with MAGE-A derived peptides in the presence of autologous LPS matured dendritic cells (DC). CTL specific for MAGE-A TAA could be generated in a limited number of cases (3/32). Within this project we also designed and constructed novel rVVs encoding multiple MAGE-A epitopes and co-stimulatory molecules and we demonstrated that these reagents induce specific CTL responses, in patients and healthy donors. Regarding urological cancers we first focused on bladder cancers and we showed that MAGE-A TAA expression is associated with poor prognosis in transitional cell carcinomas. More recently, we addressed discovery of antigenic epitopes in p53 binding domains of the large T antigen from BKV, a polyoma virus suggested to be associated with oncogenic transformation in prostate. HLA-A0201 restricted epitopes inducing high responsiveness in healthy donors were successfully identified.

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Angiogenesis
Cancer
Lymphangiogenesis
Metastasis
Signal transduction
Tumorigenesis

Tumor Biology

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Molecular dissection of tumor angiogenesis, lymphangiogenesis, and metastasis

The major objective of our research is the identification and characterization of molecular events involved in late stage tumorigenesis. In particular, we focus on the contribution of tumor angiogenesis and lymphangiogenesis to tumor progression and on the molecular mechanisms underlying the transition from benign neoplasia to malignant cancers and the metastatic dissemination of tumor cells. In addition to tumor cell lines in vitro, we employ transgenic mouse models of tumorigenesis to determine causal connections between the expression of particular genes and tumor progression in vivo.
The development of malignant tumors is in part characterized by a tumor cell's capability to overcome cell-cell adhesion and to invade surrounding tissue. E-cadherin is the main adhesion molecule of epithelia, and it has been implicated in carcinogenesis, because it is lost in almost all human epithelial cancers. Using transgenic complementation experiments in transgenic mouse models of multistage carcinogenesis, we have shown that the loss of E-cadherin-mediated cell-cell adhesion is causally involved in the transition from well-differentiated adenoma to invasive carcinoma. Currently, we are investigating the signal transduction pathways that are activated by the loss of E-cadherin function and that induce tumor cell migration, invasion and metastatic dissemination. Thereby, we aim at the identification and characterization of genes and factors that are contributing to epithelial-mesenchymal-transition (EMT) of tumor cells during tumor progression. Finally, we are conducting experiments in cultured breast cancer cells and in transgenic mouse models of breast cancer to dissect the molecular processes underlying organ-specific metastasis.
A second major effort in our laboratory focusses on the molecular regulation of tumor blood vessel angiogenesis and lymphangiogenesis. We have generated a number of mouse models in which the angiogenic activity of different angiogenic factors can be evaluated in a direct comparison. These mouse models offer the unique opportunity to study the pathological, physiological and molecular consequences of different qualities and quantities of angiogenesis and lymphangiogenesis for tumor progression and metastasis. For example, employing these mouse models we have also initiated experiments to identify surrogate markers for tumor angiogenesis and lymphangiogenesis, markers that are desperately needed for the diagnosis, prognosis and clinical monitoring of cancer patients that are being treated with anti-angiogenic therapies. Moreover, we also investigate the contribution of bone marrow-derived cells to tumor angiogenesis and lymphangiogenesis.
The regulation of tumor angiogenesis involves a variety of receptor tyrosine kinase (RTK)-mediated signal transduction pathways. Sprouty proteins, recently identified antagonists of RTK signaling, inhibit endothelial cell proliferation and differentiation by repressing the activation of mitogen-activated protein kinase (MAPK) pathway. Sproutys are anchored to membranes by palmitoylation and themselves are also a target of the MAPK signaling cascade, for example by regulation of their subcellular localization and by phosphorylation. Currently, we are investigating the mechanism by which Sproutys intersect RTK signaling in endothelial cells in vitro and in our transgenic mouse models in vivo.
Finally, we employ various transgenic mouse models for the design and testing of innovative cancer therapies, either based on anti-angiogenic strategies or by directly targeting cancer cells. For example, in collaboration with Prof. Christoph Rochlitz and Dr. Christoph Mamot, Clinical Oncology, University Hospital Basel, we are testing immunoliposomes that are designed to target the tumor vasculature. In collaboration with Prof. Helmut Mäcke, Radiological Chemistry, University Hospital Basel, we investigate the use of radiolabeled peptide antagonists for glucagon-like peptide receptor 1 for the imaging and therapy of malignant insulinoma. Both of these approaches have been highly successful in the preclinical setting and are now being adapted for clinical use.

Figure 1

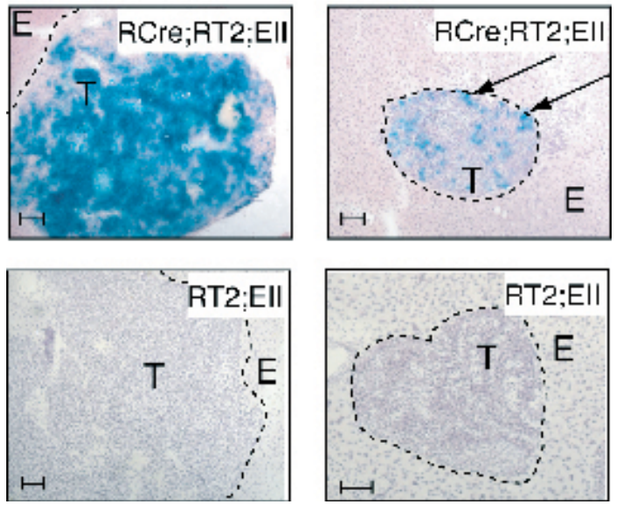


Fig. 1: β1-integrin-mediated cell adhesion prevents senescence of pancreatic β tumor cells. Genetic ablation of β1-integrin function in tumor cells of Rip1Tag2 transgenic mice results in senescence of tumor cells as visualized by the expression of senescence-associated β-galactosidase (top panels; blue staining). Control tumors with intact β1-integrin function do not senesce (bottom panels). E = exocrine pancreas; T = tumor. Scale bar = 50 μm.

Fig. 2: Podoplanin induces loss of actin stress fibers, filopodia formation and cell migration in the absence of epithelial-mesenchymal-transition (EMT). MCF7 human breast cancer cells have been transfected with control vector (left panel) or with a vector encoding podoplanin (right panel) and stained for actin (green) and podoplanin (red).

Fig. 3: Tumor lymphangiogenesis induced by transgenic expression of the lymphangiogenic factor VEGF-C in the Rip1Tag2 x Rip1VEGF-C double-transgenic mouse model of pancreatic β cell carcinogenesis. Lymphatic vessels are stained with antibodies against LYVE-1 and Podoplanin, while CD31 delineates blood vessels. E = exocrine pancreas; T = tumor.

Figure 2

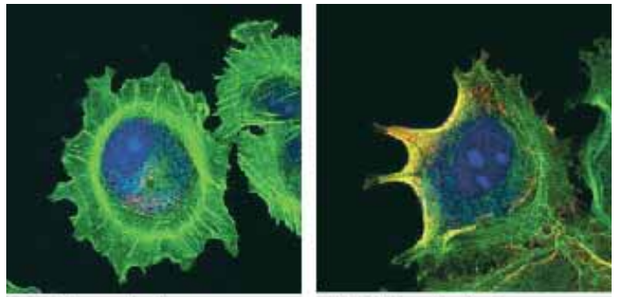
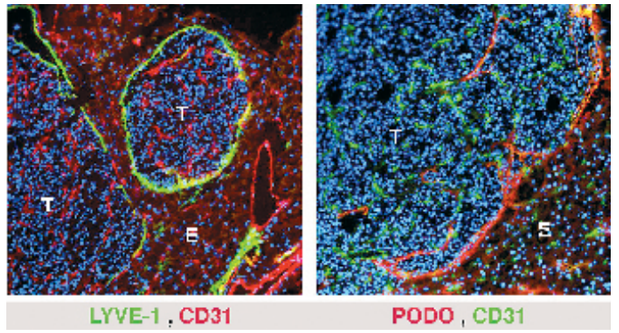


Figure 3



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DBM Focal Area Immunology

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With the evolution from a single-cellular to a multi-cellular organism, an innate and an adaptive immune system have been created for the protection against life-threatening infections. However, the high versatility and a notable complexity of the immune system also harbor the danger of immunodeficiency and autoimmunity as a consequence of a defective development and an aberrant immune response, respectively.

The Immunology Focus of the Department of Biomedicine is comprised of 14 research groups. Four of these groups focus their efforts on developmental aspects of the immune system. In particular, these studies seek (i) to unravel the molecular and cellular mechanisms that of B and T cell development focusing on how central and peripheral B cell tolerance is achieved; and (ii) to analyze the molecular control of primary and secondary lymphoid tissue organogenesis. These studies will not only provide insight into the development of the different types of effector cells but should also shed light on how the microenvironment supports their differentiation.

Complement is required to combat infections and to clear the body from necrotic and apoptotic cells. To prevent excessive tissue damage, the complement activation cascade has to be tightly controlled. Research by one of the groups in the Immunology Focus seeks to unravel how this control is effected. Complement deficiencies in man and mice can result in the development of autoimmunity. For example, the occurrence of systemic lupus erythematosis (SLE) is strongly associated with the presence of auto-antibodies against the complement component C1q. The pathogenic role of C1q specific antibodies is investigated by one of the research groups within the Immunology Focus.

Inflammation of the heart muscle (i.e. myocarditis) is caused by infections, toxic substances or autoimmunity. The ensuing myocardial destruction can lead to the loss of cardiac function secondary to a dilatation of the heart muscle. Two research groups within the Immunology Focus study in a mouse model the molecular and cellular mechanisms underlying the development of autoimmune myocarditis and the cellular mechanisms that lead to atherosclerotic lesions, respectively.

Pattern recognition receptors such as the family of Toll-like receptors play a crucial role in the defense against bacterial infections. The analysis of the role of these receptors in the context of Streptococcus pneumoniae infections constitutes another research focus in the Department.

T cells expressing an α/β T cell antigen receptor may either recognize MHC/peptide complexes or, alternatively, may interact with lipids, phosporylated metabolites and sugar presented by CD1. One research group within the Immunology Focus analyses the physiology of these latter, non-conventional T cells and seek to elucidate their role in immunopathology.

In individuals with primary or secondary immunodeficiency, viral infections constitute a life-threatening challenge. Four research groups within the Department of Biomedicine focus their efforts on the immune system's early recognition of viral infections and ways by which an anti-viral response can be enhanced.

Taken together, a wide variety of basic and translational immunological research activities have been successfully established within the Department. This has created a network of laboratory-based research with strong links to clinical medicine and to other institutes of the life-science focus on the University of Basel.

Complement

Autoantibodies

Systemic lupus erythematosus

Clinical Immunology

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The pathogenic role of complement MBL and autoantibodies against complement C1q in human diseases

Systemic lupus erythematosus (SLE) is considered an archetype of systemic autoimmune diseases. However, the causes and pathogenic mechanisms of SLE are still not fully understood. A major hypothesis of the pathogenesis of SLE assumes that the disease is driven by a defective clearance of dead and dying cells. In the context of an altered clearance these dying cells could become antigenic and initiate an autoimmune response. The complement system has been shown to play an important role in the clearance of apoptotic cells and the deficiency of one of the early components of the classical pathway of complement is strongly associated with the development of SLE. However, most SLE patients have no primary complement deficiency. In contrast, hypocomplementemia in SLE patients is a secondary event and most often associated with antibodies against the first component of the classical pathway of complement (C1q). Autoantibodies against C1q (anti-C1q) strongly correlate with renal flares in SLE patients. Previous studies suggest that the occurrence of anti-C1q in SLE patients is necessary but not essential for the development of proliferative lupus nephritis. It is possible that anti-C1q interfere with the normal function of the complement system including the clearance of apoptotic cells. However, the role of anti-C1q in other diseases is not yet established and the potential pathogenic mechanism of anti-C1q remains to be elucidated. Furthermore, the importance of regular anti-C1q measurements as a clinical follow-up marker in SLE patients is not yet established.

Therefore, our group aims to further examine the pathological role and the clinical relevance of anti-C1q antibodies in a double approach based on experimental studies of anti-C1q and clinical studies of patients with SLE. The experimental part includes i) the investigation of the correlation of anti-C1q with the occurrence of a glomerulonephritis in lupus-prone mice and ii) the generation of human monoclonal anti-C1q and the investigation of their interference with physiologic functions of the complement system in vitro and in vivo. In our clinical studies we aim to establish anti-C1q as an important follow-up parameter in SLE patients and to investigate its role in related renal diseases such as acute post-streptococcal glomerulonephritis.

Complement MBL, that is strongly related to complement C1q, has been shown to play an important role in the defence against infectious agents. However, more recent studies suggest that MBL also binds to apoptotic cells and plays a pro-inflammatory role in experimental settings of ischemia-reperfusion injury. The high frequency of functional MBL deficiency in the general population (about 25%) predestinates MBL for clinical studies investigating its role in human diseases. Thus, we are investigating the role of complement MBL in different settings of human ischemia-reperfusion injury.

Connection to Clinical Practice

According to the name of the laboratory and as outlined above, our research is focussed on questions relevant to clinics. Experimental as well as clinical research projects are directly linked to each other. Performing both types of studies, it is our aim to improve the understanding of pathogenic mechanisms in autoimmune and other diseases as well as to provide more detailed information on diagnostic parameters that can be used in the clinical routine.

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Lymphocyte progenitors
Developmental plasticity
IL-7
Notch
c-Kit
FLT3L

Developmental and Molecular Immunology

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Molecular mechanisms guiding mouse lymphocyte development

Haematopoietic stem cells (HSCs), a very rare bone marrow cell type, are responsible for the life-long production of all blood cells including T and B lymphocytes. Until recently, it was thought that the differentiation of HSCs into the various haematopoietic cells was a rather hierarchical process with differentiation along a particular lineage associated with a progressive loss of potential of generating other lineages. The recent development of very sensitive and quantitative in vitro assays, together with the identification of new progenitor subpopulations, has challenged this idea. Thus, we have identified in the mouse bone marrow a novel population of cells, representing 0.2% of all nucleated cells which can differentiate in vitro into B and T lymphocytes as well as myeloid cells. Based on these findings, we have called these cells “early progenitors with lymphoid and myeloid developmental potential”, or EPLM. Phenotypically, EPLM are B220+, CD117+, CD93+, CD127+ and CD135+ but are CD19- and NK1.1- and are therefore very similar to pro B cells found in Pax5-deficient mice which can also differentiate into many cell types. The in vitro requirements for the differentiation of EPLM into the various hematopoietic lineages are summarized in figure 1. In collaboration with Prof. Daniela Finke, we have shown that TSLP can replace IL-7 in the differentiation of EPLM into B and T lymphocytes.
During pregnancy, EPLM numbers in the bone marrow are normal whereas pro-B/pre-B I cell numbers are reduced more then 10-fold. This block in B cell development is due to a transient decrease in IL-7 production, a consequence of the elevated estrogen levels during pregnancy.
EPLM express CD135, the receptor for Flt3 ligand (Flt3L). Daily treatment for 7-10 days of mice with 5-10 mg Flt3L increased EPLM numbers 50-fold and in vitro analysis revealed that they fully retained myeloid and T but had lost B cell developmental potential. The latter finding accounts for reduced bone marrow precursor B cell compartment of Flt3L-treated mice. Thus, bone marrow EPLM numbers seem to be controlled by Flt3L with high levels impairing their B cell developmental potential.
In vivo transplantation studies showed that low numbers (2-5 x103) of EPLM could only generate B cells whereas with higher numbers (2 x 104) both T and B cells were seen. Despite the efficient myeloid potential of EPLM in vitro, we could not reveal this potential in vivo. Based on these findings, we favor the idea that physiologically, EPLM are primarily B-committed cells.
Based on the differential expression of CD25, CD44, CD117 and CD135, early thymocyte progenitors can be subdivided into several subpopulations. Currently, the consensus idea is that the thymus is colonized by a primitive cell type called a “thymus seeding progenitor” (TSP) which is CD44+, CD117+, CD135+ and CD25-. TSP’s are the direct progenitors of the double negative (DN) thymocytes which are subdivided into four subsets: CD44+, CD117+, CD25-, CD135- (DN1), CD44+, CD117+, CD25+, CD135- (DN2) CD44-, CD117low, CD25+, CD135- (DN3) and CD44-, CD117low, CD25-, CD135- (DN4) cells.
In vitro analysis, using culture systems to test B, T, NK and myeloid/dendritic cell development revealed that TSP can still generate all lineages whereas DN1 and DN2 have lost the capacity to generate B cells (fig. 2). On the other hand, DN3 cells can only generate T cells, indicating that during the transition from DN2 to DN3, commitment to the T cell lineage takes place.
The growth and differentiation of TSP, DN1 and DN2 into downstream stages of T cell development requires Notch, IL-7 (or TSLP) and SCF (fig. 2). SCF signaling seems to be downstream of Notch since the expression of its receptor c-Kit (CD117) is under Notch control. DN3 cells on the other hand only require a Notch signal to proliferate and differentiate into double positive thymocytes.

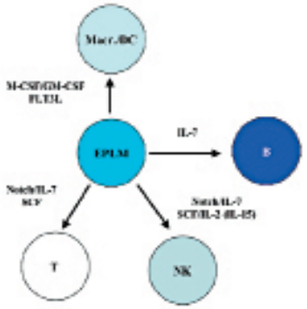

Fig. 1:
Early stages of B cell development in the bone marrow

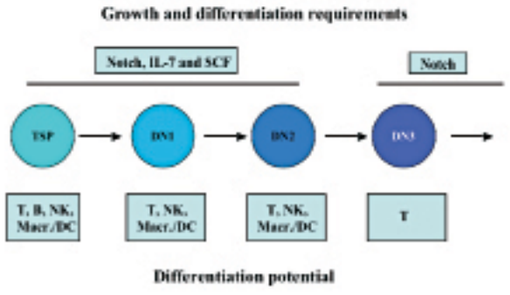

Fig. 2:
Early stages of T cell differentiation in the thymus

Connection to Clinical Practice

Molecular mechanisms guiding human lymphocyte development

The establishment of so-called humanized mouse models has dramatically facilitated experimental research on human hematopoiesis. In collaboration with Prof. Dr. Wolfgang Holzgreve (University Women’s Hospital, Basel) we have established the RAG-2 x common g double deficient humanized mouse model system in our laboratory. Thus, upon transplantation with human CD34+ cord blood stem cells, newborn RAG-2/gc mice show a robust and long-lasting reconstitution of the primary and secondary lymphoid organs by progenitor and mature human lymphocytes. Currently, we are using this model system to analyze the molecular mechanisms underlying human lymphocyte development. Moreover, we are testing the role of the TNF-family member BAFF in late stages of B cell development and its potential involvement in the development of systemic lupus erythematosis.

Selected Publications

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– Benard, A., Ceredig, R., and Rolink, A. G. (2006). Regulatory T cells control autoimmunity following syngeneic bone marrow transplantation. Eur J Immunol 36, 2324-2335.
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– Ceredig, R., Rauch, M., Balciunaite, G., and Rolink, A. G. (2006). Increasing Flt3L availability alters composition of a novel bone marrow lymphoid progenitor compartment. Blood 108, 1216-1222.
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Lymph node
Peyer's patch
Lymphoid tissue inducer cell
Interleukin
Thymic stromal lymphopoietin
Organogenesis

Developmental Immunology

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Lymphoid tissue formation in ontogeny and disease

Adaptive immune responses are generated in secondary lymphoid organs such as the spleen, lymph nodes (LNs) and Peyer's patches (PPs). The majority of infections lead to remodeling of lymphoid organs but the mechanism is unknown. Viral and parasite infections (e.g. HIV, LCMV, P. berghei ANKA) can destroy the architecture of secondary lymphoid organs, thus contributing to the failure of protective host responses. Extranodal lymphoid tissues form in inflammatory lesions of chronic infections, autoimmune diseases, allergic reactions and chronic graft rejection. These so-called "tertiary lymphoid tissues" have been suggested to play a role in presenting non-self and self antigens and in maintaining chronic inflammation and autoimmune responses. In mouse models detailed studies on the induction of lymphoid tissues during fetal and adult life therefore provide important information regarding the remodeling and induction of lymphoid microenvironments in infection and chronic inflammation.

We have identified fetal CD4+ IL-7Rα+ CD3- cells as lymphoid tissue inducer (LTi) cells. During fetal life, LTi cells interact with mesenchymal cells via adhesion molecules (α4β1-integrin/VCAM-1) and TNF family member molecules (Lymphotoxin (LT) αβ/LTβR) (Figure 1). The engagement of LTβR on mesenchymal cells by LTi cells is crucial for the expression of homeostatic chemokines, which then allow the recruitment of lymphocytes and the organization of functional lymphoid compartments.

Ectopic lymphoid follicles are found in patients with rheumatoid arthritis, and disease development positively correlates with high concentrations of serum and synovial IL-7. Moreover, both IL-7 and its receptor are implicated in multiple sclerosis pathogenesis. We hence studied the function of IL-7 in normal and ectopic lymphoid tissue development using a transgenic (Tg) mouse model. In mice ubiquitously overexpressing IL-7, we observed a striking neoformation of PPs, ectopic LNs (Figure 2) and cecal lymphoid patches. Ectopic LNs had a normal architecture, were connected to the lymphatic system and responded normally to antigenic challenge. We also found numerous ectopic lymphoid follicles in autoimmune target organs such as the pancreas and the salivary gland. The development of additional lymphoid organs and tertiary lymphoid tissues in organs correlated with an increased life span and LTαβ expression of LTi cells. In IL-7Tg mice, which were deficient for LTαβ or LTi cells due to a deletion of LT or RORγ, the formation of additional PPs and LNs did not occur. These data demonstrate that normal and ectopic lymphoid tissue development was dependent on LTi cells and LTαβ. Taken together, high levels of IL-7 can induced the formation of additional secondary and tertiary lymphoid organs.

Known for its role in triggering allergic diseases in human and mice, thymic stromal lymphopoietin (TSLP) is a cytokine, which binds to the IL-7Rα chain. Using TSLP Tg mice we found that IL-7 and TSLP had overlapping functions in lymphoid development. Both cytokines were important for the generation and maintenance of B and T cells in primary and secondary lymphoid organs. TSLP Tg expression rescued the disorganization of the thymic lymphoid architecture seen in IL-7-/- mice (Figure 3). Finally, increased availability of TSLP restored LN and PP development in IL-7-/- mice. Altogether, our results suggest that TSLP could replace the function of IL-7 in lympho-organogenesis and lymphopoiesis.

Future studies in collaboration with A. Fontana (Zurich) and F. Ponchel (Leeds, UK) aim at investigating the link between cytokine production, tertiary lymphoid tissue development and progression of chronic inflammations in autoimmune diseases. We will investigate the cellular subsets that are involved in lympho-organogenesis in mice and humans. In addition, we will test the molecular requirements for transition of mesenchymal cells into lymphoid stromal cells. Our study will provide important information on how alteration of lymphoid tissue development can modulate immunological disorder such as chronic inflammation or immunosuppression in infectious diseases.

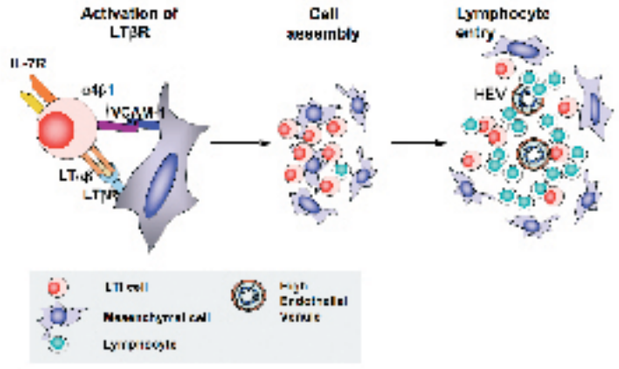

Fig. 1: Model of lymphoid development in fetal life.

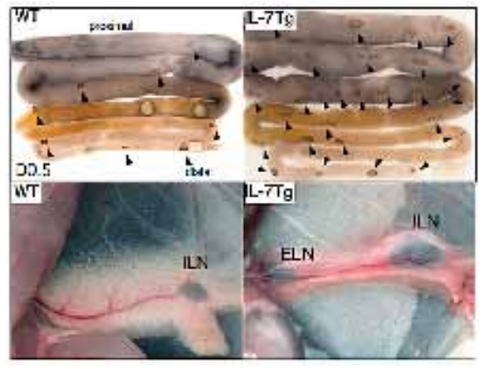

Fig. 2: IL-7 Tg expression induces the de novo formation of additional PPs and LNs. PPs are identified in neonatal (day 0.5) intestine of WT and IL-7Tg mice by whole mount immunohistochemistry with anti-VCAM-1 Ab staining. ILN=inguinal lymph node, ELN=ectopic lymph node.

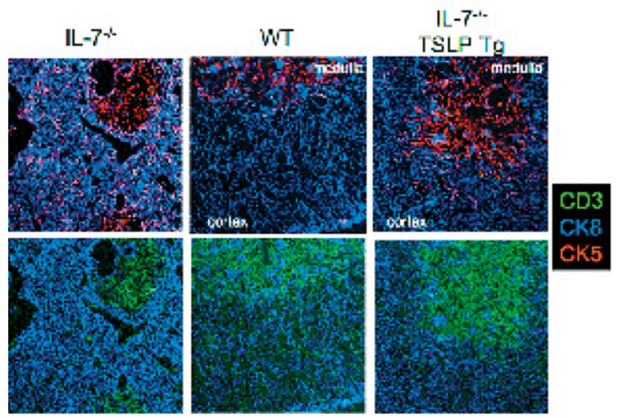

Fig. 3: TSLP Tg restores thymic organization. Immunofluorescence analysis of thymi from IL-7 $^{-/-}$ littermates, WT controls and IL-7 $^{-/-}$ K14-TSLP Tg mice, stained for CD3 (green), Cytokeratin (CK) 5 (red) expressed by medullary thymic epithelial cells and Cytokeratin (CK) 8 (blue) expressed by cortical thymic epithelial cells. Original magnification: x20.

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Heart Failure
Myocarditis
Autoimmunity
Inflammatory Dilated Cardiomyopathy
Cytokine
Stem cells

Experimental Critical Care Medicine

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Targeting Inflammatory Mechanisms of Heart Failure

Besides genetic susceptibility, viral infections triggering autoimmunity have been implicated in the pathogenesis of dilated cardiomyopathy, the commonest cause of heart failure in young patients. We have shown that dendritic cells loaded with heart specific self-peptides induce T-cell mediated myocarditis in naïve, non-transgenic mice (Fig. 1). After resolution of acute myocarditis, mice develop heart failure. This model of autoimmune myocarditis (EAM) reflects a unifying theory as to how tissue damage and activation of Toll-like receptors (TLRs) during infections can induce autoimmunity, autoimmune relapses, and cardiomyopathy (Fig. 2). This model offers a nice approach to study inflammatory mechanisms and the pathophysiological response of the failing ventricle to volume or pressure overload. It is a valuable tool to the development of novel treatment strategies and will be of help in refining current therapeutic options for inflammatory heart disease and heart failure.In particular, induction of autoimmune myocarditis requires priming of autoreactive CD4+ T-cells that migrate to the heart and interact with resident tissue cells expressing self-antigen. The autoreactive CD4 T cell response results in the IL-17 dependent recruitment of inflammatory monocytes to the heart. Monocytes represent the major infiltrating cells in acute myocarditis and represent a double-edged sword in inflammatory heart disease: on one hand they contribute to tissue injury and pathological remodeling, on the other hand they represent a key element in an Interferon-gamma dependent negative feedback mechanism confining autoreactive CD4+ T cell responses. Our projects address the roles of specific mediators and cytokines involved in the progression and resolution of cardiac inflammation. In our experiments we take advantage of knockout mice lacking specific cytokines, chemokines and/or their receptors. We hope that this approach will allow us to develop novel treatment strategies based on in vivo blocking of specific pro-inflammatory mediators (Fig. 2). Inflammatory mechanisms are also critically involved in the pathogenesis of atherosclerosis and coronary heart disease the most prevalent heart disease worldwide. Accordingly, we are also interested in inflammatory aspects of atherosclerosis (the “vulnerable plaque”).Emphasis is given that our working hypotheses are guided by clinical observations. In turn, we are interested in a straight transfer of our experimental findings to clinical practice. Therefore, our group established close national and international collaborations with clinicians and basic researchers.
Our partners are not only at the Department of Biomedicine but also located at the Basel University Hospital (Internal Medicine, Critical Care and Emergency Medicine, Cardiology and Pneumology), at the Swiss Federal Institute in Zürich, at the IMBA in Vienna, and at the Harvard Medical School in Boston. At the moment we are one of the worldwide leading groups in the field of autoimmune myocarditis.
Taken together, our research focuses on inflammatory heart diseases such as myocarditis, postviral cardiomyopathy, and atherosclerosis. Taking advantages of mouse models we contribute to the development of novel treatment strategies and vaccine design for devastating heart diseases.

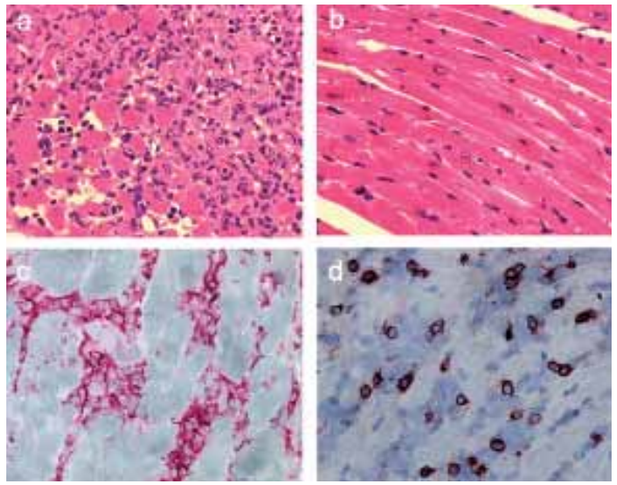

Fig. 1: Immunization of mice with cardiac myosin together with Freund’s complete adjuvant results in severe autoimmune cardiac inflammation (a). Freund`s complete adjuvant alone does not affect the heart (b). Inflammatory infiltrates include monocytes, dendritic cells expressing MHC class II molecules (c), and lymphocytes. IL-17 producing $CD4^+$ T lymphocytes (d) are critical for disease development.

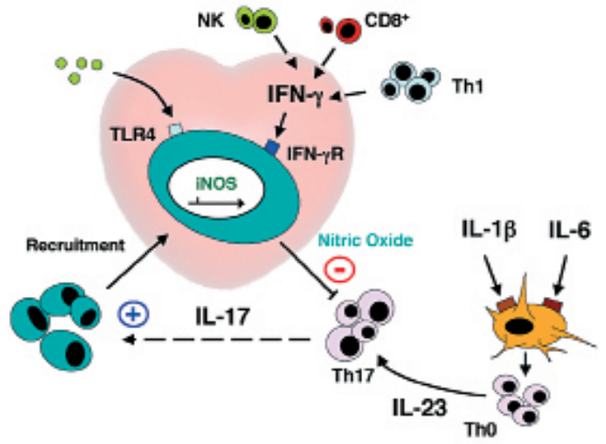

Fig. 2: Activated dendritic cells expressing cardiac self-antigen then prime autoreactive, IL-17 releasing $CD4^+$ T cells in an IL-6 and IL-1 dependent manner. IL-23 promotes the expansion of autoreactive IL-17 releasing T cells, which promote the recruitment of monocytes to the heart. Heart-infiltrating monocytes on the other hand represent a key element in a negative, Interferon gamma dependent and nitric oxide mediated feedback mechanism confining the autoreactive T cell response.

Connection to Clinical Practice

Prof. Dr. Christian Müller
Division of Internal Medicine
University Hospital Basel

To recognize and understand heart failure: a joint venture between basic and clinical research

The laboratory of Experimental Critical Care Medicine focuses on the pathogenesis of inflammatory mechanisms in heart failure and atherosclerosis. At the bench, we are developing novel treatment strategies and diagnostic approaches. Our partners at the Division of Medicine A (Head: Prof. A.P. Perruchoud), Department of Internal Medicine are directly transferring our research progess “bedside” to the patients of the University Hospital. Prof. Christian Müller and his team at the Division of Medicine A are taking advantage of the so called B-type natriuretic peptide (BNP) as a specific marker for volume overload of the heart. Prof. Müller recently showed that BNP measurements clearly discriminate between heart failure and other causes of acute dyspnea. At the moment several studies addressing the relevance of neuroendocrine and inflammatory mediators as diagnostic and prognostic markers in patients with proven or suspected heart diseases are under way.

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T lymphocyte
Antigen presentation
Infection
Vaccine
Autoimmunity
Cancer

Experimental Immunology

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Recognition of non-peptidic antigens by T lymphocytes

The recognition of peptides, lipids, phosphorylated metabolites and sugars allows the immune system to detect a wide range of antigenic moieties. We are studying the recognition of non-peptidic antigens by human T lymphocytes in physiological and pathological conditions including infection, cancer, atherosclerosis and autoimmune diseases.

Participation of CD1e in processing of microbial glycolipid antigens
A subset of T cells recognizes complexes formed by CD1 antigen-presenting molecules associated with self or microbial glycolipid antigens. CD1e is the only human CD1 isoform which is soluble and, because it does not reach the cell surface, is not an antigen-presenting molecule. We have shown that CD1e is required for processing complex glycolipid antigens like the hexamannosylated-phosphatidyl-myo-inositol (PIM6) present in the cell wall of Mycobacterium tuberculosis. This antigen stimulates CD1b-restricted T cells only after processing consisting of the digestion of terminal mannoses by alpha-mannosidase occurs in the lysosomal compartment. Recombinant CD1e binds to glycolipids and assists the digestion of PIM6 by alpha-mannosidase. Thus, CD1e is directly involved in the editing and the expansion of the repertoire of glycolipidic T cell antigens and can therefore optimize the anti-microbial immune responses.

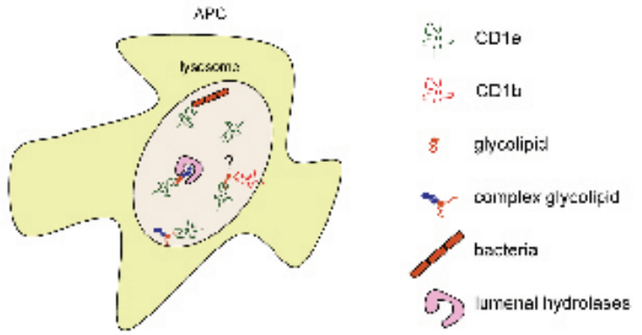

Fig. 1: A model of CD1e function. Soluble CD1e molecules (green) are present inside the endosomal compartment of professional antigen presenting cells (APC). They extract microbial lipids (complex glycolipids) from bacterial cells (red), or bind to self lipids (complex glycolipids) localized in the endosomal limiting membrane, and offer them to luminal hydrolases (pink), thus facilitating antigen processing. CD1e might participate in transfer processed glycolipid antigens to CD1b antigen presenting molecule.

Bacterial infections promote T cell recognition of self-glycolipids
We have described an alternative way by which bacterial infections provoke the activation of glycolipid-specific T cells. The mechanism is not through direct recognition of microbial lipids derived from the infecting agent, but through the stimulation of the glycosphingolipid (GSL) metabolism of the infected cells. CD1+ APC infected with several bacteria (Escherichia coli, Bacillus subtilis, Staphylococcus aureus, or Mycobacterium bovis BCG) or treated with bacterial components (LPS, lipoteichoic acid or Pam3CysSer-Lys4 lipopeptide), acquire the capacity to stimulate self-GSL specific T cells due to the increase in the endogenous GSL synthesis. This stimulation may contribute to inflammatory responses during bacterial infections and may predispose to autoimmune diseases.

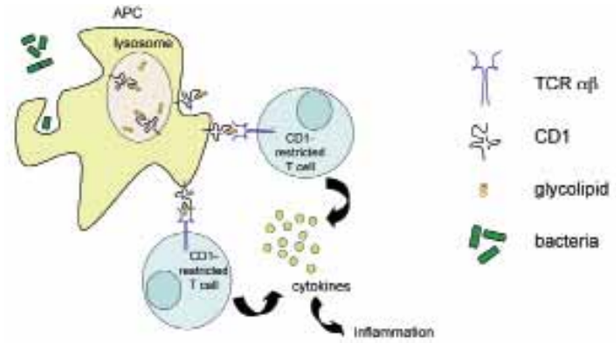

Fig. 2: CD1-restricted T cells detect bacterial infection through self-recognition. Bacterial components released during bacterial infection (green) of APC increase the synthesis of glycolipid autoantigens (yellow), which associate to CD1 molecules and result in the activation of CD1-restricted T cells.

The dysregulation of the mevalonate pathway occurring during bacterial infection activates TCR gamma/delta cells
Primates, but not rodents, have TCR Vgamma9/Vdelta2 T cells bridging innate and adaptive antimicrobial immunity. We have previously shown that these cells recognize small phosphorylated non-peptidic metabolites generated by the mevalonate pathway of both eukaryotic and prokaryotic cells. Tumor cells such as Burkitt's lymphomas are one example of cells that accumulate large amounts of isopentenylpyrophosphate (IPP), the metabolite that stimulates TCR Vgamma9/Vdelta2 T cell response.
We have found that TCR Vgamma9/Vdelta2 cells become activated during the initial phases of infections with Gram-positive and Gram-negative bacteria. Infection upregulates the production and accumulation of host-derived TCR gamma/delta stimulatory antigens which are metabolites produced in the host mevalonate pathway. Accumulation, dephosphorylation and increased activity of the hydroxymethylglutaryl-Coenzyme-A-reductase, the rate-limiting enzyme of the mevalonate pathway, are induced early during infection and cause accumulation of stimulatory metabolites. Thus, as a mechanism of immediate antimicrobial immunity, primates have evolved the ability to readily respond to bacterial infection by sensing the dysregulation of the mevalonate pathway.

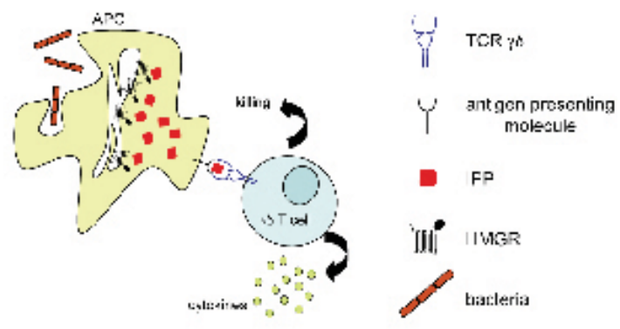

Fig. 3: The mechanism how bacterial infection stimulates TCR gamma/delta cells. Early after bacterial infection (orange) the infected APC upregulates hydroxymethylglutaryl-Coenzyme-A-reductase (HMGR), and accumulates isopentenylpyrophosphate (IPP, red). This endogenous metabolite is responsible for the activation of the TCR gamma/delta cells.

Connection to Clinical Practice

New vaccination strategies using lipid antigens

Our laboratory is exploiting lipid antigens in novel vaccination strategies, with the aim of preventing and curing cancer, autoimmune and inflammatory diseases as well as such infectious diseases as tuberculosis.
Identification of T cells specific for lipids of self and microbial origin has opened new perspectives for the generation of novel vaccines. The strategy of using lipids as vaccines has two advantages. Firstly, because lipids are presented to T cells by CD1 antigen-presenting molecules, which are functionally non-polymorphic, the entire human population will react to the same immunogenic lipids. Secondly, in contrast to proteins, lipids cannot be modified under selective pressure in bacteria or in tumor cells.
Our current experiments are revealing the possible use of mycobacterial lipids as efficacious vaccines inducing long-term protection in M. tuberculosis infection.
Other experiments are aimed at the identification of lipid antigens specifically expressed by human tumor cells and in dysmetabolic diseases.

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Skin cancer
Apoptosis
RNA interference
Gene therapy
HIV-pathogenesis

Experimental Immunology

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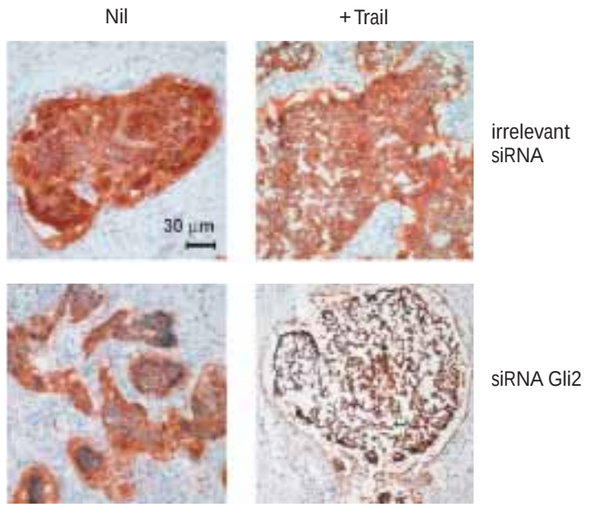
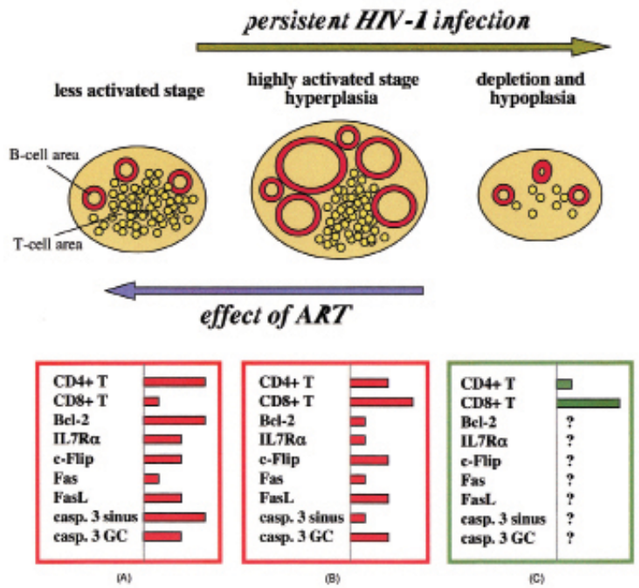
Gli2 renders basal cell carcinoma cells resistant to apoptosis by upregulation of the anti- apoptotic molecules cFlip and bcl-2

According to the World Health Organization between 2 and 3 million non-melanoma [basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)] and over 130'000 melanoma skin cancers globally occur each year with a strong tendency of rising incidences. The risk to develop skin cancer is usually based on constitutional and inherited factors combined with environmental factors, mainly exposition to UV light through sun exposure. Sunlight and its underlying UVB radiation has two major effects, it suppresses the immune response in the skin, and it may initate transformation of skin epidermal cells. Thus, UV light can either mutate the p53 tumor suppressor gene and/or genes of the hedgehog (HH) signaling pathway, e.g. the patched (ptch) or smoothened (smo) genes which control transcription via the downstream Gli genes. Ptch is the transmembrane receptor for the HH protein family. If the Hedgehog peptides are absent, ptch inhibits smo, another transmembrane protein functioning as a G-protein coupled-like receptor, and prevents it to signal. In the reverse situation, when the HH peptides bind to and inactivate ptch, smo exerts its activity leading to a hyperphosphorylation of the proteins bound to the complex with Gli. This allows full length Gli proteins to translocate to the nucleus where they induce transcription of HH target genes. Thus, loss-of-function mutations of ptch or gain-of-function mutations of smo are often associated with BCC development, and this is linked with the fact that these mutations result in a continuous overactivation of the Gli transcription factors. Three Gli genes (Gli1, Gli2 and Gli3) have been identified in humans. Among these, Gli2 seems to be the primary positive transducer of hedgehog signaling. Therefore, we investigated the role of Gli2 in BCC formation. In gene chip analysis we tested which genes are up- or downregulated if Gli2 expression is high. We found that, beside bcl-2, the caspase 8 inhibitor cFlip which antagonizes the extrinsic apoptotic pathway was upregulated by high Gli2 expression. Using a keratinocytic cell line, HaCat NHis-Gli2, in which Gli2 expression is under the control of tetracycline, we indeed found, that Gli2 overexpression increased apoptosis-resistance of the cells through the upregulation of bcl-2 and cFlip. Investigating the cFlip promoter, we identified and confirmed Gli2 binding sites on the promoter. Gli2 gene silencing by RNA interference reduced the apoptosis resistance via cFlip downregulation. This direct functional link between Gli2 and cFlip was not only demonstrated in the keratinocytic cell line but also in BCC tissue. In general, BCCs demonstrate a strong resistance to the extrinsic and intrinsic apoptotic pathway. We found that this is based on a high cFlip and bcl-2 expression as a consequence of Gli2 overexpression. Moreover, we could demonstrate, that Gli2 gene silencing in BCC tissues made the tumor cells sensitive to TRAIL-mediated apoptosis by downregulating cFlip. As Gli2 silencing does not only downregulate cFlip, but also bcl-2, Gli2 could be a key target for a novel therapeutic approach in tumors with dysregulated hedgehog signaling.

Additional support for the key role of Gli2 in BCC formation comes from in vivo studies in a mouse tumor allograft model. A constitutively Gli2 expressing mouse tumor cell line originating from a trichoblastoma was stably transfected with Gli2-specific shRNA to induce Gli2 gene silencing or with control shRNA. Injecting the Gli2 gene silenced cells into nude mice for tumor formation we obtained a strongly retarded tumor growth compared with control tumor cells. Investigating the mechanisms we found that Gli2 gene silencing has led to the complete disruption of the tumor structure. Two main reasons for the tumor destruction were identified. We found that apoptosis was markedly increased while vascularization was strongly decreased in these tumors. Thus, important functions of the transcription factor Gli2 in this tumor model are not only the prevention of apoptosis but also the promotion of microvascularization.





Connection to Clinical Practice

Antiretroviral therapy applied early in HIV-infection halts irreversible lymph nodes destruction

CD4+ T cell depletion and destruction and the involution of lymphoid tissue are hallmarks of HIV infection. Although the underlying mechanisms are still unclear, disordered apoptosis appears to play a central role. We investigated the effect of antiretroviral therapy on lymph node tissue, with particular respect to morphology and apoptosis. Two inguinal lymph nodes were excised from 31 previously untreated individuals who were in an early stage of HIV infection, the first one prior to treatment and the second after 16 to 20 months of treatment. Paraffine sections were investigated for lymph node architecture, distribution of cellular and viral markers, apoptosis and expression of apoptotic key molecules. After 16-20 months of antiretroviral therapy, we found a significant decrease in the highly activated HIV-driven immune response in the lymph node tissue, which is mainly responsible for the lymph node destruction. This was evidenced by a marked reduction in follicular hyperplasia, a normalization of the follicular dendritic cell network, a significant increase in the number of CD4+ T cells paralleled by a significant decrease in the number of CD8+ T cells. In addition, several pro-apoptotic (Fas, TRAIL, active caspase 3) and anti-apoptotic (bcl-2, IL-7Ralpha) molecules were reconstituted in the tissues during therapy resembling an expression pattern very similar as in lymph nodes of HIV-negative individuals. This lead in parallel to a marked decrease of apoptosis. Thus, antiretroviral therapy initiated in the early stages in HIV infection may halt the irreversible destruction of lymph node tissue and may partially normalize disordered apoptotic processes.

Selected Publications

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Virus-specific CD4+ and CD8+ T cells

Epstein-Barr virus

Cytomegalovirus

HIV-infection

Solid-organ transplantation

Chemotaxis

Immunobiology

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Correlates of Efficient T Cell-Immunity: Lessons from the Immunocompromised Host

The general hypothesis fundamental to our work states that establishing and maintaining adequate CD8+ T cell-immunity depends on appropriate CD4+ T cell-help.

We aim at testing this hypothesis by investigating various models of impaired T cell-immunity, such as HIV-infection and iatrogenic immunosuppression after solid-organ transplantation.

A deficit in CD4+ T cell-help may perceptibly arise due to low absolute CD4+ T cell-counts or via a pathogen-selective CD4+ T cell-deficit. Indeed some HIV-infected individuals continue to be at risk for opportunistic diseases –such as EBV-associated primary central nervous system lymphoma (PCNS-lymphoma)– despite prolonged normalization of CD4+ T cell counts. In these patients we recently were able to demonstrate that irrespective of absolute CD4+ T cell counts EBV-specific CD4+ T cell function was lacking (Figure 1). We now investigate how such defective CD4+ T cell-function is linked to impaired CD8+ T cell-mediated immunity.

Iatrogenic immuno-suppression as installed at the time of solid organ transplantation signifies a temporally well defined, and clinically relevant shift in the immune-competence of transplant recipients. Characterizing correlates of efficient cellular immunity in this setting has both basic immunological and clinical implications. Defining characteristics of immunological competence of CD8+ T cells, and the requirements for CD4+ T cell-help in their generation, are central goals in immunology. From a clinical point-of-view, elucidating the cellular/molecular pathogenesis of EBV- and CMV-associated disease may identify biomarkers/profiles allowing for individual risk-stratification, thus helping clinicians to identify individuals at risk for EBV- and/or CMV-associated disease. This work is ongoing.

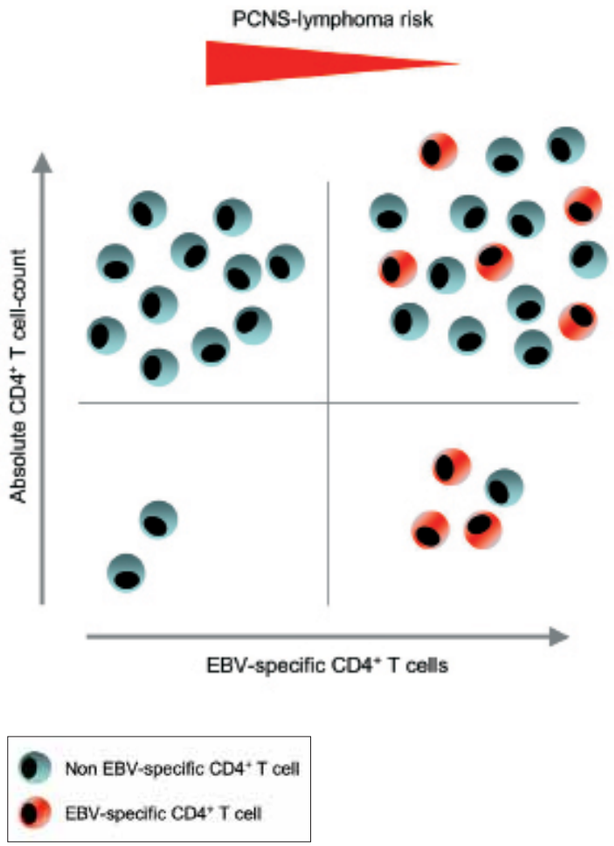

Fig. 1:
Dissociation of absolute CD4+ T cell-counts and EBV-specific CD4+ T cell-function

Selected Publications

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Innate immunity

Ectosome

Macrophages

Complement

Complement C2 inhibitor trispanning (CRIT)

Inflammation

Immuno-nephrology

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Corinne Lochmatter (PhD student)

Complement and inflammation

Complement is required to combat infections, and to get rid of necrotic and apoptotic material without producing harm to self. Thus, it needs a powerful activation cascade producing inflammation and enhancing the immune response on one hand, but strong mechanisms to control excess damage as well. Our present investigations are based on our recent observations made on complement modulating directly and indirectly inflammation.

CRIT, a regulator of complement in Schistosome

Complement C2 receptor inhibitor trispanning (CRIT) is a transmembrane protein of Schistosomes, initially described as a trispanning orphan receptor (TOR) by Jameel Inal in 1999. A fragment of its first extracellular domain has homologies with the beta chain of complement C4. The corresponding peptide binds C2, inhibits the cleavage of C2 by C1s, and blocks classical pathway activation. Although less active the same peptide binds factor B of the alternative pathway and inhibits the activity of the alternative pathway C3 convertase. In vivo the same peptide reduces inflammation in a mouse model of reverse Arthus reaction. The biology of CRIT (TOR) as inhibitor of complement activation on Schistosome is unknown and is the field we want to further investigate:

1) To define the exact structure of CRIT, and which cells express it and at which stage of the Schistosome biological cycle.
2) To analyse and compare the CRIT function from different schistosomes (mansoni, japonicum, and haematobium), since the sequence responsible for complement inhibition in S. mansoni differs in the two other species.
3) To define the capacity of CRIT to inhibit human complement on the Schistosome surface.
4) To see whether CRIT is a target of the immune response in humans, and if so, whether anti-CRIT specific antibodies interfere with the function of CRIT.

Ectosomes – particles with biological functions

Ectocytosis describes the release of small vesicles (ectosomes) by budding from the surface of many different cell types (polymorphonuclear leukocytes [PMNs], erythrocytes, etc). Beside different cell surface and intracellular proteins, ectosomes express phosphatidylserine (PS) like apoptotic cells. PS serves as a receptor for many proteins including C1q, which may bridge ectosomes to phagocytes. Our initial in vitro data suggest that ectosomes of PMNs and erythrocytes may down-modulate inflammation and immune response, as apoptotic cells do.

Our present goals are to define the properties of ectosomes, in particular their capacity to interfere with the function of cells involved in inflammation and immunity. The specific aims are:

1) To define the uptake and intracellular processing of ectosomes by different cells.
2) To study the cellular responses induced by ectosomes.
3) To study the changes in the cellular programs induced by ectosomes (gene expression).
4) To analyze the possible anti-inflammatory properties of ectosomes in vivo in mice, as well as their capacity to block an immune response.
5) To follow the fate of erythrocyte-derived ectosomes in vivo in humans.

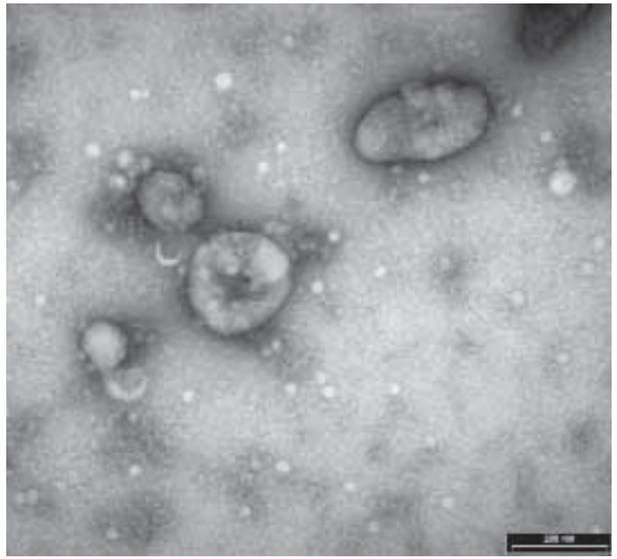

Fig. 1: Electron microscopy of Red Blood Cells (RBCs)-derived ectosomes released during storage.
Ectosomes purified from the supernatant of leukocyte-depleted packed RBCs. Heterogeneity in size 30-500nm. Size bar: 200nm

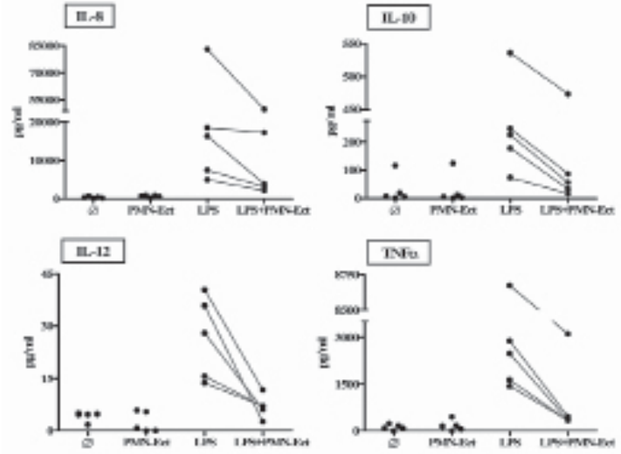

Fig. 2: PMN-derived ectosomes inhibit the release of inflammatory cytokines by LPS-matured human monocyte-derived DCs. iMoDCs were incubated for 24 h with (i) medium alone (Ø), (ii) medium & PMN-Ect, (iii) medium & LPS (10 ng/ml), and (iv) medium & LPS & PMN-Ect. Concentrations of IL-8, IL-10, IL-12 and TNFα were analyzed in supernatants.

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Toll like receptor 2

CD14

Streptococcus pneumoniae meningitis

Infection Biology

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The role of pattern recognition receptors in *Streptococcus pneumoniae* meningitis

The pattern recognition receptors TLR2 and CD14 mediate host responses to Gram-positive bacterial components. Infection with live Gram-positive bacteria does not require TLR2/CD14, but is regulated by activation of these receptors. After binding to bacterial lipopeptide and lipoteichoic acid, CD14 associates with TLR2, which is mediating inflammatory signals via NF-kB. Our theme is understanding TLR2/CD14 function in regulating host response to infection with the Gram-positive bacterium Streptococcus (S.) pneumoniae. We used a subarachnoidal infection model with a clinical isolate of S. pneumonie serotype 3 in C57BL/6, TLR2-/-, CD14-/- and Double Knockout mice.

a) We investigated the effects of TLR2 and CD14 on the course of meningitis and disease outcome.
b) We identified the cell types expressing TLR2 and CD14 in meningitis.
c) We studied TLR2 regulation of inflammatory gene and protein expression during meningitis.
d) We assessed TLR2 effects on bacterial localization in meningitis, and on phagocytosis and killing of S. pneumoniae by granulocytes.

a) We previously demonstrated that TLR2 protects mice from early death in meningitis by reducing brain bacterial load and downmodulating TNF in the cerebrospinal fluid (CSF) (Echchannaoui et al., J infect Dis., 2002). We then investigated the role of CD14 in this model of meningitis. We showed that leukocyte migration into CSF is slowed by CD14 via MIP-2 receptor (CXCR2) and MIP-2 downmodulation (Echchannaoui et al., J. Leukocyte Biol. 2005). Results obtained in TLR2-/- and CD14-/- mice indicate that both receptors participate in innate host defense by downmodulating inflammation during meningitis, although by different mechanisms. To understand the effects of both receptors combined, meningitis was performed in TLR2-/-/CD14-/- mice. They had more bacteria, yet less TNF and infiltrating cells in CSF than single Knockouts and a similar disease course as single Knockouts (Echchannaoui et al., BMC Infect Dis. 2007). At the present time it remains an interesting question, how the two coreceptors interact in their immunomodulatory action.
b) Next we wanted to know the blood and brain cell types expressing TLR2 and/or CD14. We demonstrated surface TLR2 in all blood granulocytes and CD14 in a small fraction of this population only. In CSF the great majority of infiltrating cells was double-positive for TLR2 and CD14, thus CD14 was acquired after blood brain barrier passage. We showed by in situ hybridization that transcripts of TLR2 and of the comolecules CD14, MD-2, TLR1/6 strongly increase after infection in infiltrating, not in resident cells.
c) We were interested to associate a function to TLR2 and CD14 in infiltrating cells. Interestingly the pattern receptors colocalized with TNF in CD45+ infiltrating cells in the ventricles, corpus callosum and the meninges. TNFmRNA was restricted to infiltrating cells since it was abolished in leukocyte depleted mice with meningitis. TNF gene and protein expression was stronger in TLR2-/- than wild type brains and was associated with increased IkB gene expression suggesting that TLR2 is controlling inflammation via TNF regulation in cis (Letiembre et al., J Neuroimmunol, 2005).
d) We finally were interested to understand the mechanisms, by which TLR2 is controlling bacterial clearance. We found brain bacterial load decreased early in infection by TLR2 via reduced adherence to and uptake into plexus choroideus epithelia (Echchannaoui et al., Immunobiol. 2005). In addition, we found that TLR2 accelerates bacterial uptake into granulocytes and increases granulocyte-dependent oxidative killing in vitro (Letiembre et al., Infect.& Immunity, 2005).

In summary, our results show that in pneumococcal meningitis TLR2 and CD14 are restricted to infiltrating cells, where they downmodulate TNF and CXCR2 respectively and permit a better bacterial clearing by reducing bacterial adherence to plexus choroideus epithelial cells and by accelerating bacterial phagocytosis and killing. In future the role of pneumococcal surface lipoproteins as virulence factors and ligands for TLR2 are investigated.

Connection to Clinical Practice

Infectious Diseases
Adjuvant treatment of *Streptococcus pneumoniae* meningitis

Streptococcus (S.) pneumoniae meningitis has a high lethality despite antibiotic treatment. Inflammation is a major pathogenetic factor, which is unresponsive to antibiotics. Therefore adjunctive therapies with antiinflammatory compounds have been developed. TNF484 is a TNF-alpha converting enzyme (TACE) inhibitor and has been found efficacious in experimental meningitis. Toll-like receptor 2 (TLR2) and CD14 contribute to host response in pneumococcal meningitis by enhancing bacterial clearing and downmodulating inflammation. From our in vivo studies using single knockout mice, it appears that TLR2 and CD14 both are protective, although by different mechanisms. Because both knockout strains showed excessive TNF, we compared the treatment response in wt, TLR2-/-, CD14-/-, and CD14-/-/TLR2-/-double knockout mice with meningitis to the antibiotic ceftriaxone and/or anti-inflammatory treatment with TNF 484. With antibiotic therapy all wt, CD14-/- and TLR2-/-/CD14-/- mice, but only 79% of TLR2-/- mice, were rescued. TACE inhibitor treatment alone did not rescue, but prolonged survival in wt mice and in TLR2-/- and CD14-/- mice to the values observed in untreated wt mice. By combined antibiotic and TACE inhibitor treatment 95% of TLR2-/- mice were rescued. In conclusion, during pneumococcal meningitis strong inflammation in TLR2-deficiency was associated with incomplete responsiveness to antibiotics and complete response to combined antibiotic and TACE inhibitor treatment. TACE inhibitor treatment offers a promising adjuvant therapeutic strategy in pneumococcal meningitis, especially in case of excessive inflammation.

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Diagnosis and treatment
of implant-associated infection
Sonication
Calorimetry
Radiolabeled vitamin B12
Molecular methods for diagnosis of sepsis

Infectious Diseases

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New and innovative approaches for diagnosis and treatment of implant-associated infections

Modern medicine has developed a variety of artificial devices to assist in the performance of physiological functions, functioning as short-term devices (e.g. catheters, osteosynthetic material) or permanent devices (e.g. prosthetic joints, artificial cardiac valves, pacemakers, neurosurgical shunts). After implantation, an interface is created between human tissues and prosthetic materials, which is associated with an increased risk of infection (1). Infections associated with implanted devices are typically caused by microorganisms attached to surfaces, embedded in an extracellular matrix and entering a non-growing (stationary) phase, a microbial structure called biofilm (Fig. 1).

Once an infection is established, it is both difficult to diagnose and difficult to treat. As a consequence, implant infections are frequently diagnosed late, when chronic inflammation around the implant causes failure of the implant and requires removal of the device. Repeated surgical interventions and prolonged antimicrobial treatment are often required to control the infection, causing high morbidity and consume substantial proportion of healthcare expenditures. Therefore, improved diagnostic and treatment approaches are needed.

We focused on improvement of diagnosis by employing ultrasound (sonication) to remove adherent biofilm microorganisms from implant surfaces (2) (Fig. 2). In addition to conventional cultures, detection of microbial nucleic acid with broad-range or multiplex polymerase chain reaction (PCR) amplification is investigated. For accurate and rapid diagnosis of infection, detection of heat production by microbial replication and metabolism using microcalorimetry was investigated (3, 4). For this purpose, an isothermal batch calorimeter was used and a miniaturized flow chip calorimeter was designed. A further approach to diagnose infection before surgery non-invasively is imaging using bacteria-specific tracers. For this purpose we investigated radiolabeled vitamin B12-derivatives in a cage mouse model, followed by imaging using SPECT/CT (Fig. 3). Vitamin B12-derivatives which are not binding to transcobolamin II are not taken up by eukaryotic cells, but are accumulated in rapidly replicating cells, such as microorganisms. This approach and may improve the detection of infection, potentially distinguishing from a sterile inflammation.

The second focus of the research group is the evaluation of new antimicrobial and antibiofilm agents and their combinations against implant-associated infections in vitro and in animal models. For this purpose, a guinea pig model with subcutaneously implanted tissue cages is used to determine the pharmacokinetic parameters and antimicrobial treatment efficacy of biofilm infections (5). Research on biofilms may lead to novel, innovative strategies for early detection and effective treatment of implant-associated infections, resulting in a significant improvement of patient management and public health. This is particular important, since the number of implanted devices and materials in the aging population is continuously increasing.

Fig. 1: Ultrastructure of microbial biofilms.
Microorganisms attached to surface change their growth characteristics from a free-floating (planktonic) to a sessile (biofilm) mode, as part of their survival strategy. In the biofilm, bacteria are more resistant against antibiotics or host immune defenses, representing a treatment challenge in modern medicine involving implants.

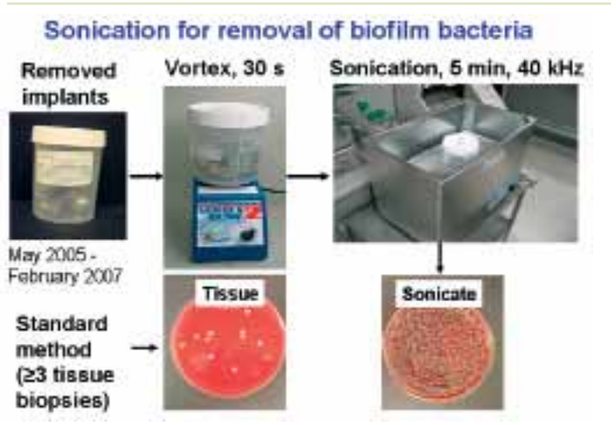

Fig. 2: Sonication procedure of removed implants.
By subjecting explanted device to appropriate ultrasound intensity (40 kHz, 0.2 W/cm^2), viable bacteria in biofilms are detached from the surface in order to be detected in culture or by molecular methods.

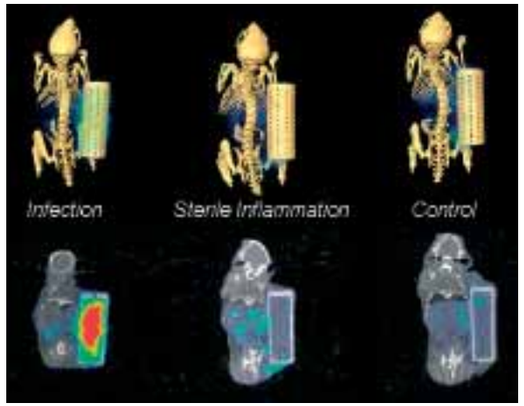

Fig. 3: Imaging of infection by SPECT/CT using a radiolabeled vitamin B12-derivative in mouse model.
A cage was implanted subcutaneously, which was either infected with Staphylococcus aureus (left), injected lipopolysachharide to induce sterile inflammation (middle) or used a control (right). 48 hours after in-cage injection of ^{99m}Tc-labeled cobalamin derivative (20 µCi), the infected cage showed a positive signal, whereas sterile non-infected cages were negative.

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Clinical Infectious Diseases – an interdisciplinary research field

Infectious diseases cover a vast area of topics. The often critical balance between host and pathogen is more and more influenced by modern therapeutic interventions such as immunosuppressive therapy, transplantations or artificial devices, i.e. prosthetic joint replacement, pacemakers or artificial heart valves. Life expectancy has much increased; hence age interferes with aspects of disease occurrence. Our research in the field of infectious diseases focuses on several aspects.

Optimal diagnostic and therapeutic procedures and use of correct antibiotic application and duration

In 2003 our division started an antibiotic stewardship program. A first study was published which analyzed the empirical and adjusted antibiotic use in the emergency room. Inadequate antibiotics were given in 22% of empirical and in 27% of adjusted therapy. As a consequence interventions should focus on both initial empirical therapy and streamlining and adjustment of therapy once microbiological results become available. A further study is ongoing, investigating the switch of an i.v. to an oral antibiotic therapy to shorten hospital stay and to reduce costs. In a more specific study we investigated the antibiotic treatment in over 300 hospital episodes of drug addicted patients and showed that 80% of these patients could be treated according to international guidelines or recommendations by infectious disease specialists.

S. aureus infection and pathogens relevant for infectious diseases epidemiology

In several studies we described aspects of clinically and epidemiologically relevant pathogens such as S. aureus and Mycobacterium tuberculosis. Pathophysiologic and molecular aspects of S. aureus were investigated comparing the expression of PIA and ica specific transcripts in vitro and in an animal model. The clinical description of S. aureus infection is very important, due to its high pathogenicity and frequency. Analyzing 308 episodes of S. aureus bacteraemia showed that the overall hospital-associated mortality was still 20%. Hence, it may be important to reliably detect carriers of S. aureus as carrier state may be associated with a higher risk for latter infections. By screening close to 3000 individuals for S. aureus-carriage we showed that screening of throat swaps significantly increases the sensitivity of detection for S. aureus carriage by 25.7%. Regarding tuberculosis (Tb), we showed that new immigrants suffering from active infection are often quite asymptomatic. For this study we investigated 111 of 42'601 new immigrants with chest radiographs suspicious for Tb and compared the symptoms and course to foreign born residents and native residents with Tb.

Transplant infectious diseases (viral and fungal diseases)

The last years were very much dedicated to have a stringent scientific set-up for transplantation infectious diseases. A long standing interest of the group led by Hans Hirsch is the research on the pathogenesis of polyoma viruses, in particular the BK and JC-virus. In a recent study, investigating the dynamics of polyoma type BK virus in renal transplant recipients, a collaborative study with the Division of Kidney Diseases, we demonstrated that high-level BK-virus replication is a major pathogenetic factor that may have implications for genome rearrangements, immune invasion and antiviral resistance. This was followed up by study on the characterization of highly frequent epitope-specific T-cells as well as a detailed analysis on polyoma BK-specific cellular immune response to VP1 and large T-antigen in kidney transplant recipients.
In hematological stem cell patients diagnostic procedures are investigated for fungal and viral diseases such as aspergillosis or RSV infection. E.g. the value of galactomannan for the diagnosis of invasive aspergillosis was investigated. The structured collaboration with the Division of haematology has and will allow a more comprehensive view on infectious diseases encountered by stem cell transplanted patients.

HIV/AIDS – treatment response and immune reconstitution

In the past years HIV research concentrated on aspects of potent antiretroviral therapies dramatically improving prognosis, in particular immune response and specific immune reconstitution. The Swiss HIV-Cohort Study following up more than 14'000 patient so far allows to specifically target patient groups of interest and to reliably compare them to control patients. In one case control study, led by Christoph Hess of the Immunology Group, we demonstrated that irrespective of absolute CD4 T-cell counts, HIV-positive patients, who subsequently developed primary CNS lymphoma lacked EBV-specific CD4 T-cell function. Further studies within this research period investigated optimal therapies regarding functional and absolute increases of CD4 T-cells.

Prosthesis associated infections and osteomyelitis

Hip or knee replacements and osteosynthesis may improve quality of life. Rarely, these procedures are associated with prosthetic-joint infection. Novel methods for the detection of bacteria were analyzed such as microcalorimetry or sonication of the prothesis. The latter method showed more sensitive than conventional periprothetic-tissue culture. The microcalorimetry measures heat from replicating microorganisms and is a promising novel method to detect bacteria.

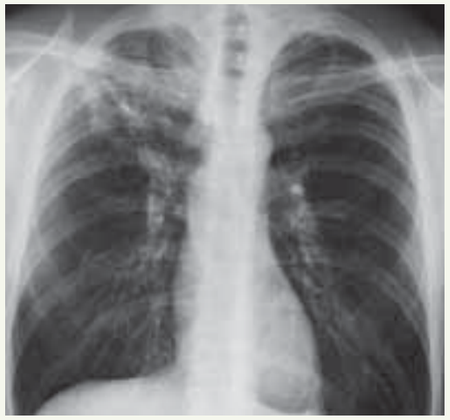

Fig. 1: Patient with pulmonary tuberculosis

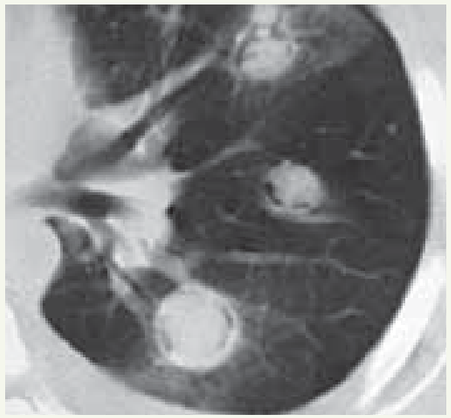

Fig. 2: Multiple pulmonary infiltrates with air crescent sign associated with invasive aspergillosis in a patient with leukemia.

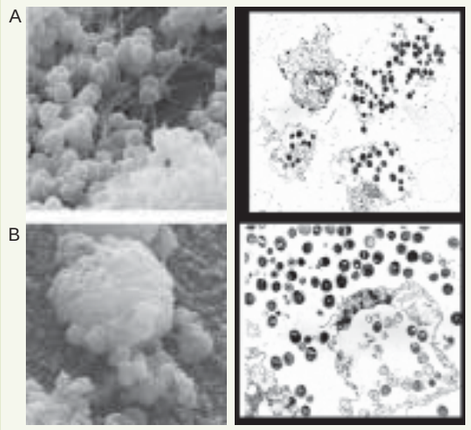

Fig. 3: Adherent staphylococci typical for biofilm formation

HIV minorities
Resistance
Coreceptors
Gleevec resistance
Targeted cancer therapy

Molecular Diagnostics

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Systems to detect HIV minorities, to assess viral tropism for new inhibitor classes and to follow treatment success in cancer therapy

Even now more than 15 years into treatment of the HIV epidemic new central research questions arise and new inhibitors emerge for clinical use. It has been this dynamic field that has turned HIV into an unprecedented model system for many clinically relevant aspects of therapy and disease management. Our group has begun to translate systems into other diseases and our previous work on selected projects in oncology (CML) as well as in virology (HBV, HCV) begins to yield.
Aspects of the unprecedented dynamics and flexibility of HIV are reflected in its wide sequence variation, for which we could demonstrate consequences in the replicative capacity of the virus (Holguin et al.,2006), and which reveals limits of conventional resistance testing by sequencing as under treatment HIV emerges to a growing extent as ever-adapting pathogen with its growing number of variations deposited in the genome of the infected patient. This new understanding precipitates in growing proof that therapy-experienced patients may particularly benefit from the high dissecting power of replicative phenotyping systems, a trend that we were recently able to demonstrate analyzing patients' profiles in our database (Hirsch et al., 2005).
Research questions in a busy last year with fruitful scientific poster discussions at various conferences during 2006 concentrated on the multiple aspects of sensitive detection of HIV minorities and mixtures, on database-driven concepts of viral resistance and new ways to utilize resistance-information for optimizing therapy (to corner the virus), and on next steps in the production of a phenotyping system for kinase targets in oncology.

detection of clinically critical HIV minorities in human plasma

The matched pairs of genotypic and phenotypic informations for each patient, today >30'000 sets in PhenoBase, a database jointly produced by the IMM and InPheno allow to perform statistics of concordances/discordances between genotypic and phenotypic evaluations. We identified the interesting case of 3TC, for which several patients presented a discordance geno-S/pheno-R. As for this drug already single mutation typically confer resistance (M184V), we hypothesized that such discordance could, due to the greater sensitivity of the replicative format of PhenoTecT, hint the presence of genotypically undetected viral variants in the original clinical sample.
We aimed at determining the limit of detection of the phenotypic assay for the single mutant M184V. Defined mixes of wild-type and mutated proviral plasmids were therefore evaluated. Mixes were denoted according to the respective proportion of mutated virus they contained: 0% (containing only pNL4-3 as reference provirus), 0.1%, 0.3%, 1%, 3%, 10%, ..., and 100% (containing only the M184V-mutated provirus). Following four-days of viral replication, reporter gene activity (beta-Gal) was determined and expressed as percent of viral production. The representation shown in Figure 1 represents a resistance factor (RF) as the ratio between the IC50 of the sample and the one of the wild type. The results demonstrate that already at a proportion of 1% of the mutant M184V decreased susceptibility to 3TC can be detected by replicative phenotyping.
To quantitatively estimate the true proportion of M184V mutant in discordant patients' samples we developed a real-time PCR protocol distinguishing between wild-type and 184V-mutant. The introduction of internal destabilizing mismatches in the mutant-specific primer improved discrimination between the wild-type sequence and mutant M184V. As shown in Figure 2 the number of threshold cycles (deltaCt) was equal to 13 cycles between mutant and wild-type. A deltaCt for 0.1% and 0% mutant still more than two cycles demonstrates that the system reliably and quantitatively identifies even very small sub-populations of mutants in a mixed HIV infection.

Our new system to assess HIV coreceptor tropism or on a phenotyping system for molecularly targeting cancer therapeutics are currently being validated.

Connection to Clinical Practice

New tools for HIV therapy, monitoring in treatment of HBV and HCV; molecular diagnostics in oncology

My research group is committed to and has engaged in the development of new molecular-biology-based tools for the accompaniment of new drugs and therapeutic concepts. HIV has been a very instructive model system defining the needs but also the viral potency in escaping therapy. This viral resistance via different escape routes has served as basis for new laboratory diagnostic concepts, has inspired next generation drug discovery, and will be a valuable starting point for our research towards optimizing treatment and addressing resistance mechanisms for hepatitis viruses B and C, for which selective drugs are in pharmaceutical development or have recently reached the clinics.
In the context of cancers that can be selectively targeted with new drugs such as Gleevec and successors a precise molecular understanding of a relationship between mutations and escape-route is still incomplete. Research of my group intends to contribute to providing new, predictive diagnostic means for clinical therapy optimization and for future establishment of genotype-based routine diagnostics.

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Vascular Endothelium
Cytotoxic T Lymphocytes
Arteriosclerosis
Graft vs Host Disease

Molecular Nephrology

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Antigen presentation by human vascular endothelial cells

Vascular endothelial cells (EC) are an exposed target tissue for immune-mediated injury. However, acute and widespread endothelial cell death in the course of cytotoxic T lymphocyte (CTL)-mediated immune responses is an uncommon event, even after stem cell or solid organ transplantation. We compared EC with epithelial and leukocyte derived cell lines as target cells in cytotoxicity assays using peptide-specific CTL clones as effector cells. B lymphoplastoid cells (BLC) and epithelial cells were susceptible but EC were resistant against CTL-mediated cell death. EC presented more than ten-fold lower levels of immunodominant minor histocompatibility peptides than BLC from the same donor. This suggested that EC present a different repertoire of MHC class I-ligands and by this mechanism might escape a CTL-mediated immune attack. Therefore we compared the HLA-A*02 restricted peptide repertoire of human EC with syngeneic BLC. We found that EC present MHC ligands that are unique for the quantitative predominance of certain uncommon peptide species found at low levels on other epithelial cell lines, but never on BLC. The abundance of these MHC class I ligands on EC was only partially explained by the preferential expression of the precursor proteins in these cells. In healthy, human HLA-A*02-positive blood donors, CTL specific for certain ubiquitously expressed HLA-A*02 presented self-peptides were reproducibly induced by peptide-pulsed dendritic cells, but CTL specific for EC-selective HLA-A*02 ligands did never emerge. This suggests that abundant MHC class I restricted peptides that are selectively expressed on EC induce effective tolerance and may further contribute to the relative immune privilege of these cells. These cell-type selective differences in the spectrum of MHC class I ligands influence the outcome of CTL-EC interactions in autoimmune and alloimmune diseases and determines tissue injury in the course of antimicrobial immune responses.

Connection to Clinical Practice

Panarterial and focal signs of symptomatic arteriosclerosis

Arteriosclerosis is a common disease among elderly people. We have developed the arterial tissue microarrays to investigate the structural, cellular and molecular composition of the vascular wall in the course of the disease. Symptomatic, active disease is defined as arteriosclerosis that is characterized by cardiovascular events, e.g. myocardial infarction or stroke. Patients who suffered from these events are called vulnerable patients. When different vascular beds from the carotid, the coronary, the renal and the iliac artery were analyzed in vulnerable patients and in individuals free of cardiovascular events, we identified both panarterial but also focal morphological signs of arteriosclerosis. For example, the hyperplasia of vasa vasorum or the subendothelial deposition of apolipoproteins in the intima are panarterial, early signs of symptomatic, active disease. Currently, we are further characterizing the inflammatory infiltrate in atheroclerotic lesions of vulnerable patients to identify novel targets for molecular diagnosis or therapy. For example, we found activated, cytotoxic T lymphocytes in advanced atherosclerotic lesions of individuals with symptomatic arteriosclerosis. This T cell subset is absent from the normal arterial intima.

The UW-Madsion Rapacz Familial Hypercholesterolemia Swine Model is a large animal model for arteriosclerosis. We extended our tissue microarray analysis of atherosclerosis to this animal model in order to determine similarities and differences between human and pig arteriosclerosis. This knowledge is important to design preclinical tests to validate innovative diagnostic or therapeutic procedures.

We developed a method for clinical disease phenotyping that is based on the rules of differential display. In a non-selected group of patients, this tool represents a valid surrogate marker for the assessment and classification of arteriosclerosis in man. Its predictive strength needs to be assessed prospectively.

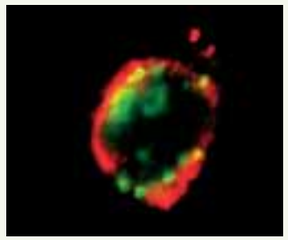

Fig. 1:
Activated cytotoxic T lymphocyte in the arterial intima.
Red: CD3. Green: TIA-1

Fig. 2:
Advanced coronary lesion in a 4 year old Rapacz pig.

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Thymus
Development
T cell
Signaling
Autoimmunity
Transplantation

Pediatric Immunology

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Development and function of the thymic microenvironment: Cellular and molecular studies in health and disease

The thymus achieves two interrelated functions essential for the adaptive immune system; the life-long generation of new T cells and the production of a repertoire of T cells tolerant to harmless self-antigens but reactive to injurious foreign antigens. This formation of mature T cells is the result of intricate interactions between maturing lymphoid cells and stromal cells. The latter consist particularly of thymic epithelial cells (TECs) which provide factors critical for survival, expansion, differentiation and selection of T cells. Derived from the endodermal lining of the 3rd pharyngeal pouch, epithelial cells committed to a thymic cell fate separate from the pharynx and, together with mesenchyme, are organised to form the thymus anlage. Concurrent with the homing of lymphoid precursors to this primordium, TEC proliferation and maturation are stimulated resulting in the formation of distinct cortical and medullary stromal compartments. There is a division of labour among the different TEC subsets. Cortical epithelial cells assist in the initial attraction of blood-borne lymphoid precursor cells, control the maturation of the T cell lineage and positively select thymocytes with an T cell antigen receptor (TcR) of sufficient affinity for a self-MHC/peptide complexes. Subsequently, the selected TcR repertoire is refined by stromal cells including medullary (m) TEC to assure the purging of thymocytes with reactivity to self-antigens. In mTEC, the transcription factor autoimmune regulator (Aire) contributes to the expression of tissue-restricted antigens (TRA) that are typically detected in peripheral organs. The congenital or acquired absence of Aire expression is associated with an altered presentation of TRA, with changes in the composition and organisation of the medullary stroma, and, consequently, with a distorted T cell repertoire selection.

Whereas the cellular origins of the thymic primordium are now well established, the precise molecular nature of the signals responsible for thymic epithelial cell commitment, proliferation, differentiation and homeostatic maintenance are still only incompletely identified. We have therefore investigated in vitro and in vivo (i) the genetic control of the TEC commitment and maintenance, (ii) the signaling pathways involved in TEC differentiation and function, (iii) the phenotypic nature and developmental potential of adult TEC precursor cells, (iv) the control of Aire expression by TEC as well as (v) the cellular and molecular mechanisms following TEC injury in a clinical model of hematopoietic stem cell transplantation.

Using gene targeted mice and the TEC-directed overexpression of gain-of-function mutants, we have been able to demonstrate that canonical Wnt signaling is required at the time of TEC commitment for the formation of a regular thymus anlage whereas constitutive stimulation of this pathway eliminates TEC identity and blocks T cell development. Preventing signaling via the classical pathway triggered by TGF-β family members distorts the regular architecture and composition of TEC resulting in a severely hypocellular thymus. This effect, however, appears to be largely independent of TGF-β as mice deficient for TGFβRII signaling in TEC display a normal thymus that is interestingly largely resistant to age-related involution. Moreover, signaling via bone morphogenetic proteins, fibroblast growth factors and sonic hedgehog is also necessary for regular thymus organogenesis.

We have as well been able to isolate adult TEC that can be maintained in culture or used as tissue replacement in vivo while maintaining their capacity to support thymopoiesis.

This system is presently used to assess TEC function and development in vitro using different physiological stimuli and pathological perturbations. Following hematopoietic stem cell transplantation, thymic epithelial cells are recognized by allogeneic T cells of donor origin and are thus subjected to cytotoxic damage and functional alterations. Based on our knowledge concerning TEC development, we are now investigating different therapeutic strategies to repair/replace damaged thymic stromal tissue.

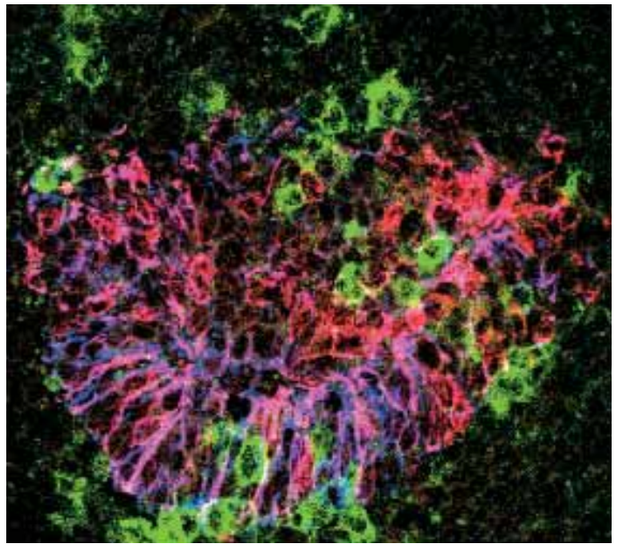

Fig. 1: Thymus anlage in the developing mouse (day 12.5 of gestation) displaying blood borne lymphoid precursor cells (CD45,green) entering the epithelial primordium (cytokeratin 5 red and cytokeratin 18, blue)

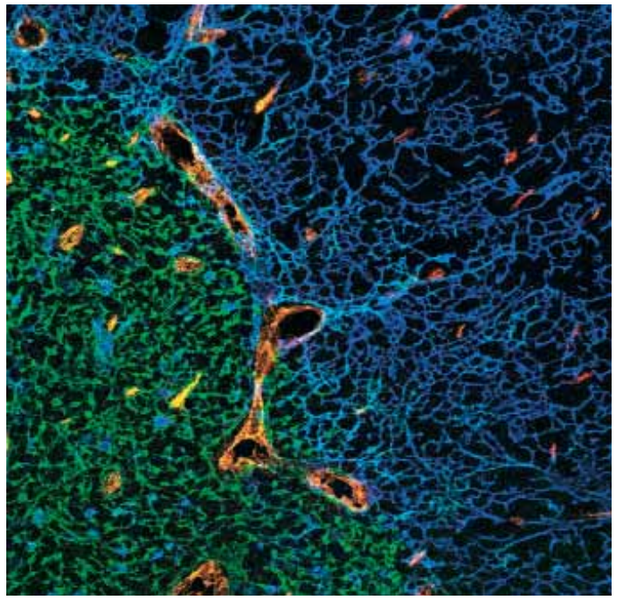

Fig. 2: Architecture and composition of the cortico-medullary junction of the adult thymic microenvironment. Staining for endothelial cells (CD31, red), medullary (cytokeratin 5, green) and cortical epithelial cells (Cytoteratin 18, blue)

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T cell receptor
Tolerance
Transplantation
Signaling
Regulatory T cells
Cross-match

Transplantation Immunology and Nephrology

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T cell receptor signaling: the basis of central tolerance and peripheral regulation

While transplantation has been successfully used to treat many types of organ failure, it is limited in the long term by the immune system's intolerance of the graft. Graft rejection is initiated by T lymphocytes and reflects the immune systems' pre-occupation with eliminating cells, which are "non-self". Controlling graft rejection in the long term depends on understanding how T cells distinguish between "self" and "non-self".

The T cell receptor: a decision maker for the T lymphocyte

T cells develop in the thymus, where thymocytes undergo two forms of selection. Positive selection generates functional T cells, which express low affinity receptors for the host's MHC. Negative selection eliminates thymocytes expressing high affinity TCRs for self-MHC; this is a critical step in avoiding auto-immunity. The molecular bases of positive and negative selection are not understood. The distinction between self and non-self is based on the affinity of a thymocyte's TCR, where thymocytes with low affinity TCRs are positively selected, while those with high affinity TCRs are negatively selected. Where then is threshold between low and high affinity recognition? By studying several different TCRs, we established that the threshold affinity demarcating positive and negative selection. This receptor affinity is defined by a $K_D = 6\ \mu M$ and a $T_{½} \sim 2$ sec. This universal affinity constant also defines the minimal receptor affinity to activate a peripheral T cell. Therefore, the affinity threshold used during T cell development is maintained in peripheral CTLs. We are presently determining the affinity threshold for eliminating autoimmune $CD4^+$ helper T cells to determine whether this lineage is selected by different or similar affinity parameters. We are also investigating the receptor affinity requirements for selecting regulatory T cells.

We studied the interactions between CD8 and the TCR by generating versions of these molecules tagged with fluorescent proteins (CD8-YFP and TCR ζ-CFP) capable of displaying fluorescence resonance energy transfer. High affinity ligands induce a rapid formation of FRET, while low affinity ligands under the selection threshold induce FRET signals much more slowly. Receptor/co-receptor interactions are severely compromised in cells expressing a TCR, lacking a conserved motif in the TCR α chain (α-CPM). We proposed a zipper model to describe how the pMHC ligand brings the TCR and co-receptor together. TCRs without the α-CPM lack some teeth in the zipper and cannot efficiently carry out the kinase reactions to initiate a TCR signal. Current work examines the organization and activation of ZAP-70 in the immune synapse by ligands of varying affinity.

We also studied how the MAPK pathways are activated in thymocytes during positive and negative selection. Negative selecting ligands activate Ras and ERK at the plasma membrane, while positive selecting ligands activate these signaling pathways within the Golgi compartment. By retaining activated ERK at the plasma membrane, negative selectors prevent pERK from entering the nucleus to deliver a survival signal. Ras activation in the Golgi observed with positive selectors allows pERK to enter the nucleus and rescue the thymocyte from cell death. The ability to differentially compartmentalize Ras activation is a key part of the mechanism underlying T cell tolerance. We are currently trying to elucidate how the MAPK pathways are activated in mature T cells.

We have also examined the signals required to generate memory CTLs. A mutation in the transmembrane domain of the TCR β chain generates a TCR, which fails to develop memory T cells. The mutant receptor is not efficiently recruited to the immunological synapse and cannot efficiently.

Finally, we have also developed a model of graft rejection by transplanting MHC-disparate skin onto mice, which are then challenged with T cells expressing a graft specific TCR. Rejection in this model can be completely suppressed with regulatory T cells (Tregs). We are trying to understand how Tregs control graft rejection and whether these cells could be used in a clinical setting.

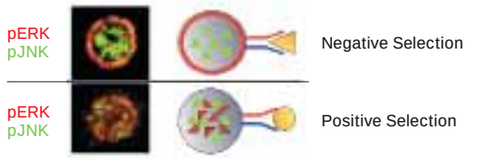

Fig. 1: MAPKs are differentially compartmentalized in thymocytes undergoing positive or negative selection. During negative selection, engagement of the T cell receptor with a high affinity ligand signals Ras activation and pERK retention at the plasma membrane; this eliminates an autoimmune T cell specificity via apoptosis. During positive selection both pJNK and pERK are found in the nucleus, leading to thymocyte survival and maturation.

Fig. 2: Stimulation of a T cell with an antigen loaded presenting cell recruits the TCR (ζ-CFP) and the CD8 co-receptor (CD8β-YFP) into the immunological synapse. The molecular interaction between these two molecules was followed using fluorescence resonance energy transfer (FRET) between the CFP and YFP tags.

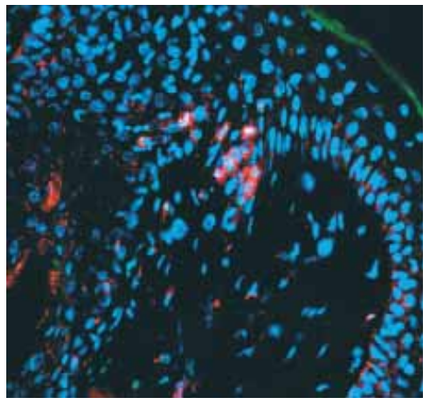

Fig. 3: Histological analysis of regulatory T cells (stained in red and white) in a tolerated skin allograft.

Connection to Clinical Practice

The clinical work has focused on minimizing the transplant rejection and identifying markers of rejection following transplantation.
A study was carried out to determine the pre-transplantation risk of a recipient developing a donor specific antibody mediated rejection (AMR) following renal transplantation. Recipient sera were analyzed for the presence of donor specific antibodies (DSA) using flow beads is called virtual cross-matching. This is a method where HLA antigens, bound to beads are incubated with patient sera and the resulting anti-HLA antibodies are identified by flow cytometry. Only 2 of 56 patients without DSA developed antibody mediated rejection. The 9 patients displaying DSA pre-transplantation, were treated with induction therapy including anti-T lymphocyte globulin and IV immunoglobulins. Despite this additional therapy, 4 patients had clinical/subclinical AMR. The study shows that virtual cross-matching can accurately define absence or presence of DSA and may become an invaluable tool for organ allocation and pre-transplant risk assessment.
Another study correlated the presence of protein markers in the urine of transplant recipients with the presence of tubulointerstitial injury. None of the investigated biomarkers allow a clear differentiation between stable transplants with normal tubular histology and stable transplants with subclinical tubulitis. Protocol allograft biopsy currently remains the preferred tool to screen for subclinical tubulitis. A final study examined the importance of finding C4d in the urine. Urinary C4d did not correlate with C4d staining in the peritubular capillaries, but rather reflected nonspecific glomerular injury.

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Polyomavirus
Cytomegalovirus
Viral load
Cellular immunity
Replication capacity
Viral dynamics

Transplantation Virology

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Transplantation Virology: Clinical, virological, and immunological studies

Virus infection, replication, and disease describe different entities in a host, which do not necessarily overlap. This is particular obvious for viruses with a propensity for silent, non-replicative states called latency such as herpes- or polyomaviruses. Viral diseases become more likely when the immune system is impaired. After solid organ transplantation (SOT), immune functions are deliberately suppressed by drugs to protect the transplant from 'host-versus-graft' injury (rejection) opening a window of opportunity for viral replication and severe disease. After hematopoietic stem cell transplant (HSCT), patients are vulnerable to viral infections following conditioning for prolonged times after engraftment. Transplantation virology investigates virological, immunological, and clinical factors influencing the altered virus-host balance and its outcome. Thus, we investigate polyomavirus BK (BKV) and JC (JCV) as well as cytomegalovirus (CMV) and respiratory syncytial virus (RSV) in SOT and HSCT patients.
BKV has become a paradigm for viral complications posttransplant. Polyomavirus BK-associated nephropathy (PVAN) is an emerging disease affecting 1-10% of kidney transplant (KT) recipients with graft loss in roughly 50%. Intervention is difficult due to the lack of specific antivirals and relies on improving immune control by decreasing immunosuppression. Immunosuppression is a prerequisite for BK viremia and PVAN in KT patients. This pathogenesis differs from the BKV-associated hemorrhagic cystitis in HSCT patients.

Clinical studies
In an international study comprising 682 de novo kidney transplant recipients in 15 centers on 3 continents, the calcineurin inhibitors cyclosporine and tacrolimus were directly compared in a randomized double-blinded study of patients receiving a backbone of mycophenolate and prednisone. We found that BKV viremia is significantly more frequent and at higher level in patients receiving tacrolimus compared to cyclosporine.

Virological studies
To investigate the role of viral determinants, we examined BKV noncoding control regions (NCCR), which coordinate viral gene expression and replication of the viral DNA genome. We found that rearranged (rr-)NCCRs were more frequent in plasma than in urine (22% versus 4%) yielding discordant NCCR in the two compartments. rr-NCCR BKV variants were associated with 20-fold higher plasma BKV loads with histological PVAN diagnosis and more advanced disease (PVAN pattern B). Cloning of rr-NCCRs revealed diverse duplications or deletions in different NCCR subregions, but all conferred increased early gene expression (Fig. 1), increased replication capacity, and more pronounced cytopathology of recombinant BKV in vitro. Thus, emergence of rr-NCCR confers increased BKV replication capacity and accelerated disease in kidney transplants.

Mathematical modeling
Fast BKV replication dynamics in renal tubular epithelial cells drive PVAN to premature KT failure. Onset and resolution of PVAN closely correlates with plasma BKV loads. BKV also replicates in urothelial cells, but remains asymptomatic in more than 2/3 of KT patients. Urine BKV loads were ~3000-fold higher than plasma viral loads with >90% resulting from urothelial replication. Minimal estimates of BKV half-life in urine indicated rapid dynamics (~12h) similar to rates in plasma. Viral expansion was best explained by mathematical models where BKV replication started in the kidney, followed by urothelial – tubular epithelial cell cross-feeding reaching a dynamic equilibrium after ~70 days. Curtailing intrarenal replication by 50% decreased, but did not clear viremia, without affecting viruria. Reduction by 80% was required for clearing of viremia within 50 days, but viruria persisted for >100 days, while >90% reduction cleared viremia and viruria by 3 and 10 weeks (Fig. 2), respectively. Our 2-compartment models emphasize plasma BKV load for primary monitoring and define goals for optimal therapeutic interventions.

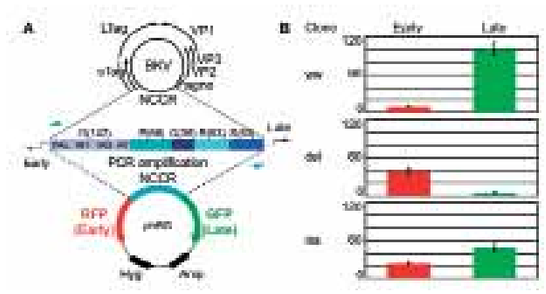

Fig. 1: BKV variants with rearranged noncoding control region increase viral early gene expression, replicative capacity and cytopathology in vitro and in vivo compared to archetype NCCR.

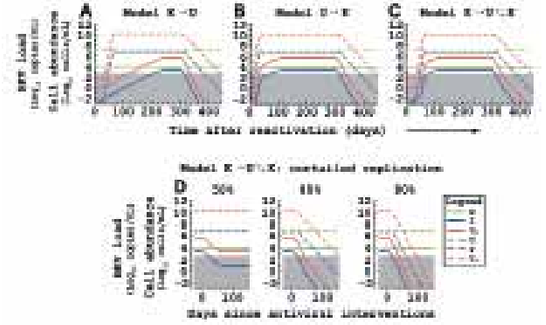

Fig. 2: Mathematical modeling indicates two independent BKV replication compartments in urothelial and tubular epithelial cells of KT patients, which are partially linked (K⟶U⟷K). Replication starts in the kidney, reaches the urinary tract followed by bidirectional viral flux cross-feeding into both replication compartments.

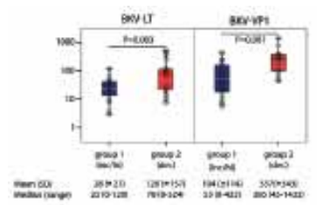

Fig. 3: Frequency of BKV-specific IFN-γ T-cells in PBMCs by ELISPOT analysis. BKV-LT: Large T-antigen, BKV-VP1: Viral capsid protein 1.

Connection to Clinical Practice

A failing balance between BKV replication and BKV-specific cellular immune functions has been suspected as the common denominator of PVAN pathogenesis. Likely, this balance can be perturbed at different points of the patient, the graft, and the virus interaction. In immunological studies using ELISPOT and flow cytometry with intracellular cytokine staining, directly or after in vitro expansion of patient T-cells, we compared the frequency of interferon-γ (IFN-γ) secreting peripheral blood mononuclear cells (PBMC) after stimulation with overlapping peptide pools covering BKV large T-antigen (LT) and VP1 capsid protein (VP1). In KT patients with current or recent plasma BKV loads, median LT and VP1 responses were 25 (range 7-113) and 114 (range 0-1432) spot forming units (SFU), respectively. In patients with decreasing or past plasma BKV loads, significantly higher median BKV-specific IFN-γ responses were detected compared to patients with increasing or persisting BKV loads (Fig. 3). Overall, VP1-specific IFN-γ responses were higher and more likely to involve CD4+T-cells, while CD8+T-cells were more frequently directed against LT. Thus, control of BKV replication coincides with T-cell expansion differentially directed against BKV antigens.

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Calcium homeostasis
Neuromuscular disorders
Ryanodine receptor
Calcium channel
Excitation-contraction coupling

Perioperative Patient Safety

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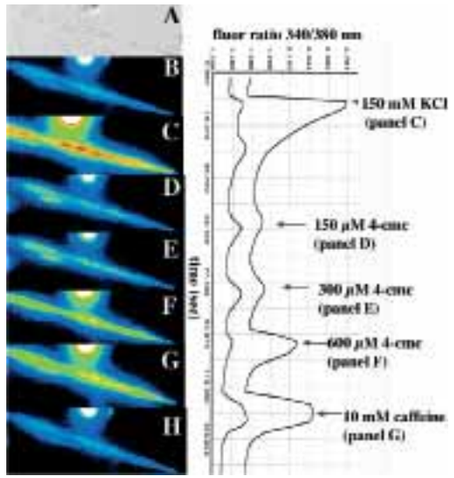
Calcium homeostasis under normal and pathological conditions

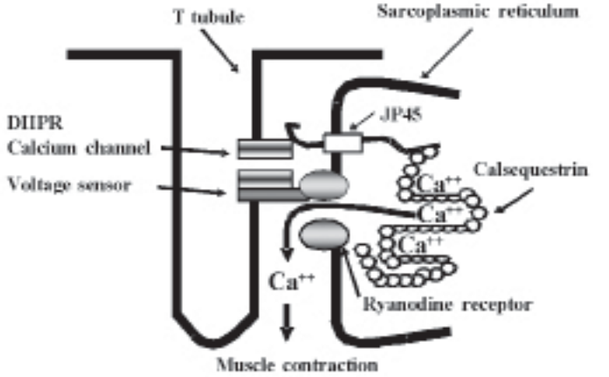
Changes in the intracellular free calcium concentration underlie a variety of biological phenomenon, such as neuronal excitability, muscle contraction, gene expression and metabolism. Under resting conditions, eukaryotic cells maintain the cytoplasmic calcium concentration ([Ca^{2+}]) at very low levels (about 100 nM), but upon stimulation its concentration raises dramatically (more than 10000-fold) in just a few milliseconds; these changes are sensed by specialized proteins, resulting in a cellular response. Because of the importance of Ca^{2+} in cell physiology, eukaryotic cells have developed specialized organelles (or subregions of organelles) to finely control the cellular [Ca^{2+}] and many proteins (from channels on the plasma membrane which allow Ca^{2+} ions to flux into the cytoplasm from the extracellular milieu, to intracellular calcium storing proteins, intracellular calcium channels and Ca^{2+} pumps or CaATPases), are devoted to calcium homeostasis. The most specialized organelle involved in Ca^{2+} regulation is the skeletal muscle sarcoplasmic reticulum; this organelle has a finely structured architecture. In fact the protein(s) sensing the action potential generated by the nerve impulse on one subspecialized membrane (the transverse tubular membrane), can physically interact with the calcium release channel (also known as the ryanodine receptor, RyR1) present on another specialized membrane, the terminal cisternae. The importance of the fine regulation of [Ca^{2+}] is illustrated by several groups of neuromuscular diseases, which are linked to mutations in the ryanodine receptor calcium channel, namely malignant hyperthermia (MH), central core disease (CCD) and multi-minicore disease MmD. To date more than 100 missense mutations in the RYR1 gene have been identified in patients and associated and linked to the CCD, MmD and/or MHS phenotype.

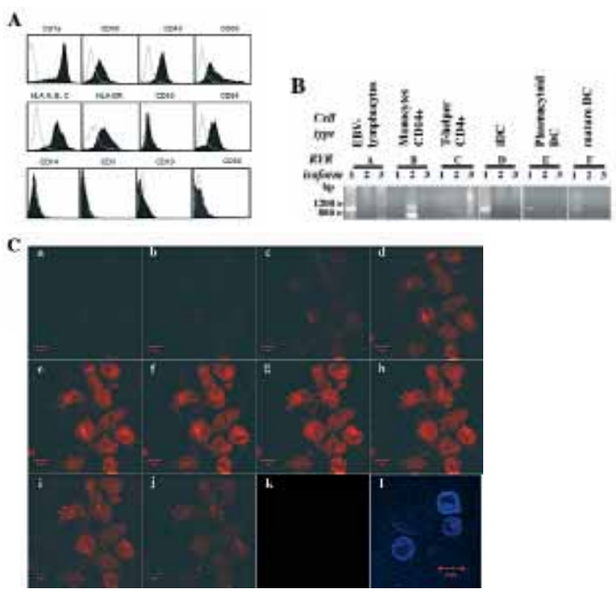
Our research focuses on several aspects of intracellular calcium homeostasis and how its dysregulation may bring about pathological phenotypes. In fact, understanding the basis of calcium dysregulation is of fundamental importance if one is to develop a pharmacological strategy aimed at improving the quality of life of patients suffering from such diseases such as malignant hyperthermia (MH), central core disease (CCD) and multi-minicore disease (MmD). In particular we study the functional effects of RYR1 mutations on intracellular calcium homeostasis in human myotubes carrying the endogenous mutation; in addition we are testing the effect in vitro of pharmacological tools to see whether they could be of potential use in patients by reverting the clinical phenotype.

Other aspects of our research focus on the proteomics of the organelle responsible for calcium homeostasis in muscle and non-muscle cells. This aspect of our research is also important in light of the fact that mutations in yet to be identified proteins of the sarcoplasmic reticulum, may potentially lead to neuromuscular disorders. In this context we have identified several novel proteins at the molecular level (junctate, JP-45, SRP-27) and are currently establishing their functional role, either by exploiting cellular systems (overexpression, knock down) or animal models in which the gene of interest has been ablated (JP-45).

Finally another line of research which has emerged during the past years is the role of the RyR1 in cells of the immune system, specifically in B-lymphocytes and dendritic cells, two types of immune cells which are involved in antigen presentation and cytokine production. The latter point is important because it may indicate a link between muscle function and some aspects of immune response







Connection to Clinical Practice

PD Dr. Thierry Girard
Department of Biomedicine
Division of Anesthesia
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Pharmacogenetics in Anaesthesia

Our research focuses on identifying mutations which are responsible for altered responses to commonly used anesthetics or muscle relaxants; in particular we are currently investigating how mutations in the ryanodine receptor gene (RYR1) cause Malignant Hyperthermia (MH) and the relationship between Butyrylcholinesterase isoforms and prolonged neuromuscular block. MH is a rare potentially fatal hypermetabolic pharmacogenetic disease which develops in genetically predisposed individuals when they come into contact with volatile anaesthetics and/or succinylcholine. This hypermetabolic reaction is caused by an alteration of calcium homeostasis, leading to muscle breakdown, elevated oxygen consumption, severe metabolic acidosis, hyperthermia and electrolyte disturbances, eventually leading to death. The past two decades of research have identified the skeletal muscle RYR1 as the main locus of MH: one of the aims of our research is to identify novel RYR1 mutations in order for them to be used for the molecular diagnosis of MH susceptibility. MH shows a considerable heterogeneity and only about 70% of MH families are known to carry RYR1 mutations. Our ongoing research aims to identify other proteins involved in excitation-contraction coupling which upon mutation may lead to MH susceptibility. Butyrylcholinesterase (BCHE, pseudocholinesterase) is responsible for the short duration of action of the neuromuscular blocking drug succinylcholine. Several BCHE variants have been identified in patients with prolonged neuromuscular block. Another of our research projects focuses on the effect of different BCHE variants on the clinical duration of action of succinylcholine

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Feral Pigeon *Columba livia*

Evolution

Behavioral Ecology

Zoonoses

Transmission of *Chlamydophila psittaci*

Integrative Biology

Prof. Dr. Daniel Haag-Wackernagel
Department of Biomedicine
Institute of Anatomy
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Group Members
Ila Geigenfeind (PhD student)
Adrian Schlageter (PhD student)
Andreas Ochsenbein (technician)

Biology and public health implications of the Feral Pigeon *Columba livia*

The feral pigeon is an interesting model for studying evolutionary processes in urban ecosystems. In the Vienna population we proved 23 hereditary factors that influence plumage colour and pattern (Fig. 1). In our study, we suggest the existence of colour based selection processes on juvenile feral pigeons by comparing the differences in colour morph frequencies between juvenile and adult feral pigeons. We could demonstrate that melanic (dark-coloured) forms have a significantly better survival rate in the urban habitat than the comparison group of wild type birds. One colouration called Checkers, clearly seems to be handicapped. There is evidence that melanic feral pigeons are more resistant against diseases like for example ornithosis. Additionally, melanic pigeons seem to have a better resistance against physical influences.

Many features of how feral pigeons use the urban habitat remain unknown or controversial. In our GPS-project we studied the spatio-temporal use of the urban habitat by feral pigeons in Basel. We equipped feral pigeons with GPS-receivers that calculated and stored the position of the individual birds. The results showed that pigeons in Basel cover distances up to 5.3 km and individual home ranges cover up to 150 ha (Fig. 2). We additionally could prove that our feral pigeons show very individual feeding strategies and the composition of feeding flocks varies at different times and days. Home ranges of the lofts overlap partially and the use of the city varies according to season, breeding status, sex, and affiliation to a loft. The total ranges of the lofts showed an existing overlap between the various pigeon populations. This overlap explains the occurrence of epidemics in feral pigeon populations. Diseases can be transmitted at important feeding sites that are meeting points for pigeons from different parts of the city. Detailed information on the transmission routes are of human concern, since feral pigeons are a reservoir of human pathogenic diseases and parasites.

Feral pigeons can harbour at least 110 microorganisms pathogenic for humans. Seven of these have evidentially been transmitted to humans (Salmonella, Chlamydophila psittaci, Aspergillus, Candida, Cryptococcus neoformans, Histoplasma capsulatum and Toxiplasma gondii). Whirled up dust particles can cause allergic reactions to pigeon antigens in humans (Pigeon Breeder's Lung). The most important ectoparasites of feral pigeons are the Red Blood Mite, Dermanyssus gallinae, the Pigeon Tick, Argas reflexus and the Pigeon Flea Ceratophyllus gallinae that can migrate into human living space when they lose their natural hosts. We described a case where a single pigeon nest was the source of a flea infestation in a couple that resulted in severe psychological distress.

In our project "Transmission of Chlamydophila psittaci from feral pigeons to humans in the urban environment" we study the possible transmission routes of Cp. psittaci from feral pigeons and their excreta to humans in the city of Basel. Cp. psittaci is the most significant zoonotic agent that can be acquired from feral pigeons. Until now 101 cases of transmission from feral pigeons to humans have been published. 43 of these infections (42.5%) have been attributed to loose or transient contacts to feral pigeons. We analyze faecal samples, water surface film samples from public fountains and air samples from public areas collected by bioaerosol-sampling. For analysis we use well-established PCR methods (ompA Nested-PCR and 16S-PCR). Additionally, we want to investigate the seasonal chlamydial shedding in individual birds. In our feral pigeon lofts of the "Pigeon Action of Basel" we have the unique possibility to investigate up to nine feral pigeon flocks in different areas of the city of Basel to perform a monitoring of Cp. psittaci under wildlife conditions. Thus we want to learn more about the epidemiology of this widespread zoonotic disease.

Fig. 1: Feral pigeons show a high genetic variation. Our study suggests the existence of colour-based selection. Dark coloured forms seem to have a better survival rate in the urban habitat. These birds seem to have better resistance against diseases and physical influences.

Fig. 2: Feral pigeons in Basel cover distances of up to 5.3 km and they own partially overlapping home ranges of up to 150ha. This interconnectedness of the flocks has an important impact on the transmission routes of diseases and parasites.

Fig. 3: Pigeon droppings can be a source of more than 110 microorganisms pathogenic for humans.
In our project we investigate the transmission routes of Chlamydophila psittaci, the pathogenic agent of ornithosis, in the urban environment.

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Mesenchymal stem cell
Autoimmune diseases
The Immunomodulatory Properties
of Mesenchymal Stem Cells

Rheumatology

Research Group associated with the DBM

Prof. Dr. Alan Tyndall
University Hospital Basel
and Felix Platter Hospital Basel

Senior Scientist
Dr. Chiara Bocelli Tyndall (PhD)

Adult Stem Cells in Autoimmune Diseases

The mechanism of immunomodulatory effect of bone marrow derived mesenchymal stem cells (BM-MSCs) or other sources (eg adipose tissue) is not clear, though both cell contact and soluble factors including indoleamine 2,3 dioxygenase, TGFβ1, IL-10, PG E2 and IL-1 receptor antagonists play a role. MSCs appear to home to distressed/damaged tissue, a potentially important therapeutic property after IVI infusion. Acute toxicity appears minimal, but questions remain concerning long term effects on tumor surveillance. MSCs may be expanded ex vivo and reinfused intravenously or locally delivered intraarterially or intramuscularly. Most human experience is with allogeneic MSC IVI. Some patients with severe inflammatory autoimmune disease do not respond to conventional therapy and risk losing vital organs or life. Examples include systemic vasculitis, SLE, systemic sclerosis and Crohn's disease. As with severe aGVHD, it is proposed that MSC infusion as adjunct therapy could reverse such a situation allowing part or full resolution. It was not known if MSCs obtained from bone marrow aspirates of patients suffering from autoimmune disease would retain their immunomodulatory properties, nor if autologous MSCs would be as potently immunosuppressive as it has been shown for allogeneic MSCs. Recently, presumed immunoprivileged state of allogeneic MSCs has been challenged, in particular in a non immunoablated host animal. Although this suggests that autologous MSCs could be a better alternative, several groups have shown impaired proliferation, differentiation and survival in bone marrow derived MSC from rheumatoid arthritis (RA) and systemic sclerosis patients. Another emerging issue concerning MSC in vitro expansion for clinical use concerns both BSE and allergy relating to the use of bovine serum which is being assessed by many groups incl. our own. We have shown for the first time that autologous healthy bone marrow derived MSC (BM-MSC) suppressed lymphocyte proliferation in vitro equally well in an autologous setting as already known in allogeneic experiments. Autologous MSCs derived from the bone marrow of AD patients retained this property. A range of AD was tested including RA, Sjoegren's syndrome, SLE and systemic sclerosis (Fig. 1). BM-MSCs from 12 patients with systemic sclerosis have shown no impairment in proliferation, differentiation, ability to support as stromal cells in vitro hematopoiesis and antiproliferative potential (in MLR and on CD3/CD28 monoclonal antibody stimulated lymphocytes, when compared with normals (coll. with Prof. D. Farge, Paris). We have compared the antiproliferative/immunosuppressive potential of BM-MSCs with that of other differentiated cells of mesodermal origin such as mature chondrocytes and skin fibroblasts (coll. with Prof. I. Martin, Basel), and found that these are as immuno suppressive as BM-MSCs (Fig. 2). Fetal bone and skin cells have also been investigated for their immunsuppression on stimulated lymphocytes, showing in this novel variable patterns (coll. with Dr. M.O. Montjovent, Lausanne). Currently we address the issue of MHC class II expression of BM-MSC – normally absent, they may be expressed under certain conditions such as exposure to interferon gamma (IFN-γ). We are investigating the expression of MHC class II in the presence of growth factors commonly used for cell expansion in vitro but also active in vivo (TGFβ1, FGF-2, PDGF BB). Recent work from others suggests that MSCs can acquire antigen presenting function, an important issue to further define regarding human MSC transplants for AD. We are exploring the observation that BM-MSCs under some conditions increase rather than suppress the proliferation of stimulated lymphocytes, with the homeostatic cytokines IL2, IL7 and IL15 (coll. with Prof. G. Spagnoli, Basel). We are also exploring the functional properties of MSCs expanded in animal protein free media such as human platelet lysate, since this is becoming a GMP regulatory issue in the EC and may become obligatory. The eventual aim is the move forward to phase I clinical trials with MSCs in patients with severe active inflammatory AD.

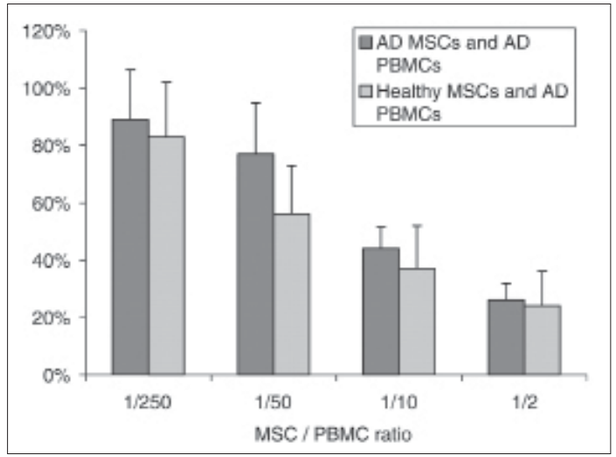

Fig. 1: Residual proliferation of PBMCs from AD patients in presence of autologous BM-MSC or BM-MSCs from healthy donors. Each bar represents the percentage of proliferation of 100,000 PBMC from AD patients in the presence of increasing numbers of BM-MSCs from healthy and AD donors. The cpm values at each cell concentration were normalized to the cpm of PBMCs without BM-MSCs. Each bar represents the average of multiple experiments (each point being in triplicate).

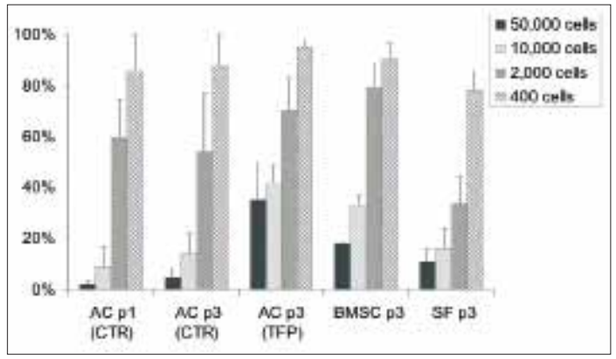

Fig. 2: Residual proliferation of anti-CD3 antibody stimulated peripheral blood mononuclear cells (PBMC) from healthy donors in the presence of increasing numbers of human AC, BMSC, and DF. The proliferation of 100,000 stimulated PBMC was measured by ^{3}H-thymidine uptake. Such proliferation in the presence of increasing numbers of mesenchymal cells (residual proliferation) was normalized to that of the stimulated PBMC alone. Each bar (y axis) representts the percentage of residual proliferation of the PBMC at each concentration (50,000-400) of the co-cultured mesenchymal cells. AC were cultured in medium without (Ctrl) or with the growth factor combination TGF 1/FGF-2/PDGF-BB (TFP). Primary cells werde expanded for one (p1) or three (p3) passages. Asterisks (*) indicate statistically significant differences from both AC (TFP) and BMSC at the equivalent cell dose.

Connection to Clinical Practice

The application of expanded autologous bone marrow derived MSC to treat severe autoimmune diseases

Ex vivo expanded MSC derived from various sources (bone marrow aspirate, adipose tissue, cord blood) are being tested as antiproliferative, immunosuppressive and tissue protecting agents in various human conditions. These include acute graft versus host disease (GvHD), Crohn's disease, acute renal failure and multiple sclerosis. We are currently determining the optimal MSC product characteristics (MSC source, isolation technique and expansion media) with a view to initiating GMP clinical phase I/II studies in patients with organ or life threatening AD not responding to conventional therapy. The apparent low acute toxicity and positive early results in a similar situation (acute GvHD) are encouraging. Our expectations are that a serious inflammatory state may be partially reversed to allow other therapeutic modalities to take effect. This programm is consistent with the Basel stem cell initiative and will be in coll. with Prof. Gratwohl, Basel. It is anticipated that GMP facilities will become available in Basel. The decade long collaboration between Rheumatology and Hematology re hematopoietic stem cell transplantation, pioneered in Basel, sets the scene for direct links between clinical material and suitable potential patients for MSC transplantation. Parts of this program are included in an FP7 translational project application called SCLERAID, based on the EULAR Scleroderma Trials and Research (EUSTAR) group. Prof. Tyndall is secretary and a founding member of EUSTAR. Further definition of patients selection eg vasculitis, SLE, systemic sclerosis will take place at an interdisciplinary meeting in Genoa Oct 26-28, 2007.

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Index

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Banfi Andrea, Dr.	abanfi@uhbs.ch	16, 44
Battegay Edouard, Prof. Dr.	edouard.battegay@usz.ch	84
Battegay Manuel, Prof. Dr.	mbattegay@uhbs.ch	128, 131
Beglinger Christoph, Prof. Dr.	beglinger@tmr.ch	60
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Biedermann Barbara, PD Dr.	barbara.biedermann@unibas.ch	134
Bodmer Daniel, Prof. Dr.	bodmerd@uhbs.ch	66
Brenner Hans Rudolf, Prof. Dr.	hans-rudolf.brenner@unibas.ch	30
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Buser Peter, Prof. Dr.	pbuser@uhbs.ch	43
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De Geyter Christian, Prof. Dr.	cdegeyter@uhbs.ch	62
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Drewe Jürgen, Prof. Dr.	drewej@uhbs.ch	46
Eberle Alex N., Prof. Dr.	alex-n.eberle@unibas.ch	54
Erb Peter, Prof. Dr.	peter.erb@unibas.ch	120
Eriksson Urs, Prof. Dr.	urs.eriksson@usz.ch	116
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Flückiger Ursula, Prof. Dr.	flueckigeru@uhbs.ch	131
Girard Thierry, PD Dr.	thierry.girard@unibas.ch	143
Gratwohl Alois, Prof. Dr.	agratwohl@uhbs.ch	40
Haag-Wackernagel Daniel, Prof. Dr.	daniel.haag@unibas.ch	144
Hahn Sinuhe, Prof. Dr.	shahn@uhbs.ch	78
Hausmann Oliver, PD Dr. med.	o.hausmann@gmx.ch	38
Heberer Michael, Prof. Dr.	mheberer@uhbs.ch	44, 82, 104
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Heinimann Karl, PD Dr.	karl.heinimann@unibas.ch	98
Herrmann Richard, Prof. Dr.	herrmannr@uhbs.ch	86
Hess Christoph, Prof. Dr.	christoph.hess@unibas.ch	122
Hirsch Hans H., Prof. Dr.	hans.hirsch@unibas.ch	140
Holländer Georg, Prof. Dr.	georg-a.hollaender@unibas.ch	10, 108, 136
Holzgreve Wolfgang , Prof. Dr. mult.	wolfgang.holzgreve@unibas.ch	10, 78
Itin Peter, Prof. Dr.	itinp@uhbs.ch	16, 50
Jakob Marcel, PD Dr.	jakobm@uhbs.ch	83
Kapfhammer Josef, Prof. Dr.	josef.kapfhammer@unibas.ch	24
Kappos Ludwig, Prof. Dr.	lkappos@uhbs.ch	22
Keller Ulrich, Prof. Dr.	ulrich.keller@unibas.ch	68
Klimkait Thomas, PD Dr.	thomas.klimkait@unibas.ch	132
Krähenbühl Stephan, Prof. Dr.	kraehenbuehl@uhbs.ch	48
Krapf Reto, Prof. Dr.	reto.krapf@ksbh.ch	134
Kuster Pfister Gabriela, Dr.	kusterg@uhbs.ch	16, 72
Landmann Regine, Prof. Dr.	regine.landmann@unibas.ch	126
Lindberg Raija, Prof. Dr.	raija.lindberg@unibas.ch	22
Martin Ivan, Prof. Dr.	imartin@uhbs.ch	16, 82
Merlo Adrian, Prof. Dr.	amerlo@uhbs.ch	33, 36
Meyer Peter, PD Dr.	peter.meyer@unibas.ch	16, 74
Miserez André, Prof. Dr.	andre-r.miserez@unibas.ch	29
Möller-Dossenbach Caroline, Dr.	caroline.moeller@unibas.ch	10
Moroni Christoph, Prof. Dr.	christoph.moroni@unibas.ch	10, 94
Müller Beat, Prof. Dr.	happy.mueller@unibas.ch	68
Müller Christian, Prof. Dr.	muellerchr@uhbs.ch	117
Müller Hansjakob, Prof. Dr.	hansjakob.mueller@unibas.ch	98
Müller-Gerbl Magdalena, Prof. Dr.	m.mueller-gerbl@unibas.ch	16, 70
Nitsch Cordula, Prof. Dr.	cordula.nitsch@unibas.ch	26
Orend Gertraud, PD Dr.	gertraud.orend@inserm.u-strasbg.fr	96
Otten Uwe, Prof. Dr.	uwe.otten@unibas.ch	28
Palmer Ed, Prof. Dr.	ed.palmer@unibas.ch	138
Pertz Olivier, Prof. Dr.	olivier.pertz@unibas.ch	16, 90
Resink Thérèse, Prof. Dr.	therese-j.resink@unibas.ch	80
Rochlitz Christoph, Prof. Dr.	crochlitz@uhbs.ch	100
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Schär Primo, Prof. Dr.	primo.schaer@unibas.ch	102
Schären-Wiemers Nicole, Prof. Dr.	nicole.schaeren-wiemers@unibas.ch	34
Schifferli Jürg A., Prof. Dr.	j.schifferli@unibas.ch	124
Schwaller Jürg, Prof. Dr.	j.schwaller@unibas.ch	16, 92
Skoda Radek, Prof. Dr.	radek.skoda@unibas.ch	6, 10, 56
Spagnoli Giulio, Prof. Dr.	gspagnoli@uhbs.ch	10, 104
Steck A.J., Prof. Dr.	asteck@uhbs.ch	18, 35
Tamm Michael, Prof. Dr.	mtamm@uhbs.ch	76
Trampuz Andrej, PD Dr.	atrampuz@uhbs.ch	16, 128
Trendelenburg Marten, PD Dr.	marten.trendelenburg@unibas.ch	16, 110
Treves Susan, PD Dr.	susan.treves@unibas.ch	142
Tyndall Alan, Prof. Dr.	alan.tyndall@fps-basel.ch	146
Urwyler Albert, Prof. Dr.	albert.urwyler@unibas.ch	10
Wodnar-Filipowicz Aleksandra, Prof. Dr.	aleksandra.wodnar-filipowicz@unibas.ch	56
Wymann Matthias, Prof. Dr.	matthias.wymann@unibas.ch	88
Zeller Rolf, Prof. Dr.	rolf.zeller@unibas.ch	10, 40, 52
Zuniga Aimée, Dr.	aimee.zuniga@unibas.ch	52