DBM FACTS

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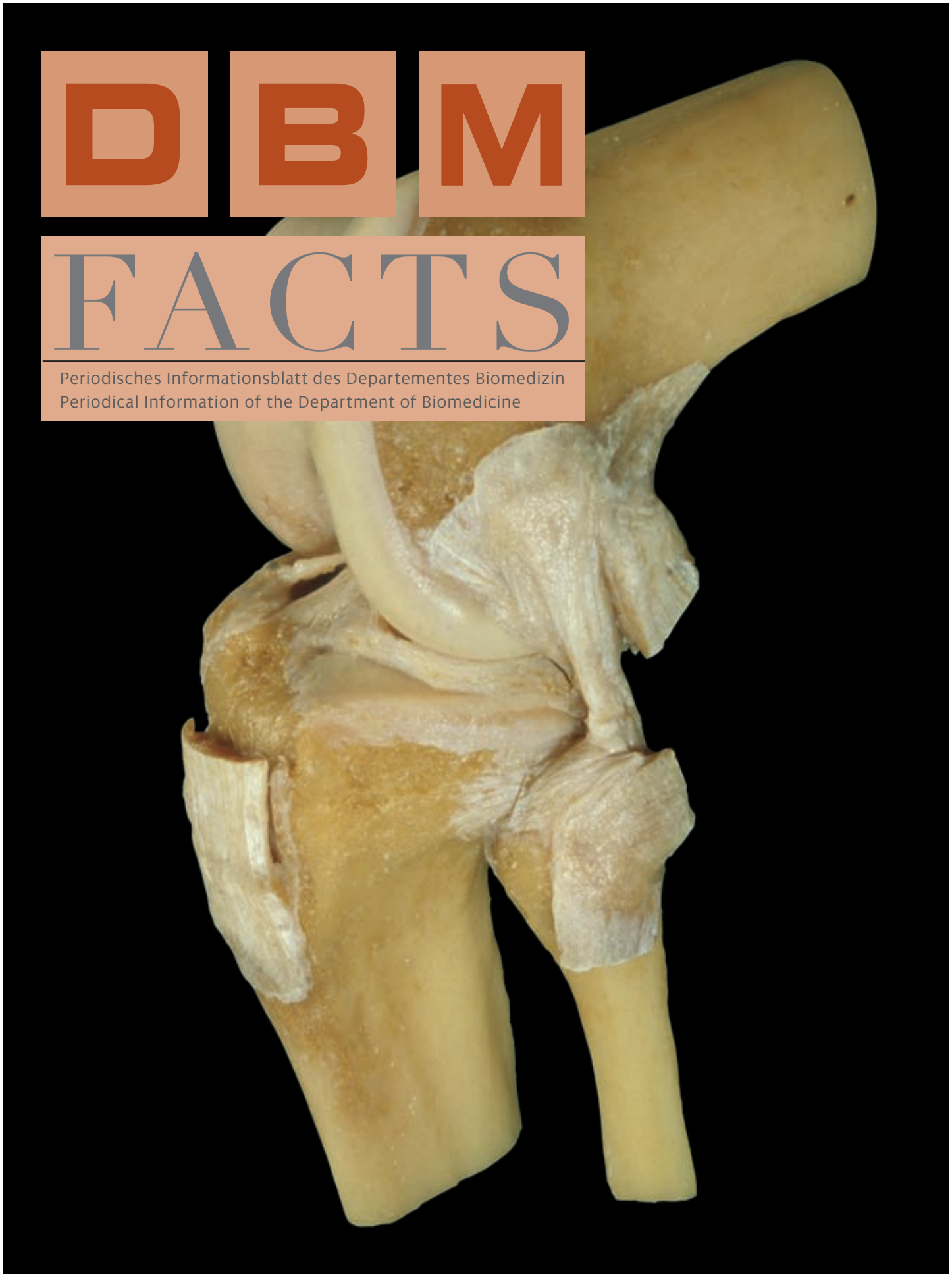

Molecular and cellular mechanisms that guide the development of cells of the immune system | DNA Repair: Of Basic Mechanisms, Resistance to 5-FU, and Unexpected Phenotypes | Midsummer in Sweden

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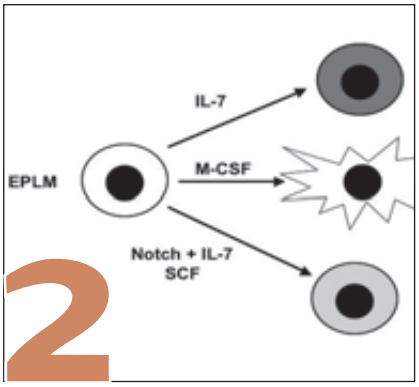
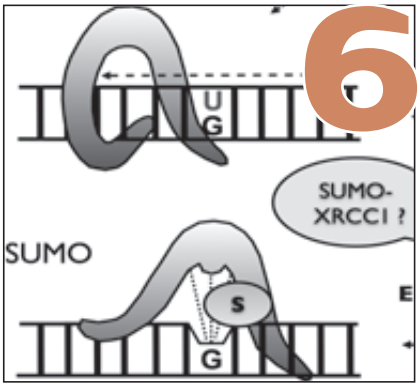
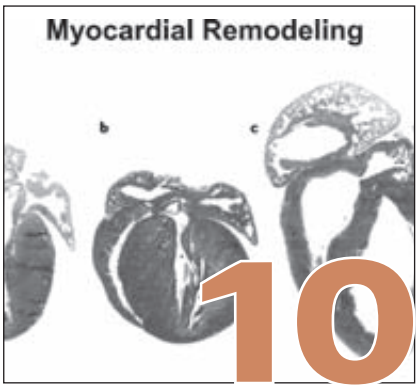


Molecular and cellular mechanisms that guide the development of cells of the immune system
from Antonius Rolink



DNA Repair: Of Basic Mechanisms, Resistance to 5-FU, and Unexpected Phenotypes
from Primo Schär



Oxidative stress and heart failure: The bumpy road from a concept to clinical pratice
from Gabriela Kuster Pfister

Midsummer in Sweden
from Lena Angmann

Chirsizyt mit dr Grossmueter
Rezepte von Verena Jäggin

Zum Titelbild:
EURO-Medienstar Articulatio genus, auf Deutsch: Kniegelenk. Es entscheidet über Sieg oder Niederlage, Ausscheiden oder Pokal – Alexander Frei, Andrea Barzaghi und Servet Çetin können ein Lied davon singen.

IMPRESSUM

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EDITORIAL

Radek Skoda
Leiter DBM

Liebe Leserinnen und Leser

In der Sommerausgabe lässt uns Ton Rolink in seinem Artikel über die molekularen und zellulären Vorgänge, welche die Entwicklung der Zellen des Immunsystems steuern, anschaulich an den Forschungsaktivitäten des Labors «Developmental and Molecular Immunology» teilhaben. Primo Schär von der Forschungsgruppe «Molecular Genetics» stellt sich im zweiten wissenschaftlichen Artikel über Mechanismen von DNA Reparatur, Resistenz gegenüber 5-Fluoruracil und unerwartete Phänotypen vor, und Gabriela Kuster Pfister gibt uns mit ihrem Bericht oxidativen Stress und Herzinsuffizienz einen näheren Einblick in die Forschungsaktivitäten des Labors «Myocardial Research». Die neuesten Publikationen aus dem Departement Biomedizin finden Sie ab Seite 16. Dass die deutsche Mannschaft nicht immer die Gewinnerin sein muss, wenn sie im Fussball auf das Schweizer Team trifft, zeigt eindrücklich das Match der PhD-Studierenden im Joggeli. DBM Facts berichtet ab Seite 30 darüber. Auf eine kulturelle Reise in ihr Heimatland Schweden geht Lena Angman mit uns (siehe Seite 28), während Susanne Blank und ihr Urgrossvater uns Basel ein ganzes Stück näher bringen (Seite 36).

Allen wünsche ich schöne und erholsame Ferientage

Radek Skoda

In the summer edition Ton Rolink gives us insight into the research activities of his laboratory „Developmental and Molecular Immunology" in his article on the molecular and cellular processes that drive the development of the cells of the immune system. Primo Schär, from the research group „Molecular Genetics" introduces himself in the second scientific article on the mechanisms of DNA repair, resistance to 5-Fluoruracil and unexpected phenotypes, and Gabriela Kuster gives us a closer look at the research activities of the „Myocardial Research" laboratory in her article on oxidative stress and heart failure. The latest publications from the Department of Biomedicine can be found from page 16 onward. A match between the PhD students in the Joggeli clearly showed that the German team must not always be the winners at football when they meet the Swiss team. DBM Facts reports on the match on page 30. Lena Angman brings us on a cultural trip through her country of origin, Sweden (see page 28), while Susanne Blank and her great-grandfather bring us a lot closer to Basel (page 36).

I wish you all lovely and restful holidays

Radek Skoda

Molecular and cellular mechanisms that guide the development of cells of the immune system

The research of the "Developmental and Molecular Immunology" group is sponsored by an endowment from F. Hoffmann – La Roche Ltd., Basel to the University of Basel and by grants from the Swiss National Science Foundation and the European community. Antonius Rolink is holder of the chair in Immunology endowed by F. Hoffmann – La Roche Ltd., Basel.

The research of the "Developmental and Molecular Immunology" group is focused on unravelling the mechanisms that control the generation of cells of the immune system in general and the development of T and B cells in particular.

For a very long time, it has been known that hematopoietic stem cells (HSCs), very rare cells in the bone marrow, are responsible for the life-long production of all the cells of the blood and until recently it was thought that their differentiation into the various hematopoietic cells was rather hierarchical. Thus, it was thought that differentiation along a given lineage was associated with progressive loss of potential to give rise to other blood cell lineages. However, the recent developments of very sensitive and quantitative in vitro assays, together with the identification of new progenitor subpopulations have challenged these ideas. Thus, lymphocyte progenitors can be shown to keep their developmental potential to give rise to myeloid, dendritic and NK cells until just prior to their final commitment stage. Here, we will summarize our contributions to these new concepts and will discuss the potential use of these early progenitors in therapy.

The developmental plasticity of Pax-5 deficient pro-B cells

Mice in which expression of the transcription factor Pax-5 had been prevented have an absolute block in B cell development at the early pro-B cell stage. The pro B cells present in the bone marrow of *Pax5*$^{-/-}$ mice express both RAG-1 and RAG-2 required for immunoglobulin (Ig) gene rearrangements, have their Ig heavy chain loci D-J rearranged and express transcripts for the B cell specific genes λ*5, VpreB, Ig*α and *Ig*β. Moreover, like wild-type pro-B cells, *Pax5*$^{-/-}$ pro B cells can be grown *in vitro* on stromal cells in the presence of IL-7 for long periods of time. However, and in marked contrast to wild type cells, *Pax5*$^{-/-}$ progenitor B cells can still develop both *in vitro* and *in vivo* into cells of other hematopoietic lineages, namely myeloid, NK, and T cells (1–3); they thus possess multi-lineage developmental potential. Very recently, Busslinger and colleagues (4) have shown that even mature B cells can regain this multi-lineage developmental potential upon conditional inactivation of the *Pax5*$^{-/-}$ gene. These findings indicate that hematopoietic differentiation has much more plasticity than previously anticipated i.e. that progenitor cells on their way to differentiate into a given lineage keep the potential to give rise to other cell types until a rather advanced stage of differentiation.

Early stages of lymphoid development in mouse bone marrow

As mentioned above, pro B cells found in the bone marrow of *Pax5*$^{-/-}$ mice show a remarkable degree of de-

Front row from left to right:
Claudia Suenderhauf, Marianne Spalinger, Roxane Tussiwand, Angèle Bénard, Antonius Rolink, Reto Ziegler, Giuseppina Capoferri

Back row from left to right:
Nicole Salvisberg, Rhodri Ceredig, Nabil Bosco, Jan Andersson, Johanna Rolink

velopmental plasticity. However, until recently it was unclear whether this type of cell was unique to the *Pax5*$^{-/-}$ mouse or whether they were also present in wild type mice. Our laboratory has analyzed in great detail whether a cell equivalent to the *Pax5*$^{-/-}$ pro B cell is present in wild type bone marrow. *Pax5*$^{-/-}$ pro B cells express B220 and c-kit (CD117) and are negative for CD19 and NK1.1. In fact, expression of CD19 is under direct transcriptional control of *Pax5* and can therefore be used as a surrogate marker for *Pax5* expression (5). In the bone marrow of wild type mice, about 0.2% of the nucleated cells have such a phenotype. Moreover, like *Pax5*$^{-/-}$ pro B cells, these cells express the IL-7Rα (CD127), Flt3 (CD135) and CD93 (6). *In vitro* analysis, using established culture systems to test B, T and myeloid developmental potential revealed that these cells from wild type bone marrow could very efficiently differentiate into all three (myeloid, NK, and T cell) lineages (6). These findings suggested that the multilineage developmental potential of these cells was similar to that of the *Pax5*$^{-/-}$ pro B cells. In fact, their plasticity was even greater in that they could still switch on the *Pax5* gene and very efficiently give rise to B lineage cells. Based on the fact that these cells from wild type mice can differentiate into B, T, NK and myeloid cells, we have called them "early progenitors with lymphoid and myeloid developmental potential", or EPLM.

EPLM in wild type bone marrow comprise about 0.2% of all nucleated cells and expressed CD135, the receptor for Flt3 ligand (Flt3L). Daily treatment of mice with 5–10 μg FLT3L for 7-10 days increased EPLM number fifty fold (7). *In vitro* analysis also revealed that EPLM from Flt3L-treated mice possessed very efficient myeloid and T cell developmental potential. However, their ability to generate B cells was dramatically reduced, thereby accounting for the decreased precursor B cell compartment in the bone marrow of Flt3L-treated mice. Thus, the number of EPLM in the bone marrow seems to be controlled by Flt3L and high levels of this seem to impair their B developmental potential.

EPLM *in vitro* can generate B, T, NK and myeloid cells. *In vivo* transplantation studies, however, showed that low numbers (2–5 x10^3) of EPLM could only give rise to B cells while higher numbers (2 x 10^4) were able to generate T and B cells (6). No myeloid development of EPLM *in vivo* has thus far been observed. Based on these findings, we strongly favour the idea that under physiological conditions, the developmental fate of EPLM is mainly to become B cells. Since, in our hands, CLPs have a very similar developmental potential to EPLMs, these cells should probably also be regarded as very early B cell progenitors (6). In figure 1 the various differentiation pathways of EPLM and the cytokines and signaling molecules involved in their development are summarized.

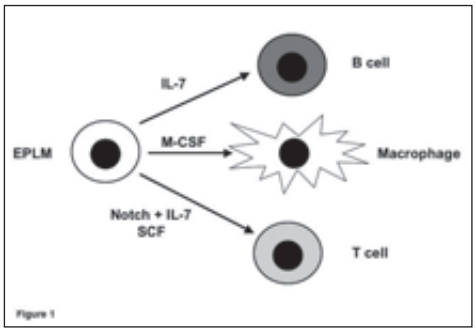

Figure 1. The developmental potential of EPLM's and the growth and differentiation involved in these processes.

Early stages of T cell development in the thymus

Early stages of T cell development in the thymus are characterized by the differential expression of the markers CD44 and CD25 and the absence of CD4, 8 and 3. Because they express neither CD4 nor CD8, these cells are usually called double negative (DN) thymocytes. DN thymocytes can be subdivided into four populations called DN1–4. DN1s are $CD44^{+}CD25^{-}$, DN2s are $CD44^{+}CD25^{+}$, DN3s are $CD44^{-}CD25^{+}$ and DN4s are $CD44^{-}CD25^{-}$. However B, NK, myeloid and dendritic cells are also present in the thymus and most of them would have a DN1-like phenotype. Therefore, the inclusion of c-kit (CD117) as a marker to define *bona fide* DN1 cells is absolutely crucial. Like DN2 cells DN1 express high levels of c-kit while DN3 cells express intermediate levels and DN4 cells are negative (8).

Because the thymus does not harbour HSC, a constant influx of progenitor cells from the bone marrow into the thymus is required in order to maintain continuous T cell production. Over the last couple of years, a whole series of papers have been published dealing with the phenotype and developmental potential of the bone marrow progenitor cell that enters the thymus (9–16). Studies by Radtke and colleagues (14) unambiguously demonstrated that signaling via the Notch-1 receptor was an absolutely crucial event in early T cell commitment and development. Thus, these authors showed that in Notch-1-deficient mice, the earliest thymocyte subpopulation was absent and that the thymus contained precursors with B cell developmental potential. Based on these findings, it was concluded that the thymus was normally seeded by a bone marrow precursor that still possessed B cell developmental potential and which would loose this potential upon Notch signaling. However, several groups, including our own, had been unable to find progenitor cells with B developmental potential in the adult thymus (12,13,17). It should also be noted, however, that other groups have found low frequencies of cells able to give rise to B cells in the thymus (10,11,15,18). Recently Sambandam and colleagues (15) showed that a small fraction of DN1 cells also expresses CD135 (Flt3) and that it is this subpopulation that contained B lineage potential. This subpopulation was called thymus-settling cells (TSP). Moreover, these cells also seem to express the chemokine receptor CCR9, which might be the receptor that guides progenitor cell homing to the thymus (12).

We have recently been able to confirm this finding. In our hands, CD135 is expressed by about 20% of DN1 cells and it has been proposed that CD135-positive DN1 cells are the precursors of the CD135-negative subpopulation. Therefore, in our schemes of thymocyte development (see figure 2) we subdivide DN1 cells into $CD135^{+}$ DN1.1 and $CD135^{-}$ DN1.2 cells. Limiting dilution analysis revealed that 1 in 1000 $CD135^{+}$ DN1.1 cells from adult mice were able to generate B lineage cells (19). Thus the TSP, as

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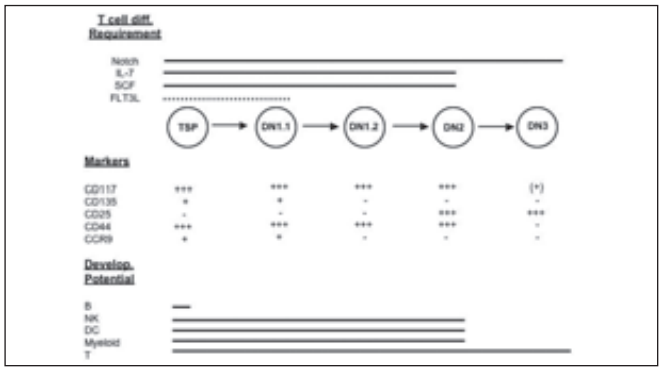

Figure 2. Early stages of T cell development in the thymus. The signals that the various stages require to progress in differentiation, the cell surface markers by which these subpopulations can be distinguished, and the developmental potential they still posses are all indicated.

defined by their potential to give rise to B lineage cells, comprises only a tiny fraction of the CD135$^+$ DN1 cells. In numerical terms, this means that the adult thymus only harbours about 5 cells with B cell developmental potential. In marked contrast, about 1 in 10 CD135$^+$ DN1.1 cells from newborn mice could generate B lineage cells, indicating that the thymus at this age has about 300 of these precursors (19). Thus the thymus might indeed be colonized by rare precursors that have T and B as well as NK and myeloid (see below) developmental potential. Using thymus grafting experiments, Jotereau and colleagues (20,21) had previously shown that the newborn mouse thymus was colonized by a wave of precursor cells. Our finding that these multi lineage cells are much more abundant in the newborn mouse supports this idea.

Recently, we have shown that DN1 and 2 thymocytes require Notch, IL-7 and c-kit signaling for their continued T lineage differentiation (22,23). Moreover, we have provided evidence that expression of c-kit by DN1 and DN2 cells is under direct control of Notch signaling. DN1 and 2 cells are not committed to the T cell lineage since they can still efficiently give rise to NK and myeloid cells (17). For the progression in T cell development, DN3 cells require Notch but not IL-7 and c-kit signaling. Even though growth of DN3 cells is still dependent on continued Notch signaling, this signaling is no longer capable of maintaining their c-kit expression. This probably means that Notch downstream signaling is altered at this important transition from DN2 to DN3 cells, a transition that also involves complete T cell commitment. In figure 2, the phenotype, developmental potential and the requirements for differentiation of the early stages of thymocyte development is summarized.

Concluding remarks

The recent development of very sensitive and quantitative *in vitro* assays, together with the identification of new progenitor subpopulations has unambiguously shown that hematopoietic development is far less hierarchic then previously thought. Thus, lymphocyte progenitors can be shown to keep their developmental potential to give rise to myeloid, dendritic and NK cells until just prior to their final commitment stage. However, the *in vivo* relevance of this plasticity is to a large part still unclear.

Finally the findings that mouse progenitor B and T cells propagated and expanded *in vitro* can upon *in vivo* transfer generate a functional adaptive immune system might offer new therapeutic strategies for patients with defects in their T and/or B cell compartments.

Antonius Rolink
Developmental and Molecular Immunology

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DNA Repair: Of Basic Mechanisms, Resistance to 5-FU, and Unexpected Phenotypes

About Fascination, Motivation and Objectives

We begin our journey through life as a single cell with two genome equivalents from our parents. In terms of DNA, this amounts to about 6 billion nucleotide pairs that would make a linear molecule of 2 meters length. By the time we are grown up, trillions of descendants of this original stem cell constitute the physical and functional makeup of our bodies, and essentially all of them carry a copy of the genome. The DNA of all these cells would now stretch over a length of 300 times the distance between earth and sun – and back! Needless to say that the magnitude of this is breath-taking, and if we consider that this lot of DNA must be synthesized and maintained in an essentially error free manner, we must appreciate that the underlying molecular mechanisms must be of the most sophisticated and fascinating developments of nature.

The faithful transmission of genetic information from mother to daughter cells depends on the accurate du-

Back row from left to right: Stefan Weis, Olivier Fritsch, Christophe Kunz, Yusuke Saito, David Schürmann

Front row from left to right: Patric Urfer, Claudia Krawczyk, Barbara Gruberski, Primo Schär, Daniel Cortazar, Frauke Focke

plication of the genomic DNA. Given the chemical reactivity of the aqueous and aerobic environment of a cell nucleus, however, the maintenance of a structurally and chemically integer DNA template for error-free replication represents a major challenge. It is therefore not surprising that cells dedicate appreciable resources to the surveillance and repair of their DNA. It is their capacity to correct damaged DNA that balances the inherent instability of our genomes, which, when elevated, is associated with premature ageing or the premature onset of age-related disease such as cancer.

The foci of our research are biological processes that enforce genome stability at the level of DNA damage response and repair. Our objective is to provide a thorough understanding of the molecular mechanisms involved in the repair of DNA base damage and broken DNA backbones, and the consequences of their dysfunction for cancer development and therapy. I will focus my account on one line of investigation to illustrate how we apply biochemistry to approach basic mechanisms of DNA repair and genetics to explore biological functions.

DNA Base Excision Repair: Insights from Studies with an Unusual DNA Glycosylase

DNA base lesions arise thousands of times every day in every single cell of our body, and occasionally they give rise to genetic mutations. Changes from C to T are frequent base substitutions in the human genome. They arise mostly through spontaneous or enzymatic deamination of 5-metyhylcytosine (5-mC) or cytosine in DNA, which generates thymine and uracil bases impaired with guanine, respectively. If mutation is to be avoided, these G•T and G•U mispairs must be corrected to G•C, and this is accomplished by a process called base excision repair (BER). BER is triggered by the action of a damage-specific DNA glycosylase that excises the irregular base from the DNA backbone, generating an abasic site (AP-site) that is further processed by an endonuclease, DNA polymerase, and DNA ligase to restore the original undamaged sequence. Human TDG is one of the BER initiating DNA glycosylases; it excises mismatched thymine and uracil from DNA as well as a number of additional base derivatives, including 5-Fluorouracil (5-FU), a chemotherapeutic base-analogue. However, TDG has attracted our attention because of some atypical biochemical and molecular features, including its physical and functional interactions with transcription factors and *de novo* DNA methyltransferases. Such peculiarities suggested that TDG might excise DNA bases in contexts other than classical DNA repair. Hence, we pursued various genetic and biochemical approaches to understand the protein and its function better. These efforts have uncovered entirely novel functional and mechanistic aspects of BER, such as a role SUMO-modification in DNA repair, a contribution of TDG dependent base excision to the DNA-directed cytotoxicity of 5-FU, and a possible association of TDG dependent processes with epigenetic events involved in stem cell maintenance and cell-fate determination.

SUMOs Make Their Way Into DNA Repair

We are not talking Japanese wrestlers here, SUMO stands for **s**mall **u**biquitin-like proteins that **mo**dify other proteins through covalent linkage to specific lysine residues. First indications for an involvement of such modifiers in DNA repair came from protein interaction studies in our laboratory; Roland Steinacher identified SUMOs as binding partners of the human TDG in yeast two-hybrid screens, and Ulrike Hardeland then showed that TDG is a specific target for covalent SUMO modification. Importantly, Ulrike also found that SUMO-conjugation significantly reduces the AP-site binding affinity of TDG, allowing the glycosylase to dissociate from the product following base release. This provided an answer to a long-standing question, namely how the endonuclease acting downstream in the repair process will gain access to the AP-site in the presence of a DNA glycosylase that binds to such intermediates with high affinity. Roland then went on to structure-function studies to provide insight into the molecular mechanisms underlying this SUMO-induced affinity change. He was able to demonstrate that unmodified TDG undergoes a conformational change upon binding to DNA, assuming a clamp-like structure that engages into a tight mode of DNA interaction upon encountering and processing a G-mismatched substrate. By showing that SUMO-modification induced opening of this DNA binding clamp, he was able to establish a novel, SUMO-based mechanism for the coordinated hand-over of DNA repair intermediates between upstream and downstream acting enzymes in DNA repair (Figure 1).

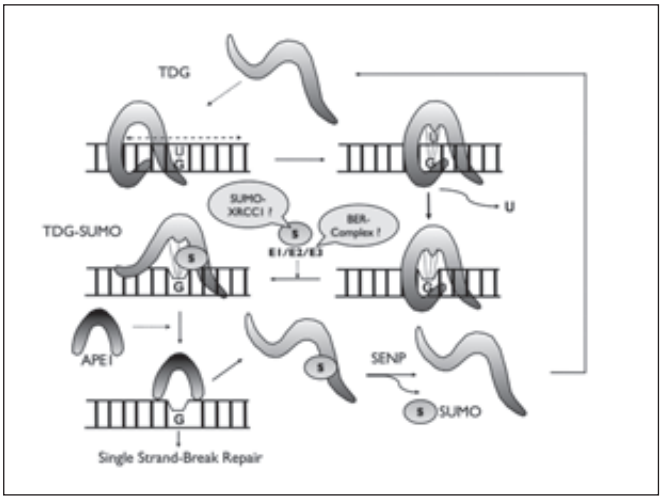

Figure 1: Base excision by TDG involves dynamic DNA interactions. Experimental evidence suggests that upon contacting DNA, the N-terminus of TDG forms a DNA binding clamp, which allows the glycosylase to slide along the duplex in search for a potential substrate. Upon encountering a substrate, the catalytic domain of TDG rotates the base (U) out of the helix and engages into specific interactions with the DNA strand opposite. Following base release, SUMOylation of TDG opens the clamp structure, facilitating its dissociation from the AP-site. APE1 gains access and carries on with the BER process.

Roland then started to investigate whether SUMO-modification might be a more general mechanistic feature of BER. Following the reconstitution of a SUMO conjugation system with purified human proteins, he and David Schürmann explored the modification of other BER proteins *in vitro*. This identified at least two more BER proteins as possible SUMO targets, among those XRCC1, a central component of the system with matchmaker function. Subsequent experiments addressing the role of TDG and XRCC1 modifications in the repair process then lead to an interesting discovery. The key observation was that SUMO modification to TDG was stimulated by the addition of DNA if performed in a nuclear extract, but inhibited in a reconstituted system, consisting of purified TDG and SUMO modification factors only. This suggested that a specificity factor, presumably a SUMO-E3 ligase type of activity, was missing in the purified system. Roland and David reasoned that specific BER proteins or complexes might provide such a function and reconstituted the BER system by adding the purified components to the SUMOylation reaction. This had little effect on TDG modification in the presence of DNA, unless they added SUMO in an XRCC1-conjugated form (instead of free SUMO1). This yielded an efficient and directed transfer of the SUMO moiety from XRCC1 to TDG, a process that was in agreement with protein interaction data showing that only SUMO1-conjugated but not free XRCC1 is capable of interacting with TDG. These findings indicate that SUMO modification might coordinate the BER process (and possibly other DNA transactions) by orchestrating the sequential recruitment, assembly and reconfiguration of multi-protein complexes as the repair reaction proceeds.

TDG and Cellular Responses to 5-FU

To explore the biological function of TDG, we generated null-mutants in yeast and mouse and analyzed the phenotype of TDG loss in both models. In the course of his work with yeast, Marc Bentele discovered that TDG is responsible for much of the DNA directed cytotoxicity of the therapeutic anti-cancer drug 5-FU. Moreover, he observed that 5-FU induces significant genomic instability in yeast and that this also depends to a large extent on active TDG. Since the DNA-directed effect of 5-FU is likely a consequence of increased misincorporation dUMP and 5-F-dUMP into DNA, we concluded from these results that the processing of uracil and 5-FU by TDG is cytotoxic and mutagenic.

Guided by the yeast studies, Christophe Kunz started to address the phenotype of TDG deficient mouse cells (ES cells and MEFs) that Yusuke Saito and himself generated in the context of a knock-out project. As predicted by the yeast phenotype, mouse cells lacking TDG showed a marked hyperresistance to treatment with 5-FU (Figure 2). This phenotype correlated with the level of Tdg activity in cells, implicating a rate-limiting role of TDG in the conversion 5-FU-induced DNA lesions into toxic intermediates. Further investigation confirmed this hypothesis and shed light onto the underlying molecular details. Christophe could show that upon 5-FU exposure, fluorouracil nucleotides get incorporated into genomic DNA with steady-state levels being significantly elevated in TDG deficient cells. Together with Frauke Focke he could further show that the excision of these bases by TDG generates DNA strand-breaks, activates DNA repair processes, and triggers DNA damage signaling in S-phase. These results provided novel

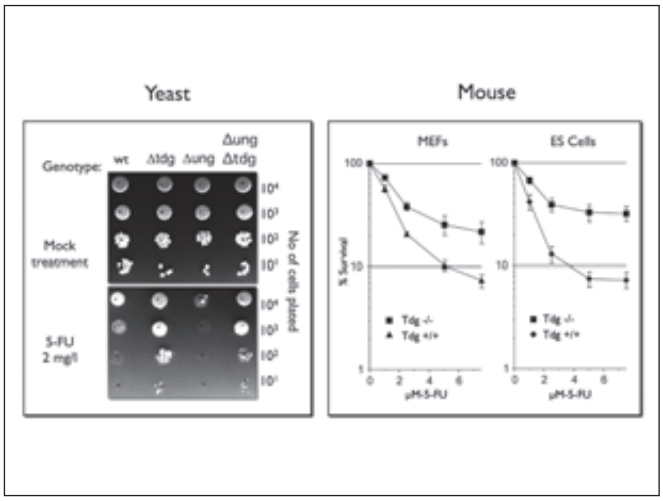

Figure 2: Disruption of TDG in fission yeast and mouse cells causes cellular hyper-resistance to 5-FU. Serial dilutions of fission yeast cells (left panel) were dropped onto media containing 5-FU (or mock plates) and colony forming was scored following incubation at 30°C as shown. Mouse embryonic fibroblasts (MEFs) and stem cells (ES cells) were exposed to increasing concentrations of 5-FU for 48 hours. Shown are survival curves as percentages of mock-treated cells (right panel).

and important insight into the molecular events underlying the DNA-directed cytotoxicity of 5-FU. Of more general significance, this work implicated that although TDG may contribute to the repair of base lesions, it does so in an inefficient way that gives rise to mutagenic and cytotoxic DNA intermediates, a property that may related to its strong interaction with the AP-site following base release (Figure 1). Apparently, TDG dependent BER is not designed to deal with frequently occurring DNA base lesions and fulfills a specialized rather than a general repair function.

Towards Understanding the Specialized Function of TDG

Attempts by Teresa Lettieri and Yusuke Saito to generate a TDG knockout mouse were exhausting, initially only because the locus was very hard to target by homologous recombination and then because the glycosylase quite unexpectedly turned out to be essential for embryogenesis. Since all other DNA glycosylase knockouts generated previously did not show any developmental defects, this phenotype corroborated our view that is a special case among this DNA glycosylases with function that may be different from the simple repair of base damage. To facilitate further functional studies, Yusuke and Christophe established a series of TDG deficient ES cells and MEFs. They performed transcriptome analyses of ES cells undergoing in vitro differentiation and found evidence for an involvement of TDG in the establishment of cell-lineage specific gene expression. Considering its interactions with transcription factors and DNMT3a and DNMT3b and its enzymatic properties, we speculated that TDG might act in concert with DNA methyltransferases to protect certain CpG sites from erroneous de novo methylation during cell differentiation. In a series of in vitro differentiation experiments with TDG proficient and deficient ES cells, Yusuke indeed observed differences in the dynamics of *de novo* methylation in promoters of pluripotency genes (e.g. Oct4) that undergo epigenetic silencing during ES cell differentiation. His data are in support of a scenario, whereby TDG is targeted to these promoters through its interaction with transcription factors or DNA methyltransferases in order to initiate excision repair of erroneously methylated cytosines. Consistently, Daniel Cortazar's recent efforts to chromatin-immunoprecipitate TDG (TDG-ChIP) yielded physical evidence for an enrichment of the glycosylase at the promoters of Oct4 and other genes.

These are exciting developments, implicating a role for DNA repair not only in genome but also in epigenome maintenance. Sure, there is still some way to go before we will be able to come up with a conclusive scenario for such a function, but it looks like studying TDG dependent BER guides us in a promising direction. One obstacle along this way seems to be phenotypic instability of TDG deficient ES cells. If this is a reflection of an inherent promiscuous epigenetic behaviour of these cells, we seem to be on the right track though.

And There is More of Us – Next Time

I must apologize to Olivier Fritsch, Claudia Krawczyk, Patric Urfer and Stefan Weis for not having included their projects on DNA double-strand break repair and cancer epigenetics in this account. I promise it will be their turn next time.

Primo Schär

Oxidative stress and heart failure: The bumpy road from a concept to clinical practice

The "Myocardial Research" laboratory (located in lab 319) was established in late 2006 based on a SCORE program from the Swiss National Science Foundation (Gabriela Kuster). In 2007, the team was joined by Stéphanie Häuselmann (PhD student), Berit Rosc-Schlüter (PhD student), Vera Lorenz (biomedical analyst) and Otmar Pfister (MD, principal investigator cardiac stem/progenitor cells). Our research has two major topics: (1) The molecular mechanisms of myocardial remodeling and repair, specifically the role of reactive oxygen species in these processes (GK, SH, BRS); and (2) Cardiac stem/progenitor cells, specifically their regulation by hematopoietic growth factors and their role in cardiac cell homeostasis and myocardial repair (OP, VL). In the present issue of the DBM Facts, I will introduce you to the first topic, give you an overview over the "oxidative stress hypothesis of heart failure" and try to explain why having a good concept might just not be good enough...

From left to right:
Vera Lorenz, Berit Rosc-Schlüter,
Stéphanie Häuselmann,
Gabriela Kuster Pfister, Otmar Pfister

The heart failure epidemic: medical and economic significance

Worldwide, roughly 1.5–2% of the population suffers from heart failure which is a major complication of virtually all types of cardiac disease and a leading cause of death and hospitalization. Amongst the elderly population (>75 yrs), the prevalence of heart failure is even higher (up to 10%). Recent advances in treatment of cardiac diseases, in particular myocardial infarction, have improved survival without, however, restoring the full contractile competence of the heart. Thus, both aging of the population and modern-time treatment of heart disease will further increase the prevalence of heart failure. In Switzerland, roughly 100 000 people are affected and 20 000 new cases are added each year. Although heart failure treatment has likewise markedly improved over the past decades – mainly due to the introduction of angiotensin-converting enzyme inhibitors, β-blockers, and aldosterone antagonists – prognosis is still poor. The one-year survival rate for advanced stage heart failure is even worse than those for most malignant cancers. Because of the high frequency of hospitalizations, the costs for treatment of heart failure are high and amount to an estimated 1 (US)-billion CHF per year.

Myocardial remodeling is a pivotal process in the development of heart failure

Myocardial injury of any kind [e.g. mechanical (arterial hypertension), hypoxic (myocardial infarction), toxic (alcohol), infectious (myocarditis), metabolic (diabetes) etc.] initiates a process that is referred to as myocardial remodeling and that plays a key role in the development of heart failure. Myocardial remodeling encompasses progressive changes in myocardial structure and function that may be protective or adaptive at the beginning (compensatory hypertrophy), but turn deleterious or maladaptive as disease progresses (ventricular dilation and dysfunction), finally leading to heart failure (Fig 1). At the level of the cardiomyocytes, these changes include myocyte growth (hypertrophy) loss of myocytes (apoptosis or necrosis), alterations in myocyte architecture and reexpression of fetal isoforms (e.g. β-myosin heavy chain) that affect contractile function and/or calcium homeostasis. Myocardial remodeling is triggered and promoted by so-called remodeling stimuli. These include cytokines and neurohormones that are released in the setting of myocardial injury.

Perturbation of the cellular redox-homeostasis can lead to oxidative stress

Reactive oxygen species (ROS) are highly reactive oxygen moieties that arise from incomplete reduction of molecular oxygen (O_2) either by a leak of electrons from the respiratory chain in the mitochondria or through the action of intracellular oxidase enzymes such as NADPH oxidase. A number of enzymatic antioxidants are in charge of removing excessive ROS and of maintaining a physiological redox-balance (Fig. 2). Myocardial remodeling stimuli can directly enhance ROS production by activating ROS-generating enzymes and/or decreasing the antioxidant defense capacities, which results in a net increase of ROS *(oxidative stress)*. Via oxidation of DNA, lipids and proteins,

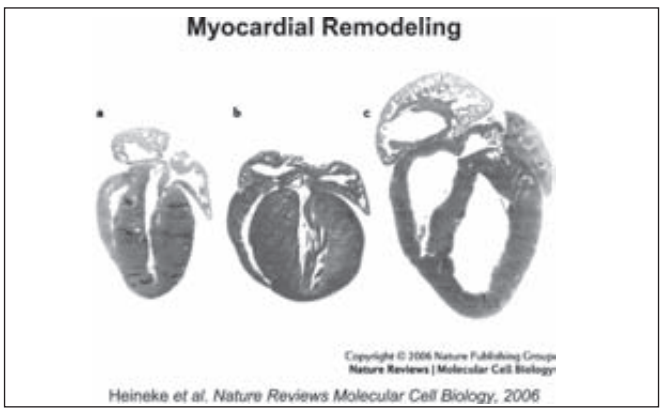

Figure 1

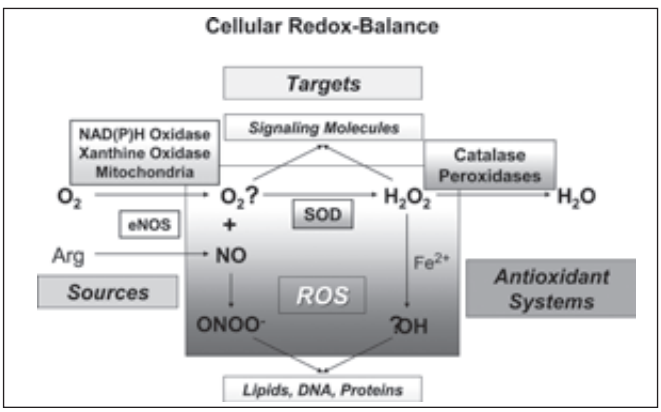

Figure 2

excessive ROS may directly induce cellular injury that leads to cell death, disease and premature aging. However, ROS can also participate in cell signaling through activation of redox-sensitive signaling cascades and thus initiate both protective (adaptive) or damaging (maladaptive) cellular events.

The oxidative stress hypothesis of heart failure

Markers of oxidative stress (e.g. lipid peroxidation products such as isoprostanes) are elevated systemically in the myocardium of humans and in animal models of heart failure. However, recent evidence suggests that ROS are not just mere by-products of heart failure. The *oxidative stress hypothesis* of heart failure postulates that ROS actively contribute to myocardial remodeling. Consistent with this hypothesis, antioxidant treatment can ameliorate adverse remodeling *in vitro* and in animal models *in vivo*. Conversely, the administration of ROS can mimic typical features observed in myocardial remodeling *in vitro*: treatment of rat cardiomyocytes with hydrogen peroxide leads to differential, concentration-dependent activation of specific kinase signaling pathways resulting in hypertrophy in response to low and apoptosis to high concentrations of hydrogen peroxide. Accordingly, ROS have been implicated as mediators of hypertrophic and apoptotic signaling in response to various remodeling stimuli, such as mechanical strain, α- and β-adrenergic receptor stimulation, TNFα, angiotensin and endothelin. Still, the mechanisms whereby ROS exert their effects remain poorly understood.

According to the redox-homeostasis model depicted in Fig. 2, the following *therapeutic strategies* to protect the heart from oxidative stress can be delineated: (1) Scavenging or neutralizing ROS by *enhancing antioxidant capacities*, (2) *Inhibiting sources* of ROS or (3) *Protecting potential ROS targets* from oxidation.

Clinical antioxidant trials and reasons for their failure

Despite overwhelming evidence from *in vitro* and *in vivo* animal studies in support of the oxidative stress hypothesis of heart failure, several clinical trials using (mostly nutritional) antioxidants in humans have yielded disappointing results. Neither vitamin C, nor vitamin E or β-carotene has proven useful in the prevention or treatment of cardiovascular disease. Notably, vitamin E in fact *increased* the incidence of heart failure events in a large study population at augmented risk of cardiovascular disease (HOPE trial). These findings cast major doubt on the use of antioxidants as a suitable strategy for the treatment of human heart failure. Inappropriate end-points, antioxidative effects of adjuvant therapies, issues in patient selection and inappropriate antioxidants may – at least to a certain degree – explain why antioxidants were ineffective in these trials. Other reasons, however, may relate to the fact that under certain circumstances, ROS-signaling may be protective rather than deleterious, or to the most recent intriguing hypothesis that "*reductive stress*" (i.e. an abnormal increase of reducing equivalents such as reduced glutathione and NADPH) itself may cause a distinct form of cardiomyopathy.

Protective ROS-signaling and the counter-hypothesis: when reductive stress causes cardiomyopathy

The effect of ROS on cell number (by affecting cell survival or proliferation), morphology (growth/hypertrophy) and function (contractility) relates to the oxidative "burden". For example, a low oxidative burden induced by low levels of ROS or by ROS of low reactivity will initiate rather regulatory or protective processes, whereas a high oxidative burden (high levels or highly reactive species) will induce cellular injury. Besides the reactivity of the species, their *sources*, the cell type (cardiomyocytes, endothelial cells or fibroblasts) and subcellular localization, as well as the respective targets influence the net response to ROS. Similarly, type and stage of the disease may play a role.

Very recently, *reductive stress* due to an unbalanced increase in activity of endogenous antioxidants has been shown to induce a distinct form of cardiomyopathy in a mouse model of a human multisystem protein aggregation disease. These animals exhibit increased recycling of oxidized to reduced glutathione that causes protein aggregation and hypertrophy, finally leading to heart failure and premature death. These findings published in *Cell* last year will change the paradigm of ROS as purely detrimental contributors to myocardial remodeling and highlight the need of a more comprehensive understanding of the role of ROS and their potential targets in the heart.

Understanding the mechanisms of ROS interactions

In our lab, we seek to understand the role of myocardial ROS from distinct sources and their mechanisms of inter-

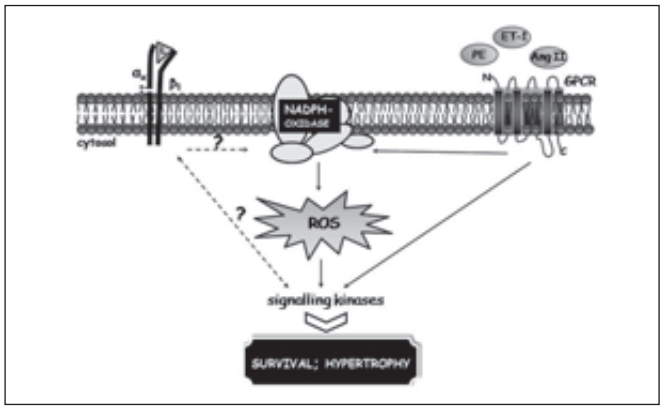

Figure 3

action with respective target molecules. ROS can induce oxidative modifications of proteins and thus alter their structure and/or function. In the laboratory of Dr. W.S. Colucci at Boston University, where I have previously worked, we found oxidatively modified (nitrotyrosinylated) protein in mice hearts after ascending aortic constriction, a condition associated with increased oxidative/nitrosative stress. Further work suggested 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. We are currently focusing our work on β_1-integrin as a potential target of ROS. Integrins are transmembrane receptors that participate in the regulation of cell growth, proliferation and death. In cardiomyocytes, β_1-integrin mediates hypertrophy and protects the cells from apoptosis. In our current project we test whether ROS participate in the control of β_1-integrin activity by regulating the amount (transcriptional regulation) and/or avidity of β_1-integrin (post-translational regulation) in cultured rat cardiomyocytes. In turn, β_1-integrin itself may exert its cell-protective effects by modifying ROS-signaling. Preliminary data suggest that β_1-integrin expression is increased in response to Gq-coupled receptor (GqCR)-stimulation in cardiomyocytes and that this increase is mediated via NADPH oxidase-derived ROS (Fig. 3). Likewise, NADPH oxidase-derived ROS seem involved in the regulation of β_1-integrin function. We are now further characterizing the NADPH oxidase-dependent pathways involved and we dissect the roles of distinct isoforms of NADPH oxidase in the regulation of β_1-integrin and its protective effects in cardiomyocytes.

The future: targeting the source rather than ROS

In view of the disappointing clinical trials using antioxidants for treatment of cardiovascular disease and heart failure in humans, inhibition of the respective sources of ROS seems a promising alternative strategy. Recently, small molecule and peptide inhibitors of NADPH oxidase have been developed and tested in various models of cardiovascular disease *in vitro* and *in vivo*. However, an increasing body of evidence suggests that NADPH oxidase-derived ROS may have distinct effects depending on the isoform, the disease state and the cell type. NADPH oxidase may thus be crucial to both cardiovascular health and disease. Our own preliminary data suggest that NADPH oxidase-derived ROS participate in the regulation of *adaptive* remodeling in cardiomyocytes via regulation of β_1-integrin. We hope that our studies will contribute to a more comprehensive understanding of the roles of ROS in myocardial remodeling, in order that better targeted strategies can be developed in the future to prevent and treat heart failure.

Gabriela M. Kuster

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Hunting the Immunology Ghosts in a Medieval Castle

There are several kind of congresses and meetings, but the Wolfsberg Meeting for Swiss Immunology PhD students is a special one.

When I started my PhD here in Switzerland all the students already in the lab where telling me fantastic stories about this meeting, but at the time I thought they were really exaggerating. Then I went there.... and it was all true!

The meeting is held in Schloss Wolfsberg, on the Lake of Constance. The building belongs to UBS who sponsor the meeting.

When you arrive there and if the weather is fine, you will have a fantastic view of the lake and of the countryside, as the castle is on a small hill surrounded by a forest. Then when the gates of the congress centre open, you see the indoor swimming pool. This is the moment in which you start to think that this meeting is ranking up in your favourite Top Ten meetings. But you still have not seen the whole package...the rooms are really stylish and I don't know how many of us PhD students are used to staying in hotels with bathrooms with a stone shower, double sink and fading lights. But there is no time to spend there, the programme schedule is very strict and soon after arrival (10 minutes later!) the meeting starts.

The welcome speech from Prof. Hengartner is a milestone of the meeting, and the best part is when he gives the rules for making a good presentation: hold the pointer firmly; don't use it like a paintbrush; point it only where you want to highlight something; explain all the graphs (especially the axes); and don't overkill the audience. It seems reasonable, doesn't it? But don't underestimate an overexcited student at their first oral presentation! The environment is so cool that very soon everybody realizes that this is not a "normal" meeting. The audience is composed only of students, more or less 80 places are available, with students coming from Basel, Zurich, Bern, St.Gallen and Bellinzona and seven Professors who lead the sessions. It's like being in a big PhD student club where you feel free to ask questions and where it is possible to speak openly about the technical problems that you might have encountered during your experiments. First year students present a poster in a poster session, while students of the second

Schloss Wolfsberg at the Lake of Constance (page 14), visiting the old mill in Tägerwilen (page 15).

and third year have a 10 minutes oral presentation. The interaction between people is greatly encouraged. During coffee breaks, lunches, and dinners it is easy to speak with the other students and exchange ideas for experiments, or to learn some tricks on how to use a technique.

The food is another plus for this meeting. Forget the tasteless gummy congress lunches and welcome in the world of three course haute cuisine. They really know how to cook and when you have to choose between the menus (fish or meat? vegi? hard choice, ice cream or chocolate mousse? or maybe both?) you already foresee that it will be good regardless. And in the evening after the last presentation session of the day (never forget why you are there!), you can stay in the lounge (with fireplace) for the last beer and the last chat. And after all these science talks don't you think it would be great to relax a little? Well, for this there is the "free sport evening" where all the sport facilities of the centre are open until 11 p.m. The swimming pool, the gym, the sauna, and the table tennis are all suitable choices!

In previous years my motivation to play either basketball or volleyball during those evenings was really high as Michel Mallaun and Marco Cavallari can witness, but last year after 3 minutes of the game I needed an oxygen mask and the following day I discovered the presence of totally unknown and forgotten muscles! So this year, which was unfortunately my last, I spent more time in the swimming pool and in the sauna than in the gym (getting older means getting wiser, or not?). In addition, this year Marco, Michel, and Heike Himmerleich organised a salsa session that was particularly appreciated by the neighbouring rooms! Moreover, Marco and Michel proved to be real iron-men at all of the meetings: every morning at 6 a.m. they were off to the swimming pool for a "wake up" swim. They tried to convince other people to go with them... with no success!

The cherry on the cake of the meeting had always been the organized excursion: there are so many nice places around the castle! This year we visited a restored mill that, in the past, harnessed water power to cut tree trunks. We were told the history of the mill and how a group of retired people decided to take on the challenge of restoring the buildings around the mill, as well as the mill itself, and to transform it in a museum. Their next challenge is to replant and regrow the Mueller-Thurgau vine at its original site. We had a wine-testing and we can say that it is really good! In the three days meeting there was also time for a lecture from UBS where the Head of Wealth Management for the Zurich Region, Werner Peyes, showed us the trends in the market and, with very interesting interactive games, showed us how and where invest our money (when we have some, one day!).

I hope that the new generation of Immunology PhD students of the institute will not miss the opportunity to attend this cool meeting. In addition to the fun that we had, there is the possibility to learn something in immunology fields different from our own, to meet people, network, start the basis for future collaborations, and have a look of what is going on here in Switzerland in the other universities and institutes.

Federica Facciotti

Criteria for selecting papers presented in "DBM Facts"

Please submit articles as pdf files to the Departmental Assistant, Manuela Bernasconi:
manuela.bernasconi@unibas.ch

We will try to include as many articles as possible in each issue. However, there are page constrains, which may force us to make a selection. The final decision will be made by the chair of the Department of Biomedicine according to following criteria:

- First priority will be given to articles published in high ranked journals that are authored by members of the Department of Biomedicine (first author, senior author and corresponding author from DBM).
- Articles published in low impact journals and articles where members of the Department of Biomedicine are only co-authors may also be included, but will receive lower priority.

The following publications do not qualify for inclusion in "DBM Facts" (=most frequent reasons for rejection):

- Articles "in press" (please wait until the pdf is available with the correct volume, page numbers etc)
- Articles without mentioning of the DBM affiliation
- Articles where neither first author, nor senior author, nor corresponding author are from DBM
- Articles with purely clinical work without a clear contribution of the DBM laboratories
- Review articles (with very few exceptions, e.g. reviews in Cell, Science, Nature etc.)
- Book chapters

Please note that each issue of DBM Facts will have a deadline for the submission of articles. Deadline for the next issue is August 15, 2008.
Radek Skoda

The Journal of Experimental Medicine THE JOURNAL OF EXPERIMENTAL MEDICINE 205, 523–531, 2008 IF 14,5

Intrathymic expression of Flt3 ligand enhances thymic recovery after irradiation

L. Kenins[1,3], J. W. Gill[2], R. L. Boyd[3], G. A. Holländer[2], and A. Wodnar-Filipowicz[1]

Abstract:
Hematopoietic stem cell transplantation (HSCT) requires conditioning treatments such as irradiation, which leads to a severely delayed recovery of T cell immunity and constitutes a major complication of this therapy. Currently, our understanding of the mechanisms regulating thymic recovery is limited. It is known that a subpopulation of bone marrow (BM)–derived thymic immigrant cells and the earliest intrathymic progenitors express the FMS-like tyrosine kinase 3 (Flt3) receptor; however, the functional significance of this expression in the thymus is not known. We used the BM transplant model to investigate the importance of Flt3 ligand (FL) for the regeneration of the T cell compartment. We show that FL is expressed in the adult mouse thymus on the surface of perivascular fibroblasts. These cells surround the proposed thymic entry site of Flt3 receptor–positive T cell progenitors. After irradiation, perivascular FL expression is up-regulated and results in an enhanced recovery of thymic cellularity. Thymic grafting experiments confirm an intrathymic requirement for FL. Collectively, these results show that thymic stromal cell–mediated FL–Flt3 receptor interactions are important in the reconstitution of thymopoiesis early after lethal irradiation and HSCT, and provide a functional relevance to the expression of the Flt3 receptor on intrathymic T cell progenitors.

Correction:
The publication „Intrathymic expression of Flt3 ligand enhances thymic recovery after irradiation" was not published in the „The New England Journal of Medicine", as was stated in the last issue, but in the „The Journal of Experimental Medicine". We apologies for this mistake.

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PNAS PNAS 105, 7034–7039, 2008 IF 9,6

Interferon signaling and treatment outcome in chronic hepatitis C

M. Sarasin-Filipowicz[1], E. J. Oakeley[2], F. H. T. Duong[1], V. Christen[1], L. Terracciano[3], W. Filipowicz[2], and M. H. Heim[1,4]

Abstract:
Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide. The current standard therapy for chronic hepatitis C (CHC) consists of a combination of pegylated IFN alpha (pegIFNα) and ribavirin. It achieves a sustained viral clearance in only 50-60% of patients. To learn more about molecular mechanisms underlying treatment failure, we investigated IFN-induced signaling in paired liver biopsies collected from CHC patients before and after administration of pegIFNα. In patients with a rapid virological response to treatment, pegIFNα induced a strong upregulation of IFN-stimulated genes (ISGs). As shown previously, nonresponders had high expression levels of ISGs before therapy. Analysis of posttreatment biopsies of these patients revealed that pegIFNα did not induce expression of ISGs above the pretreatment levels. In accordance with ISG expression data, phosphorylation, DNA binding, and nuclear localization of STAT1 indicated that the IFN signaling pathway in nonresponsive patients is preactivated and refractory to further stimulation. Some features characteristic of nonresponders were more accentuated in patients infected with HCV genotypes 1 and 4 compared with genotypes 2 and 3, providing a possible explanation for the poor response of the former group to therapy. Taken together with previous findings, our data support the concept that activation of the endogenous IFN system in CHC not only is ineffective in clearing the infection but also may impede the response to therapy, most likely by inducing a refractory state of the IFN signaling pathway.

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American Journal of Transplantation American Journal of Transplantation 8, 1312–1317, 2008 IF 6,8

The Number of Activating KIR Genes Inversely Correlates with the Rate of CMV Infection/Reactivation in Kidney Transplant Recipients

M. Stern[1], H. Elsässer[2], G. Hönger[2], J. Steiger[2], S. Schaub[2] and C. Hess[2,3]

Abstract:
Viral infection is a common complication after kidney transplantation. The role of natural killer cells (NK cells) in this setting remains unknown. NK cells express activating and inhibitory killer cell immunoglobulin-like receptors (KIR). We analyzed whether activating KIR genes carried by kidney transplant-recipients influence the rate of viral infection during the first year after transplantation. In patients with a KIR A/A genotype (n = 40, KIR2DS4 only activating KIR) the rate of cytomegalovirus (CMV) infection and reactivation was 36%, as compared to 20% in transplant recipients with more than one activating KIR gene (KIR B/X genotype, n = 82, p = 0.04). Adjusting for other risk factors in Cox regression, the relative risk of B versus A genotype patients was 0.34 (95% CI 0.15-0.76, p = 0.009). The degree of protection increased with the number of activating KIR genes. Symptomatic CMV disease was only observed in four individuals, all carrying a KIR A/A genotype. As for viral infections other than CMV, and for bacterial infections, no KIR-linked protective effect could be detected. Also, graft function and the rate-rejection episodes were similar in KIR A/A and KIR B/X genotype individuals. This study supports a role for activating KIR in the control of CMV infection after kidney transplantation.

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The Journal of Immunology | The Journal of Immunology | 180, 817–824, 2008 IF 6,3

Polymorphonuclear Neutrophil-Derived Ectosomes Interfere with the Maturation of Monocyte-Derived Dendritic Cells

C. Eken[1] O. Gasser[1], G. Zenhaeusern[2], I. Oehri[2], C. Hess[2] and J. A. Schifferli[1]

Abstract:
Polymorphonuclear neutrophils (PMNs) are a key component of the innate immune system. Their activation leads to the release of potent antimicrobial agents through degranulation. Simultaneously, PMNs release cell surface-derived microvesicles, so-called ectosomes (PMN-Ect). PMN-Ect are rightside-out vesicles with a diameter of 50-200 nm. They expose phosphatidylserine in the outer leaflet of their membrane and down-modulate monocyte/macrophage-activation in vitro. In this study, we analyzed the effects of PMN-Ect on maturation of human monocyte-derived dendritic cells (MoDCs). Intriguingly, exposing immature MoDCs to PMN-Ect modified their morphology, reduced their phagocytic activity, and increased the release of TGF-β1. When immature MoDCs were incubated with PMN-Ect and stimulated with the TLR4 ligand LPS, the maturation process was partially inhibited as evidenced by reduced expression of cell surface markers (CD40, CD80, CD83, CD86, and HLA-DP DQ DR), inhibition of cytokine-release (IL-8, IL-10, IL-12, and TNF-α), and a reduced capacity to induce T cell proliferation. Together these data provide evidence that PMN-Ect have the ability to modify MoDC maturation and function. PMN-Ect may thus represent an as yet unidentified host-factor influencing MoDC maturation at the site of injury, thereby possibly impacting on downstream MoDC-dependent immunity.

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The Journal of Infectious Diseases | The Journal of Infectious Diseases | 197, 1028–1035, 2008 IF 5,4

Biofilm Formation Induces C3a Release and Protects *Staphylococcus epidermidis* from IgG and Complement Deposition and from Neutrophil-Dependent Killing

S.A. Kristian[1], T.A. Birkenstock[3], U. Sauder[2], D. Mack[4], F. Götz[3], and R. Landmann[1]

Abstract:
BACKGROUND:
Biofilm formation is considered to be an important virulence factor of the opportunistic pathogen *Staphylococcus epidermidis*. We hypothesized that biofilm formation could interfere with the deposition of immunoglobulins and complement on the bacterial surface, leading to diminished activation of the complement system and protection from killing by human phagocytes.
METHODS:
The killing of biofilm-encased and planktonically grown wild-type (wt) *S. epidermidis* and the killing of an isogenic biofilm-negative ica mutant (ica⁻) by human polymorphonuclear neutrophils (PMNs) were compared. C3a induction and deposition of C3b and immunoglobulin G (IgG) on the bacteria after opsonization with human serum were assessed by enzyme-linked immunosorbent assay, flow cytometry, and electron microscopy. The virulence of the bacterial strains was compared in a mouse model of catheter-associated infection.
RESULTS:
Biofilm-embedded wt *S. epidermidis* was killed less well by human PMNs and induced more C3a than planktonically grown wt and ica⁻ S. epidermidis. However, the deposition of C3b and IgG on the bacterial surface was diminished in biofilm-encased staphylococci. wt *S. epidermidis* was more virulent in implant-associated infections and was killed more slowly than ica⁻ in ex vivo assays of killing by PMNs.
CONCLUSIONS:
The results indicate that prevention of C3b and IgG deposition on the bacterial surface contributes to the biofilm-mediated protection of *S. epidermidis* from killing by PMNs.

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Cellular and Molecular Life Sciences | Cellular and Molecular Life Sciences | 65, 1596–1608, 2008 | IF 4,7

Pharmacological manipulation of L-carnitine transport into L6 cells with stable overexpression of human OCTN2

L. Todesco[1], D. Bur[2], H. Brooks[1], M. Török[1], L. Landmann[3], B. Stieger[4], and S. Krähenbühl[1]

Abstract:
The high-affinity Na+-dependent carnitine transporter OCTN2 (SLC22A5) has a high renal expression and reabsorbs most filtered carnitine. To gain more insight into substrate specificity of OCTN2, we overexpressed hOCTN2 in L6 cells and characterized the structural requirements of substances acting as human OCTN2 (hOCTN2) inhibitors. A 1905-bp fragment containing the hOCTN2 complete coding sequence was introduced into the pWpiresGFP vector, and L6 cells were stably transduced using a lentiviral system. The transduced L6 cells revealed increased expression of hOCTN2 on the mRNA, protein and functional levels. Structural requirements for hOCTN2 inhibition were predicted in silico and investigated in vitro. Essential structural requirements for OCTN2 inhibition include a constantly positively charged nitrogen atom and a carboxyl, nitrile or ester group connected by a 2-4-atom linker. Our cell system is suitable for studying in vitro interactions with OCTN2, which can subsequently be investigated in vivo.

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AJP - Endocrinology and Metabolism | Amercian Journal of Physiology Endocrinology and Metabolism | 294, 241–250, 2008 | IF 4,1

TNF-α increases protein content in C_2C_{12} and primary myotubes by enhancing protein translation via the TNF-R1, PI3K, and MEK

I. Plaisance, C. Morandi, C. Murigande, and M. Brink

Abstract:
Recent evidence supports that TNF-α, long considered a catabolic factor, may also have a physiological function in skeletal muscle. The catabolic view, mainly based on correlative studies in human and in vivo animal models, was challenged by experiments with myoblasts, in which TNF-alpha induced differentiation. The biological effects of TNF-α in differentiated muscle, however, remain poorly understood. In the present study, we tested whether TNF-α has growth-promoting effects in myotubes, and we characterized the mechanisms leading to these effects. Treatment of C_2C_{12} myotubes with TNF-α for 24 h increased protein synthesis (PS) and enhanced cellular dehydrogenase activity by 22 and 26%, respectively, without changing cell numbers. These effects were confirmed in myotubes differentiated from primary rat myoblasts. TNF-α activated two signaling cascades: 1) ERK1/2 and its target eIF4E and 2) Akt and its downstream effectors GSK-3, $p70^{S6K}$, and 4E-BP1. TNF-α-induced phosphorylation of Akt, and ERK1/2 was inhibited by an antibody against TNF-α receptor 1 (TNF-R1). PD-98059 pretreatment abolished TNF-α-induced phosphorylation of ERK1/2 and eIF4E, whereas PS was only partially inhibited. LY-294002 completely abolished TNF-α-induced stimulation of PS as well as phosphorylation of Akt and its downstream targets GSK-3, $p70^{S6K}$, and 4E-BP1. Rapamycin inhibited TNF-α-induced phosphorylation of the mTOR C1 target $p70^{S6K}$ without altering TNF-α-induced PS and 4E-BP1 phosphorylation. In conclusion, our results provide evidence that TNF-α enhances PS in myotubes and that this is based on enhanced protein translation mediated by the TNF-R1 and PI3K-Akt and MEK-ERK signaling cascades.

Institute of Physiology, Department of Biomedicine, University and University Hospital of Basel, Basel, Switzerland

J Pharmacology Experimental Therapeutics | The Journal of PHARMACOLOGY And Experimental Therapeutics | 324, 568–575, 2008 | IF 4,0

Toxicity of Valproic Acid in Mice with Decreased Plasma and Tissue Carnitine Stores

A. C. Knapp[1], L. Todesco[1], K. Beier[2], L. Terracciano[3], H. Sägesser[4], J. Reichen[4], and S. Krähenbühl[1]

Abstract:
The aim of this study was to investigate whether a decrease in carnitine body stores is a risk factor for valproic acid (VPA)-associated hepatotoxicity and to explore the effects of VPA on carnitine homeostasis in mice with decreased carnitine body stores. Therefore, heterozygous juvenile visceral steatosis (jvs)$^{(+/-)}$ mice, an animal model with decreased carnitine stores caused by impaired renal reabsorption of carnitine, and the corresponding wild-type mice were treated with subtoxic oral doses of VPA (0.1 g/g b.wt./day) for 2 weeks. In jvs$^{(+/-)}$ mice, but not in wild-type mice, treatment with VPA was associated with the increased plasma activity of aspartate aminotransferase and alkaline phosphatase. Furthermore, jvs$^{(+/-)}$ mice revealed reduced palmitate metabolism assessed in vivo and microvesicular steatosis of the liver. The creatine kinase activity was not affected by treatment with VPA. In liver mitochondria isolated from mice that were treated with VPA, oxidative metabolism of l-glutamate, succinate, and palmitate, as well as beta-oxidation of palmitate, were decreased compared to vehicle-treated wild-type mice or jvs$^{(+/-)}$ mice. In comparison to vehicle-treated wild-type mice, vehicle-treated jvs$^{(+/-)}$ mice had decreased carnitine plasma and tissue levels. Treatment with VPA was associated with an additional decrease in carnitine plasma (wild-type mice and jvs$^{(+/-)}$ mice) and tissue levels (jvs$^{(+/-)}$ mice) and a shift of the carnitine pools toward short-chain acylcarnitines. We conclude that jvs$^{(+/-)}$ mice reveal a more accentuated hepatic toxicity by VPA than the corresponding wild-type mice. Therefore, decreased carnitine body stores can be regarded as a risk factor for hepatotoxicity associated with VPA.

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European Journal of Pharmacology | european journal of pharmacology | 586, 251–258, 2008 | IF 2,5

Dimethyl fumarate, a small molecule drug for psoriasis, inhibits Nuclear Factor-κB and reduces myocardial infarct size in rats

S. Meili-Butz[1], T. Niermann[1], E. Fasler-Kan[2], V. Barbosa[1], N. Butz[3], D. John[1], M. Brink[1], P. T. Buser[1], and C. E. Zaugg[1]

Abstract:
Persistent Nuclear Factor-κB (NF-κB) activation is hypothesized to contribute to myocardial injuries following ischemia–reperfusion. Because inhibition or control of NF-κB signaling in the heart probably confers cardioprotection, we determined the potency of the NF-κB inhibitor dimethyl fumarate (DMF) in cardiovascular cells, and determined whether administration of DMF translates into beneficial effects in an animal model of myocardial infarction. In rat heart endothelial cells (RHEC), we analysed inhibitory effects of DMF on NF-κB using shift assay and immunohistofluorescence. In *in vivo* experiments, male Sprague Dawley rats undergoing left coronary artery occlusion for 45 min received either DMF (10 mg/kg body weight) or vehicle 90 min before ischemia as well as immediately before ischemia. After 120 min of reperfusion, the hearts were stained with phthalocyanine blue dye and triphenyltetrazolium chloride. Additionally, acute hemodynamic and electrophysiologic effects of DMF were determined in dose–response experiments in isolated perfused rat hearts. DMF inhibited TNF-α-induced nuclear entry of NF-κB in RHEC. In *in vivo* experiments, myocardial infarct size was significantly smaller in rats that had received DMF (20.7% ± 9.7% in % of risk area; $n = 17$) than in control rats (28.2% ± 6.2%; $n = 15$). Dose–response experiments in isolated perfused rat hearts excluded acute hemodynamic or electrophysiologic effects as mechanisms for the effects of DMF. DMF inhibits nuclear entry of NF-κB in RHEC and reduces myocardial infarct size after ischemia and reperfusion in rats in vivo. There was no indication that the beneficial effects of DMF were due to acute hemodynamic or electrophysiologic influences.

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Biochemical Engineering Journal | Biochemical Engineering Journal | 39, 568–589, 2008 | IF 1,8

Assessment of the stability of TGFβ3 bioactivity for potential bioreactor applications

D. Vonwil[1], D. Wendt[1], S. Ströbel[1], H.J. Wallny[2], D. Gygax[3], M. Heberer[1], and I. Martin[1]

Abstract:
In order to develop suitable bioreactor systems and processes for automated and standardized cell cultures involving the use of bioactive factors, we determined the stability of transforming growth factor beta 3 (TGFβ3) over storage time and under conditions typically used for mammalian cell culture. Using a reporter gene assay with firefly luciferase as readout, significant reduction of TGFβ3 bioactivity was detected to occur both in serum containing medium (SCM) and serum free medium (SFM). The residual activity, quantified by parallel line assays, progressively decreased with time, down to 60% in SCM and 84% in SFM after 1 week at 37 °C, with no further decrease until 3 weeks, whereas such loss could not be predicted using a conventional ELISA method. The reduction of TGFβ3 bioactivity had a negligible influence in a typical biological assay (e.g., chondrocyte proliferation), supporting the possibility of prolonged storage of medium pre-supplemented with TGFβ3 for bioreactor-based chondrocyte expansion. With the ultimate goal of defining suitable operating protocols for automated cell culture bioreactors, the proposed approach should be extended to assessing the stability of other possibly labile medium supplements.

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Annals of Nutrition & Metabolism | Annals of Nutrition & Metabolism | 52, 136–144, 2008 | IF 1,6

Effect of Carnitine Deprivation on Carnitine Homeostasis and Energy Metabolism in Mice with Systemic Carnitine Deficiency

A. C. Knapp[1], L. Todesco[1], M. Török[1], K. Beier[2], and S. Krähenbühl[1]

Abstract:
Background/Aims:
Juvenile visceral steatosis (jvs-/-) mice lack the activity of the carnitine transporter OCTN2 and are dependent on carnitine substitution. The effects of carnitine deprivation on carnitine homeostasis and energy metabolism are not known in jvs-/- mice.
Methods:
jvs-/- mice were studied 3, 6 and 10 days after carnitine deprivation, and compared to jvs-/- mice substituted with carnitine, wild-type (jvs+/+) and jvs+/- mice. Carnitine concentrations were assessed radioenzymatically.
Results:
Compared to wild-type mice, carnitine-treated jvs-/- mice had decreased plasma β-hydroxybutyrate levels and showed hepatic fat accumulation. The carnitine levels in plasma, liver and skeletal muscle were decreased by 58, 16 and 17%, respectively. After ten days of carnitine deprivation, the plasma carnitine concentration had fallen by 87% (to 2.3 μmol/l) and the tissue carnitine levels by ≈50% compared to carnitine-treated jvs-/- mice. Carnitine deprivation was associated with a further drop in plasma β-hydroxybutyrate and increased hepatic fat. Skeletal muscle glycogen stores decreased and lactate levels increased with carnitine deprivation, whereas tissue ATP levels were maintained.
Conclusions:
In jvs-/- mice, tissue carnitine stores are more resistant than carnitine plasma concentrations to carnitine deprivation. Metabolic changes (liver steatosis and loss of muscle glycogen stores) appear also early after carnitine deprivation.

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Prenatal Diagnosis PRENATAL DIAGNOSIS 28, 203–208, 2008 IF 1,5

Noninvasive genotyping fetal Kell blood group (*KEL1*) using cell-free fetal DNA in maternal plasma by MALDI-TOF mass spectrometry

Y. Li [1], K. Finning [2], G. Daniels[2], S. Hahn[1], X. Zhong[1], and W. Holzgreve[1]

Abstract:

Background:

Alloimmunization against the fetal Kell (*KEL1*) blood group antigen is gaining importance relative to the Rhesus problem and is the second most important cause of hemolytic disease of the fetus and newborn. Molecular diagnosis for fetal *KEL1*, which currently involves invasive procedures, is routinely done for accessing whether a fetus is at risk. Here we developed a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)-based single allele-based extension reaction (SABER) to examine the fetal *KEL1* gene from *KEL1*-negative pregnant women using cell-free fetal DNA in maternal plasma.

Methods:

Thirty-two maternal plasma samples taken at the second and third trimesters of gestation (median: 21.5 weeks) were examined with MALDI-TOF MS-based SABER. The results were confirmed by serological tests on cord blood or polymerase chain reaction (PCR) typing on amniocyte-derived fetal DNA.

Results:

We were able to detect the fetal *KEL1* allele in 11 of the 13 *KEL1*-positive samples. No false positive results were scored. The paternal *KEL1* allele could be correctly determined in 94% of cases (30/32).

Conclusions:

Our results indicated that the MALDI-TOF MS-based SABER has been used successfully for the detection of the fetal *KEL1* status with the accuracy of 94%. Further, large-scale study, such as multicenter study, can now be explored for clinical application.

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[2] International Blood Group Reference Laboratory, NHSBT, Bristol, UK

Phytomedicine Phytomedicine 15, 120–131, 2008 IF 1,4

Hepatocellular toxicity of kava leaf and root extracts

S. Lüde[1], M. Török[1], S. Dieterle[1], R. Jäggi[2], K. Berger Büter[2] and , S. Krähenbühl[1]

Abstract:

Kava extracts are used widely for different purposes and were thought to be safe. Recently, several cases of hepatotoxicity have been published. To explore possible mechanisms of kava hepatotoxicity, we prepared and analyzed three different kava extracts (a methanolic and an acetonic root and a methanolic leaf extract), and investigated their toxicity on HepG2 cells and isolated rat liver mitochondria. All three extracts showed cytotoxicity starting at a concentration of 50 µg/ml (lactate dehydrogenase leakage) or 1 µg/ml (MTT test). The mitochondrial membrane potential was decreased (root extracts starting at 50 µg/ml) and the respiratory chain inhibited and uncoupled (root extracts) or only uncoupled (leaf extract) at 150 µg/ml, and mitochondrial β-oxidation was inhibited by all extracts starting at 100 µg/ml. The ratio oxidized to reduced glutathione was increased in HepG2 cells, whereas the cellular ATP content was maintained. Induction of apoptosis was demonstrated by all extracts at a concentration of 150 µg/ml. These results indicate that the kava extracts are toxic to mitochondria, leading to inhibition of the respiratory chain, increased ROS production, a decrease in the mitochondrial membrane potential and eventually to apoptosis of exposed cells. In predisposed patients, mitochondrial toxicity of kava extract may explain hepatic adverse reactions of this drug.

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[2] Institute of Anatomy and Embryology, University of Basel, Basel, Switzerland

Fischmarkt-Brunnen

Der Fischmarktbrunnen gilt in der Fachwelt als der schönste gotische Brunnen der Schweiz. Einst zierten 24 Figuren den Brunnen. Erfreulicherweise sind heute noch immer 22 (!!) davon erhalten. Es handelt sich um 13 Engel, 6 Heilige und 3 Propheten. Die drei Hauptfiguren stellen Maria mit dem Kind, ein Szepter in der Hand haltend, Petrus mit dem Schlüssel und den Evangelisten Johannes dar. Die Ecksäulen stehen für Gerechtigkeit, Beharrlichkeit und die Liebe. Der Brunnen muss regelmässig liebevoll gepflegt werden. Die letzte grössere Renovation stand zwischen 1971 und 1974 auf dem Programm, als die Vergoldung erneuert werden musste.

Dissertationen

Mit der Dissertationsprüfung am 18. April 2008 endete die Doktorandenzeit erfreulich für **Ulrich Langenkamp** von der Forschungsgruppe Exp. Hematology (Departement Biomedizin USB). Er hat sich in seiner Dissertation mit dem Thema: «Immunorecognition of Leukemic Stem Cells by NK cells: The role of HDAC inhibitors in NKG2D ligand-mediated anti-tumor responses in Acute Myeloid Leukemia» beschäftigt.

Am 9. Mai 2008 hat sich **Christine Bernsmeier** von der Forschungsgruppe Hepatology (Departement Biomedizin USB) erfolgreich den Fragen des Dissertationskomitees gestellt. Sie hat sich in ihrer Doktorarbeit mit den «Molecular mechanisms of insulin resistance in chronic liver disease» auseinandergesetzt.

Am 12. Juni 2008 durfte sich die Forschungsgruppe Hepatology (Departement Biomedizin USB) nochmals freuen. **Magdalena Sarasin-Filipowicz** verteidigte mit Erfolg ihre Dissertation. Das Thema ihrer Doktorarbeit lautete: «Interferon signaling in chronic hepatitis C: Mechanisms and implications for therapy».

Herzlichen Glückwunsch an alle!

Beförderungen

Venia legendi für Martin Buess

Dr. Martin Buess von der Forschungsgruppe Medical Oncology (Departement Biomedizin USB) hat auf Antrag der Medizinischen Fakultät von der Regenz der Universität Basel die Lehrerlaubnis für Medizinische Onkologie erhalten und darf nun den Titel PD führen.

Herzliche Gratulation!

Uninacht 2008 – ein Abend der Wissenschaft, eine Nacht der Überraschungen

Am 19. September 2008 steht Basel ganz im Zeichen der Universität und ihrer Forschung, Lehre und Dienstleistung. Zum zweiten Mal nach 2004 lädt die Universität Basel die interessierte Bevölkerung zu einem Abend der Begegnungen mit der Wissenschaft und den Menschen dahinter ein. Besonders willkommen sind Studierende und Mitarbeitende, denn die Uninacht ist auch ein Fest der Universität.

Das Einmaleins der ägyptischen Hieroglyphen lernen, Knochen bestimmen, einer Sprengung zuschauen, Gott suchen, das Neuste aus der Welt der Gesundheit erfahren, selber experimentieren – dies sind einige Themen der Uninacht vom 19. September 2008. Sie bietet Gross und Klein eine bunte Palette an lustvollen Begegnungen mit der Wissenschaft und den Menschen, die forschen und lehren. Zahlreiche Veranstaltungen laden ab 16 Uhr ein, Spannendes aus der Wissenschaft auf spielerische Art und Weise zu erfahren. Bis 22 Uhr sind rund zweihundert Vorträge, Ausstellungen, Diskussionen, Lesungen, Filme und Führungen angesagt. Die sieben Fakultäten überraschen die Gäste mit aktuellen und interessanten Themen. Anfassen und Mitmachen heisst die Devise. Auch für Essen, Trinken und viel Musik ist gesorgt.
Also, Termin vormerken!

INSTITUT FÜR BIOCHEMIE UND GENETIK

Akiko Kunita
Tumor Biology

INSTITUT FÜR MEDIZINISCHE MIKROBIOLOGIE

Omar Garcia
Mol. Res. for Diagnostics

Sabrina Köhli
Transplantation Virology

DEPARTEMENT BIOMEDIZIN USB

Manuele Muraro
Oncology Surgery

Peng Xia
Prenatal Medicine

Anke Wixmerten
Tissue Engineering

Daniel Bodmer
Inner Ear Research

Ausserdem haben angefangen:

DEPARTEMENT BIOMEDIZIN USB

Chiara Giovenzana, Oncology Surgery
Giuseppe Sconocchia, Oncology Surgery
Celine Osswald, Prenatal Medicine
Michael Petrich, Neurooncology
Yvonne Schönfelder, Clinical Pharmacology
Marta Bachmann, Experimental Critical Care Medicine
Gabriela Kania, Experimental Critical Care Medicine
Dorothy Huang, Prenatal Medicine
Antje Caelers, Inner Ear Research

INSTITUT FÜR BIOCHEMIE UND GENETIK

Daniel Feltrin, Cell migration and Neurigogenesis

INSTITUT FÜR MEDIZINISCHE MIKROBIOLOGIE

Sandra Girardin, Molecular Diagnostics
Sarah Marlot, Molecular Diagnostics

INSTITUT FÜR PHYSIOLOGIE

Sarah Wakefield, Molecular Diagnostics

Austritte:

DEPARTEMENT BIOMEDIZIN USB

Florent Baty, Pulmonary Gene Research
Michaël Facompré, Pulmonary Gene Research
Cathrin Cattelan, Infectious Diseases
Ulrich Langenkamp, Experimental Hematology
Stefania Riboldi, Tissue Engineering
Emmanuel Rossy, Experimental Immunology
Vera Schwierzeck, Experimental Immunology
Maria Lourdes Sânchez de Miguel, Vascular Biology

INSTITUT FÜR MEDIZINISCHE MIKROBIOLOGIE

Stefanie Fellmann, Transplantation Virology
Claudia Mistl, Transplantation Virology
Daniel Wegmüller, Experimental Oncology

INSTITUT FÜR PHYSIOLOGIE

Enzo Lain, Synapse Formation

Congratulations

Riccardo Stefano Volontè (Francioli)
Geboren am 17.2.2008

Julie Brüngger (Brecht)
Geboren am 10.3.2008

Caterina Giulia Forte (Lino)
Geboren am 28.3.2008

Herzlich willkommen, allerseits!

The spy must make use of the Coriolis effect

Here is the explanation of the current winner **Andreas Ochsenbein**:
The spy can fill the basin with water, which he then empties into the sink and watches the direction of the whirlpool. When the whirlpool generated by the outflow spins counterclockwise he is still in England, when it is clockwise he is in Argentina.
That's correct, therefore the two cinema cards go to **Andreas Ochsenbein**. Congratulations!

PATHÉ!

At this opportunity we thank the Pathé Küchlin AG for sponsoring the tickets.

Whoever else would like to win 2 cinema tickets must correctly solve our next puzzle. The editoral office look forward to your replies, which should be sent to **dbmfacts@unibas.ch**.

The closing date for submission is **15.8.2008**.

DBM Facts-Rätsel

Das folgende Problem sieht sehr einfach aus, doch bedenkt, dass das Gras nachwächst!
12 Kühe fressen in 16 Wochen 10 Hektar Weide komplett (also bis zur Wurzel) auf. 18 Kühe benötigen für diese 10 Hektar 8 Wochen. Wie viele Kühe schaffen das bei 40 Hektar in 6 Wochen?
Tipp: Ruft Euren ehemaligen Mathe-Lehrer an und bittet ihn um Hilfe!

Enigma

The following problem seems very easy, however remember that grass grows back!
12 cows completely eat 10 hectares of meadow (right to the roots) in 16 weeks. 18 cows need 8 weeks for 10 hectares. How many cows does it take to eat 40 hectares in 6 weeks?
Tip: Call your previous maths teacher and ask him for help!

Midsummer in Sweden

Midsummer is a very important and intense feast, that is celebrated by a large part of the Swedish population. It takes place on the weekend closest to June 24th , the weekend after the longest day of the year. The "biggest" day is always the Saturday, but the whole celebration lasts from Friday till Sunday. In the other Scandinavian countries, as well as in the Baltic region, midsummer was actually a dedication to John the Baptist. Not so in Sweden. There, it was only a happy time, spent together with family, friends, and neighbours. People are probably rejoicing at such a wonderful moment of the year, when the dark long nights of winter are so distant. The spirit of midsummer has a particularly large influence on the people in the countryside, and the celebration is probably most pronounced among the people in the area where I was born and raised.

This part of Sweden, Dalarna, is where a ski race takes place over 85 kilometres at the beginning of March, in remembrance of the attempted escape of the young Gustav Eriksson Wasa, as he discovered, in his struggle to free the country from the Danes, that he had no support. As the people of this region realized they had made a mistake, two young men were sent out to catch up with him. They did so at the Norwegian border. This historical incident is the founding story of the ski race "Wasaloppet". The freedom fighter was later to become the king of Sweden on June 6th 1523 under the name of Gutav Wasa.

"Majstàngen" and traditional costumes

People in this area are very bound to cultural traditions as well as being freedom loving. It has a wonderful landscape with green meadows, blue lakes, the famous red wooden houses with white corners and lots and lots of birch trees. When the very symbol of midsummer "Majstàngen", a high wooden pole, is raised in the middle of a green field, it carries a garland made out of birch branches and lovely wild flowers from the meadows. The garland will be carried through the main street of the village by young and old, all dressed in tra-

ditional costumes. The procession is headed by two horse riders, as it proudly moves down the people lined streets in the direction of the celebration ground. The "Majstàngen" is then decorated and raised and everybody sings the traditional songs, as they dance hand in hand around this symbol of midsummer. Many Swedes travel to this area during the midsummer days. Maybe they have roots there. Many do and are proudly declaring it. If it is possible, they will also try to get hold of a traditional costume, which nowadays are very rare and hard to come by as all the individual pieces are handcrafted, and there are very few individuals left who posses the skills to make these garments.

It is like you enter a different world during this time. After the dancing, and singing of the folksongs, and the children's songs around "Majstàngen", the program moves on to the performances of costumed show dancers and a team playing old traditional folk music on the violin. Their musical knowledge is handed down over generations, and many songs have never been put on paper, but have always been handed down from father to son. The best players are awarded an honorary title of the country "Riksspelman". There is also always a possibility for the audience to participate in the traditional dancing. All through these "happenings", people will surely also feel like having a snack now and then. There is a special bread made out of potatoes and cooked in a wood fired oven. It is served as rolls with homemade butter, filled with fresh cheese and chives, fresh brown cheese or herring. Wonderful!

Lena Angman with her traditional Midsummer costume

The festivities are held in all the villages surrounding a large lake, the Siljansee. A long time ago, when people were dependent on boats to get from one village to the other, or even from one farming area to the other, they had these big vessels rowed by 8-10 oarsmen. They were used especially when people went to church on Sundays. In the village Leksand, boats from different villages are raced, and it is a big, joyous, spectacle. On the decorated gangways on the lake, where the other boats come and go, there is music and dancing.

In their homes, people invite friends from near and far and enjoy

"smörgàsbord" which is a buffet containing all of the Swedish specialities your heart could possibly desire. There are meatballs, salad with red beets, herring in all forms, and of course "Janssons frestels", a potato gratin with onion and small salty herring, a real classic! And of course along with that there is beer and "nubbe", which is Aquavit, as well as singing and cheering.

Young girls become romantic

When finally the sun sinks down just a little bit below the horizon for a couple of hours, and the elves and the trolls appear at their forest stage, dancing and giving the night a magic touch, that is the time when the dew will form. It is said it has healing properties for both sick animals and human beings. Maybe on this wonderful night young girls become romantic and collect 7 wild flowers to put under their pillows so that they will dream of their future loved ones, the ones that will become their husbands. However, they must tell no-one about it, as otherwise their dreams will not come true.

Lena Angman

4:3

Match report on the DBM PhD Student Club friendship football game on April 26, 2008

Petrus and Fortuna were on the side of the Swiss football team on that beautiful Saturday afternoon last April, when the students of the DBM PhD student club met for a friendship game "Switzerland – Germany".

Before the game, every member of the Student Club had to decide on which team she or he wanted to play. This led to the team line-ups you can see in the pictures. The game took place on a football field in St. Jakob, which created the appropriate environment for such a promising event.

In a very equal and intense game the Swiss team beat their German competitors with 4:3 goals. After the regular time the score was 3:3 and the Swiss needed extra time and extra luck to decide the game in their favour. From the beginning the German team set the pace to a technically and physically intense match and already scored for the first time after 8 minutes. The Swiss were able to tie before the break, making use of all their individual skills as well as a solid team performance. After the break the German team took the lead twice more with their goals bringing

the score to 1:2 and 2:3. But each time the Swiss made use of their female specialists Cornelia Bigler (2:2) and Géraldine Guex (3:3) to stay in the game. In the extra time both teams had good chances to decide the game, but it was finally Simon Jörger with an irresistable long distance shot that decided the game for the Swiss. It was an exciting and very fair game, with great teams and remarkable goals

(the 3rd German goal scored by Mathias Mehling must be considered world class).

Michel Mallaun

Chirsizyt mit dr Grossmueter

Chumm, mir wei go Chirseli günne,
weiss ame Ort gar cheibe vil;
roti, schwarzi, bipeligäli,
zwöi bis drü a einem Stiil ...
(Schweizer Kinderlied)

Wilde Kirschen waren schon immer heiss begehrt. Bereits die Steinzeitmenschen haben die Früchte gesammelt und gegessen. Archäologen entdeckten bei Ausgrabungen versteinerte Kirschkerne in den Höhlen unserer Vorfahren. Erste gezüchtete Kirschen importierte der römische Feldherr Lucullus aus der pontischen Stadt Giresun. Heute sind Kirschen weltweit in den gemäßigten Klimazonen verbreitet. 80 Prozent der Welternte kommt aber aus Europa. Der Name der Frucht stammt vom iranisch-türkischen «keras» ab, wurde römisch zu «cerasus» und später althochdeutsch zu «kirsa».

Ein halber Liter Kirschsaft deckt den Vitamin-C-Bedarf eines ganzen Tages. Ein halbes Pfund Kirschen täglich kann den Harnsäurespiegel senken und vor Gicht schützen. Ausserdem sollen die in den Kirschen enthaltenen Stoffe für eine schöne Haut sorgen und Entzündungen hemmen. Die Früchte werden zudem in der Naturmedizin als Mittel gegen Parodontose und Arthritis genutzt. Nach einer Studie lindert Kirschsaft auch den Schmerz und den Kraftverlust bei Muskelkater.

Der Kirschkern ist annähernd kugelförmig und ca. 5 bis 8 mm gross. Kirschkerne enthalten einen geringen Anteil Blausäure. Im Backofen erhitzte Kirschkerne, eingeschlossen in einem Stoffbeutel, werden in der physikalischen Therapie (Wärmetherapie) eingesetzt.

Chirsigunfi

1 kg Kirschen

750g bis 1 kg Zucker

Die gewaschenen, entsteinten Kirschen mit dem Zucker vermischen und über Nacht stehen lassen. Hierauf auf mittlerem Feuer unter fleissigem Rühren kochen, bis der Saft in breiten Tropfen von der Kelle fällt. Die Konfiture abschäumen, in die Gläser füllen und diese verschliessen.

Kochzeit: 20–30 Minuten Kirschen (im Besonderen schwarze) gelieren nur schwer. Deshalb empfiehlt sich die Verwendung eines Geliermittels.

Chirsi-Chüechli

Mehl, Salz, Zucker und Zitronenschale mischen. Nach und nach Apfelsaft oder Apfelwein unterrühren, bis der Teig Blasen wirft. Mindestens 30 Minuten ruhen lassen, dann Öl dazurühren und unmittelbar vor Gebrauch die steifgeschlagenen Eiweiss darunter ziehen.

Je 3 bis 5 Kirschen am untersten Ende der Stiele mit Faden zusammenbinden, dann mit Puderzucker bestreuen. Durch den Teig ziehen und 1 bis 2 Minuten bei 170° goldgelb frittieren.

Mit Zimtzucker bestreuen und sofort auf den Tisch bringen.

Zutaten für 4 Personen:

Teig:

150 g Mehl

1 EL Öl

wenig Salz

$^1/_4$ l Apfelsaft oder Apfelwein

2 EL Zucker

abgeriebene Schale von $^1/_2$ Zitrone

2 Eiweiss, steifgeschlagen

500 g Kirschen mit Stiel

1 EL Puderzucker

Zimtzucker

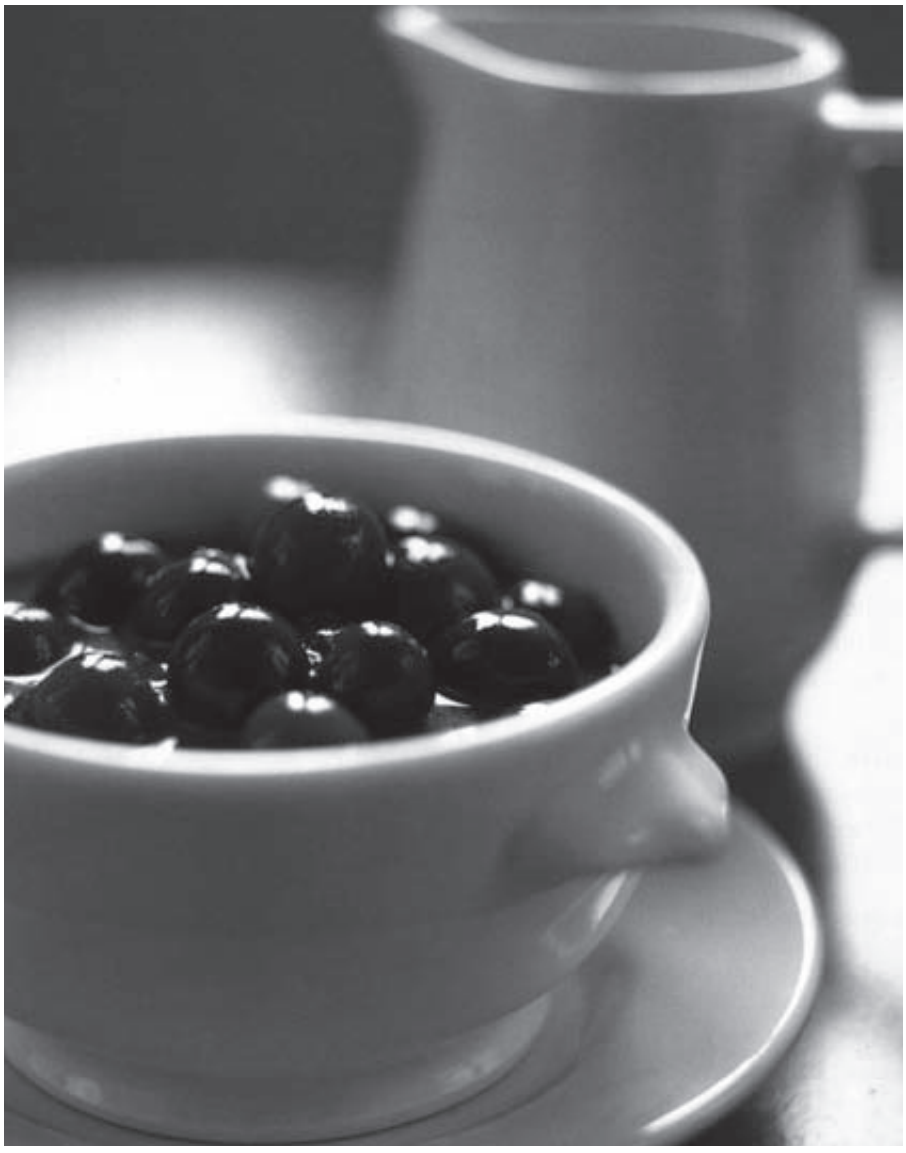
Öl zum Ausbacken

Chirsiprägel

Zutaten ür 4 Personen:
800 g entstielte Kirschen
3–4 EL Zucker
2 dl Wasser oder Apfelsaft
2 TL Maispuder (Maizena)
200 g Einback (oder anderes Brot nach Belieben)
20 g Butter,
1 kl. Stück Zimtstange
etwas Zimtzucker

Kirschen, wenn nötig entstielen, waschen und entsteinen. Den Zucker mit der Zimtstange im Wasser oder Apfelsaft auflösen und aufkochen. Die Kirschen beigeben und 3 bis 4 Minuten leicht kochen. Die Kirschen wieder entnehmen und wegstellen. Das Maispuder in wenig kaltem Wasser aufschlemmen und zur kochenden Flüssigkeit giessen. Unter ständigem Rühren weiterkochen bis die Flüssigkeit gebunden ist. Die Pfanne vom Herd nehmen und die weggestellten Kirschen wieder hinzugeben. Das Brot in mundgerechte Würfel schneiden. Die Butter in einer Bratpfanne erhitzen und das Brot darin rösten.

Das warme Kirschenkompott in vier hohen Tellern anrichten. Die Zimtstange entnehmen und die gerösteten Brotwürfel über die Kirschen verteilen. Sofort servieren und am Tisch nach Belieben Zimtzucker darüber streuen.



Tipps:

Die Grossmutter mischte das Brot noch direkt mit dem Kirschenkompott und richtete es erst dann an.

Damit zunächst noch etwas Salziges auf den Tisch kam, wurde parallel zum Chriesiprägel oft noch eine «Eierrösti» gemacht. Dazu wurden einige mit Milch verdünnte Eier verschlagen, mit Salz und Pfeffer gewürzt und mit dem gerösteten Brot vermischt. Die Mischung wurde ebenfalls nochmals in der Pfanne erhitzt.

Chriesiprägel eignen sich mit einer Kugel Zimteis auch gut als Dessert.

Likör vo schwarze Chirsi

1 kg schwarze Kirschen
$1^1/_4$ l Weingeist (90%)
500 g weisser Kandiszucker
1 l Wasser

Die Kirschen gründlich waschen, abtropfen lassen und entstielen. Dann über einer Schüssel entsteinen und die Steine in die Schüssel fallen lassen. Die Kirschen in 2 vorbereitete weithalsige Flaschen füllen.

Die Steine in ein Sieb schütten, den Saft auffangen. Etwa 10 Kirschsteine zerschlagen und zusammen mit dem Saft in die Flaschen geben. Mit dem Alkohol ganz auffüllen. Die Flaschen gut verkorken und 3 Wochen an einen sonnigen Fensterplatz stellen.

Hin und wieder schütteln. Den Kandiszucker mit dem Wasser mischen, aufkochen und etwa 10 Minuten kochen lassen. Dann in eine Schüssel giessen und vollständig erkalten lassen. Die Kirschen mit dem Alkohol dazugeben, gut durchrühren und durch ein feines Haarsieb abgiessen.

Dabei die Kirschen sanft ausdrücken. Das Sieb dann mit Milchfiltern oder einem Mulltuch auslegen und den Likör durchseihen. In vorbereitete Flaschen füllen, gut verkorken und dunkel aufbewahren.

Haltbarkeit: 6 Monate. Man kann auch den reinen Alkohol durch hochprozentigen Rum ersetzen, dann darf der Kandis jedoch nur in $^1/_2$ l Wasser aufgelöst werden und sollte etwas dicklich eingekocht werden, das dauert etwa 20 Min.

Verena Jäggin

Susanne Blank: Physiologisches Institut

Fotos: J. Claude Rohner

Wie alles begann

Ich bin in Basel geboren, spät genug, um keine 68erin mehr zu sein. Nach den Jugendjahren in Bottmingen im Haus meines Urgrossvaters und anschliessend in Binningen verbrachte ich meine Teenagerjahre in der Christoph-Merian-Siedlung auf dem Bruderholz. In einer Phase, wo mir alles zu langweilig geworden war, schmiss ich das Gymnasium und wollte «ins Leben eintreten». Ich machte eine kaufmännische Lehre bei der National-Versicherung. Dann wurde es endlich spannend: Ich reiste mit Interrail in Europa herum und genoss anschliessend einen dreimonatigen Sprachaufenthalt in Perugia - ich liebe Italien in jeder Beziehung! Zurück in der Schweiz beglückte ich die Assista Rechtsschutzversicherung in Genf, die Pax Versicherung in Basel jeweils als Sachbearbeiterin und seit 1985 bin ich für das Sekretariat des Physiologischen Instituts zuständig.

Was macht die denn eigentlich den ganzen Tag?

Bis Frühjahr 2001 war Prof. Leonhard Hösli Vorsteher des Physiologischen Instituts und mein Chef. Das Institut war zu jener Zeit im Vesalianum untergebracht. Nach der Pensionierung von Herrn Hösli übernahm im Herbst 2001 Prof. Bernhard Bettler die Leitung. Seither ist mein Arbeitsplatz im Pharmazentrum, 7. Stock, von wo ich einen wunderbaren Ausblick auf die Umgebung geniesse. Allerdings habe ich wenig Zeit, mich dem Blick in die Ferne hinzugeben, denn die Zahlen wollen gehäuft werden, die Administration muss brummen, die Buchhaltung ins Lot gebracht werden, die Anstellungen müssen klappen und diverses Material entweder ins Haus kommen oder seinen Weg in die Welt finden. Dazu stehen Abklärungen mit anderen Dienststellen und Bereichen, Auskünfte und Hilfestellungen an. Und natürlich versuche ich, die Agenda meines sympathischen Chefs im Auge zu behalten. Für mich ist es auch wichtig, dass ich von netten Arbeitskolleginnen und Arbeitskollegen umgeben bin, so dass ich mich in meiner Arbeitswelt wohl fühle.

Es gibt auch ein Leben neben der Uni

Weil ich gerne koche, hat mein Freund darauf bestanden, dass die Waage aus dem Badezimmer entfernt wird. Mode ist eine andere Leidenschaft von mir. Schönen Stoffen kann ich schlecht widerstehen, und seit es Esempio Tessuti gibt (hoffentlich honorieren sie das product placement!), komme ich mit Nähen fast nicht mehr nach. Zum Glück gibt es an der Uni ja nicht nur Arbeit, sondern auch Ferien, welche ich oft für verschiedene Reisen nutze. Über die diesjährige Auffahrt habe ich für mich Riga und Tallinn entdeckt, wunderschöne Städte im Baltikum. Lissabon und Hamburg stehen auf der Liste, aber auch Marokko und Thailand. Mexiko, Vietnam, Madagaskar, Ägypten, Sibirien, Italien, Südfrankreich habe ich schon durchwandert und befahren. Ich mag aber auch Tages- und Shoppingausflüge in der weiteren Regio. Und ansonsten hänge ich an Basel wie schon mein Urgrossvater:

My Basel

(Theobald Baerwart)

Das isch my Stadt, my Basel
Am Gnei vom wilde Rhy;
Es kennt e bitzli greesser,
Doch ‚s kennt nit lieber sy.

‚s macht vo sich nit vyl Wäse,
Blagiert nit mit der Graft,
Sy Liebi gheert der Arbet,
Sy Ruehm isch d'Wisseschaft.

Johrhundert hän's umbrandet
Mit Grieg und Gwitterstirm,
Und immer no in d'Wulgge
Dien rage d'Minschterdirm.

Und immer no duet ruusche
My liebe-n-alte Rhy,
Hit z'obe, noh de Säxe
Gang i go bade dry.

Deconvolution

Deconvolution is a process based on a mathematical algorithm to reverse the effect of convolution that occurs during image acquisition on an optical microscope. Deconvolution is therefore used during image processing to recover objects from an image that is degraded by blurring and noise.

Image Formation

An image is always the product of the real object and its point spread function (PSF; Fig. 1a). The PSF itself is a probability map: not all the photons emitted by a point source and refracted by a lens converge onto the image point corresponding to the object. The probability that they do is high, but not as high as 100%. Some photons end up in the space surrounding this point, with probabilities inversely related to distance from it. The map showing these probabilities in 3-dimensional image space represents the PSF (Fig. 2, High intensity stands for high probability of photon incidence).

The PSF is dependent on the optical system and leads to a degradation of the image. The degree of blurring is an indication for the quality of an imaging system. Imaging a point source on a confocal microscope for example will always appear as an extended blurry spot. The process that leads to the formation of such an image is called convolution.

Knowing the PSF of a microscope, an image corresponding to the original object can be constructed using a mathematical algorithm. This process – the opposite of convolution – is called deconvolution (Fig. 1b).

The Point Spread Function (PSF)

As already mentioned the PSF depends on the optical system. We can obtain it in two ways:

1. By measuring the actual PSF of a particular microscope. This is done by recording images of small beads of known size. The images are then used to calculate the PSF.
2. By calculating a theoretical PSF from the technical parameters of the microscope (numerical aperture, magnification, and wavelength).

The first method yields more accurate results, because it takes the characteristics of a particular optical system into account – lens defects, for example. On the other hand, multiple measurements have to be taken, since all the settings (lens, wavelength etc.) adopted for taking the image of the specimen have to be identical when measuring the PSF. Calculating a theoretical PSF is obviously much easier, but not as precise. However, it will do in most cases.

Sampling conditions

Images destined for deconvolution have to meet the following criteria:

Figure 1a

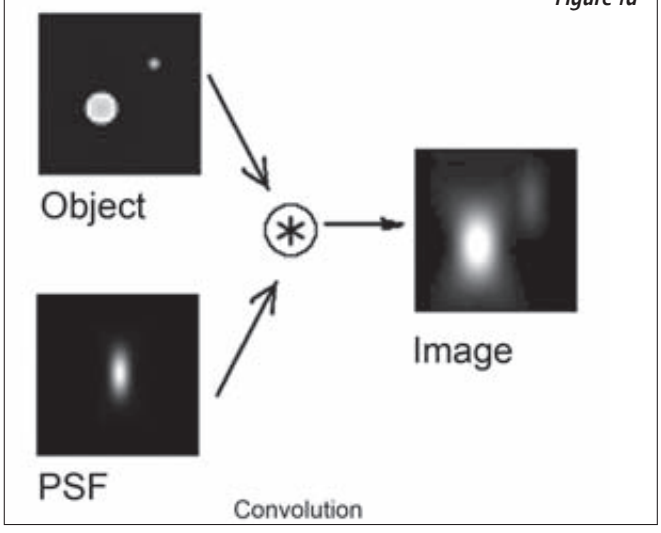
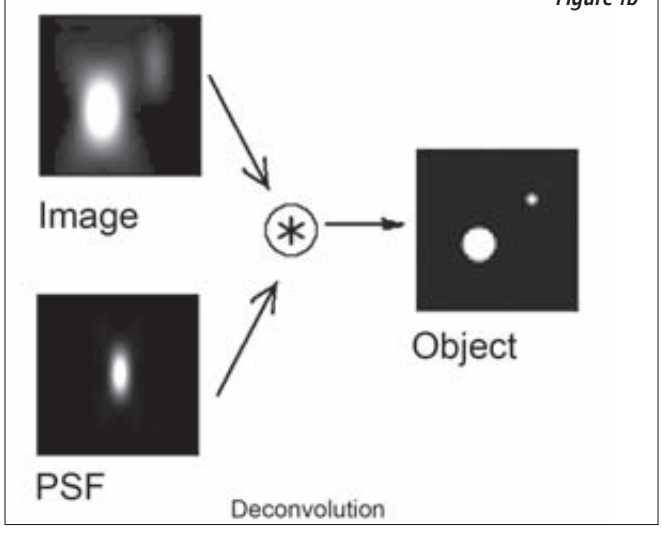
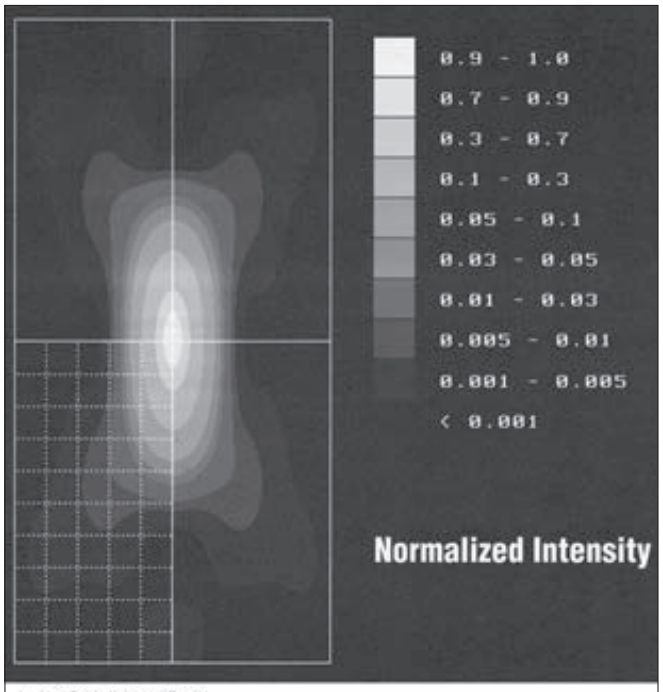


Figure 1b





Leica S.Hell (modified) ***Figure 2***

- The sampling density of an image stack has to be adequate, meaning: the pixel size and the distance between the image planes have to be small enough, close to the Nyquist rate. Therefore the image has to be sampled at least twice the maximum frequency of the pattern (Fig. 3).
- Clipping must be avoided. Clipping occurs if the dynamic range is exceeded and brightness values are either saturated (255 in an 8bit image) or black (0).
- The dimensions of an image stack have to be chosen such that the top and bottom planes lie outside the object.

Unfortunately, Nyquist sampling is often difficult to achieve. The high sampling densities may result in long acquisition times and excessive bleaching of the fluorescent marker. And the large file sizes make heavy demands on computer memory, not only during the deconvolution process itself, but also when handling the deconvolved image stacks.
It therefore stands to reason that optimal sampling is not always possible, and that compromises have to be made. Deconvolution is a nice tool, but good results are not achieved without an adequate effort!

Huygens at the DBM Mattenstrasse

We are using the Huygens Professional deconvolution software from Scientific Volume Imaging (SVI; Hilversum, NL). It is a state of the art package used by leading imaging facilities (Fig. 4).
At Mattenstrasse 28, we are running Huygens on a Linux server equipped with two Dual Core AMD Opteron 2220 processors and 16GB of DDR2 memory. This allows us to deconvolve an 800MB stack in about 30 minutes.
In the long run, we plan to establish remote access to our Huygens server such that users from other locations are able to use the Huygens package. We also help people with image acquisition, the deconvolution procedure, and subsequent image rendering. If you are interested don't hesitate to contact us!

Pascal Lorentz and Jörg Hagmann

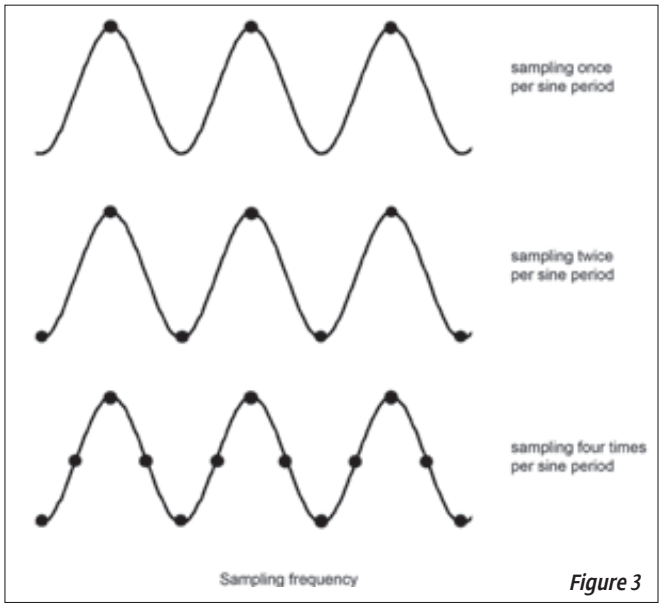
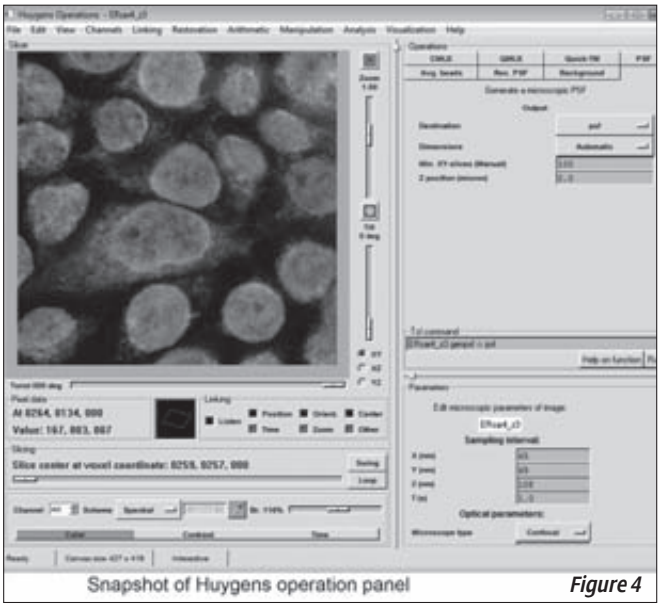


Figure 3



Snapshot of Huygens operation panel ***Figure 4***

Die versäumten Lebensziele:

Freuden, Schönheit und Natur,

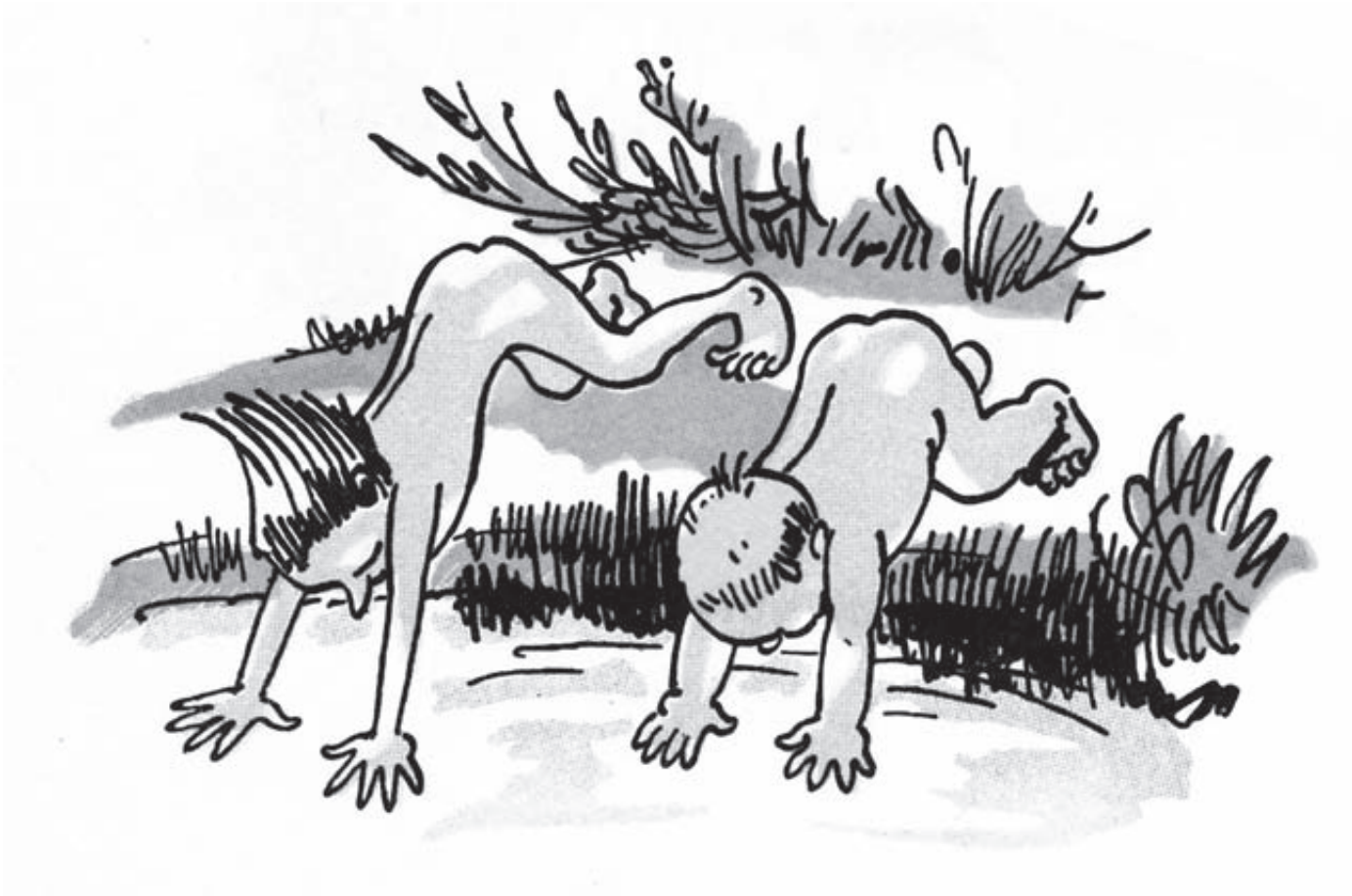
Gesundheit, Reisen und Kultur.

Drum, Mensch, sei zeitig weise!

Höchste Zeit ist's!

Reise, reise!

(Wilhelm Busch)



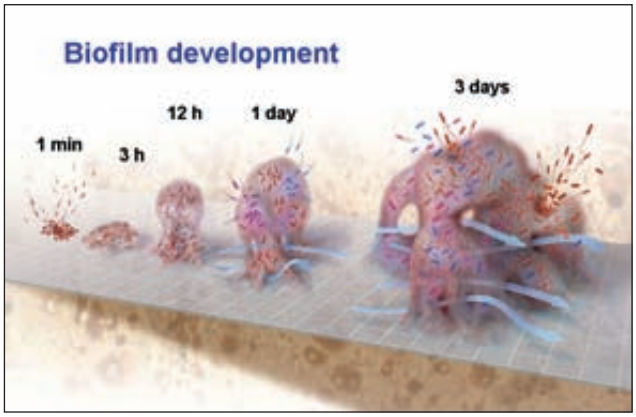
Die DBM Redaktion wünscht allen

Leserinnen und Lesern

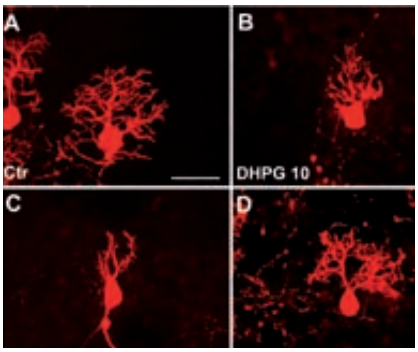
schöne und erholsame Ferien!

VORSCHAU PREVIEW

In der nächsten Ausgabe . . .



... gibt uns Andrej Trampuz einen Einblick in seine Forschungstätigkeit im Bereich Infectious Diseases



... stellt uns Josef Kapfhammer seine Forschungsgruppe Developmental Neurobiology and Regeneration vor

... stellt sich USB-Direktor Werner Kübler den Fragen der DBM Facts-Redaktion

... lässt uns Michael Heberer teilhaben an der Faszination Motorfliegen

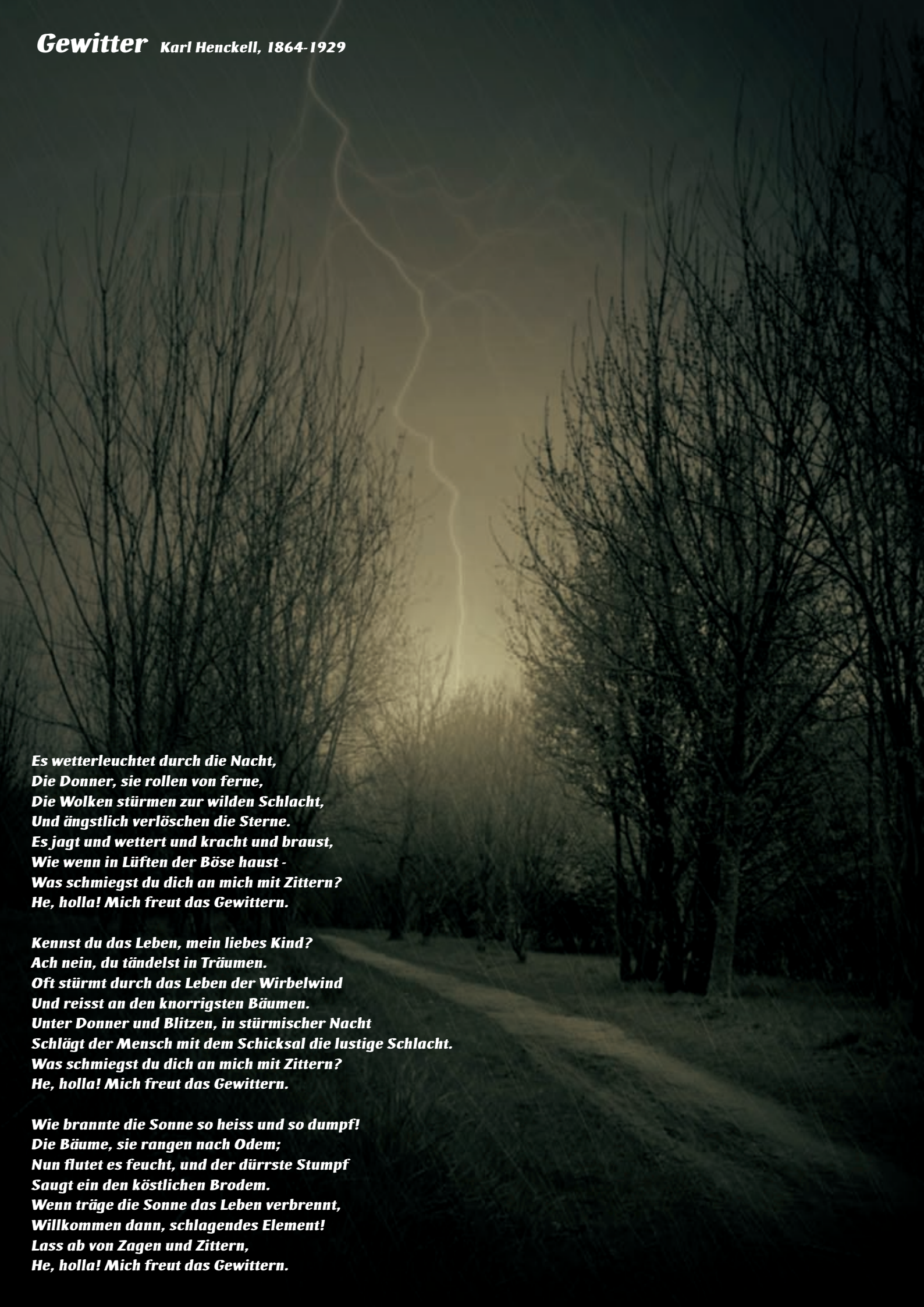
... nehmen wir Sie mit auf die schönsten Wanderrouten der Schweiz

Und ausserdem

. . . die Gewinner von 2 FCB-Tickets vom Euro 08-Wettbewerb

. . . die Gewinner der 2 Kino-Tickets vom DBM Facts-Rätsel

. . . neue Rezepte speziell für die Herbstzeit



Gewitter

Karl Henckell, 1864-1929

Es wetterleuchtet durch die Nacht,
Die Donner, sie rollen von ferne,
Die Wolken stürmen zur wilden Schlacht,
Und ängstlich verlöschen die Sterne.
Es jagt und wettert und kracht und braust,
Wie wenn in Lüften der Böse haust -
Was schmiegst du dich an mich mit Zittern?
He, holla! Mich freut das Gewittern.

Kennst du das Leben, mein liebes Kind?
Ach nein, du tändelst in Träumen.
Oft stürmt durch das Leben der Wirbelwind
Und reisst an den knorrigsten Bäumen.
Unter Donner und Blitzen, in stürmischer Nacht
Schlägt der Mensch mit dem Schicksal die lustige Schlacht.
Was schmiegst du dich an mich mit Zittern?
He, holla! Mich freut das Gewittern.

Wie brannte die Sonne so heiss und so dumpf!
Die Bäume, sie rangen nach Odem;
Nun flutet es feucht, und der dürrste Stumpf
Saugt ein den köstlichen Brodem.
Wenn träge die Sonne das Leben verbrennt,
Willkommen dann, schlagendes Element!
Lass ab von Zagen und Zittern,
He, holla! Mich freut das Gewittern.