Prenatal Medicine

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Multimodal regulation of neutrophil NETosis in pregnancy and disturbance in inflammatory conditions

Human pregnancy is associated with a mild pro-inflammatory state, characterized by activation of circulatory neutrophils. We have previously shown that this is disturbed in pathologies such as preeclampsia, leading to excessive neutrophil extracellular trap (NETs) formation. NETs are a rather unique innate immune tool employed by granulocytes, whereby their nuclear DNA is extruded into the extracellular environment to ensnare and kill a wide-array of microorganisms, ranging from bacteria, fungi to parasites. Aberrant NETs formation may damage or induce cell death of surrounding tissues, and is implicated in a number of pathologies including rheumatoid arthritis (RA), SLE, small vessel vasculitis, or coagulopathies.
The underlying signal transducing pathway initiating the NETotic process involves calcium mobilization, generation of ROS by NAPDH oxidase, nuclear localization of both neutrophil elastase (NE) and myeloperoxidase (MPO), and citrullination of histones by peptidylarginine deiminase 4 (PAD4). The latter events contribute to chromatin unfolding, a prerequisite for efficient DNA extrusion.
To date no study has examined NETs generation in normal pregnancies, nor which factors could modulate such a response during extensive period of human gestation. For this reason we recently examined NETosis in all three trimesters of normal pregnancy. Our data indicate that neutrophils from normal healthy pregnancies exhibit a distinct pro-NETotic phenotype, which increases towards term. This was characterised by an elevated response to pro-NETotic stimuli, as well as clear elevations in the expression of key signalling components required for efficient NETs formation.
In our studies to ascertain which factors drive this phenotypic change, we determined that G-CSF, the circulatory levels of which also increase in parallel during pregnancy, plays a key role in promoting a progressively enhanced NETotic state. We also noted that early in gestation (1st trimester), NETosis is augmented by the action of human chorionic gonadotropin (hCG), which boosts the action of G-CSF, in promoting a primed pro-NETotic phenotype.
As pregnancy advances, and the levels of hCG subside to be replaced by other steroid hormones expressed by the placenta, namely estrogen (E2) and progesterone (P4), we observed that maternal neutrophil activity is modulated in a considerably more complex manner. In this regard, E2 acts by being pro-NETotic. This action is antagonized by the action of P4, which serves to retain neutrophils in a highly primed state, yet hindering NETs formation. Our data suggest that the regulatory mechanism evoked by P4 involves a blockage of NE localisation to the nucleus, a step previously shown to be vital for efficient NETs formation. Since neutrophil NETs were originally described as an anti-pathogenic mechanism, our data would

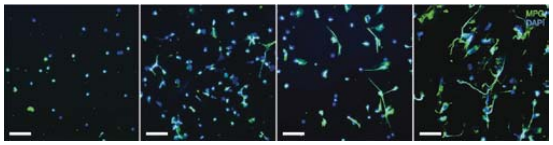

Fig. 1: NET formation and neutrophil pro-NETotic priming are augmented during pregnancy. *In vitro* spontaneous NET formation by neutrophils from healthy pregnant donors over a 3 hour time course by fluorescence microscopy using Immunofluorescence staining for MPO (green) and DNA counterstain with DAPI (blue). Scale bars: 50 µm.

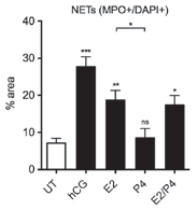

Fig. 2: Neutrophil pro-NETotic priming is regulated by pregnancy hormones.
Morphometric analysis of the NETotic (MPO+/DAPI+) neutrophils from healthy control donors after 2 hours treatment with physiologic concentrations of hCG, E2, P4 and E2/P4.

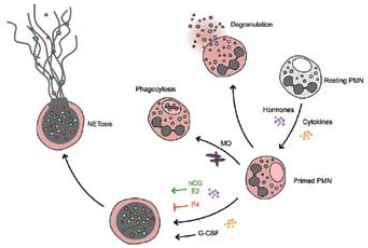

Fig. 3: Progesterone antagonises the estrogen and G-CSF driven neutrophil extracellular trap formation during pregnancy.
During pregnancy neutrophils lie under the increased influence of cytokines, e. g. G-CSF, and sex hormones. This specific milieu appears to poise the neutrophils in a stable pro-NETotic primed state. Depending on the stimulus, for instance microorganisms (MO), neutrophils react by phagocytosis or degranulation. When a different NETotic stimulus is present, such as excessive placentally derived plasma microparticles (MP) in preeclampsia (25), primed neutrophils react with overt NET release. Pro-NETotic combinations of hormones and cytokines are given in green, the most potent in bold green. Inhibitory combinations are given in red, the most potent in bold red.

suggest that this operative arm of the innate immune response is highly pro-active in human pregnancy. In this manner, by being in a highly primed pro-NETotic state, such pre-activated neutrophils could react immediately to a pathogenic threat.
Our data also provide new insight into how aberrancies in this system may contribute to the underlying aetiology of preeclampsia, in that this condition is associated with elevated levels of hCG and G-CSF, which would serve to enhance NETosis. Since there are suggestions that progesterone levels may be reduced in preeclampsia, such an imbalance may trigger an enhanced pro-NETotic response, which is exacerbated by the occurrence of inflammatory placental micro-debris, abundant in this condition.
On the other hand, our data may provide a novel insight into autoimmune conditions such as systemic lupus erythematosus, which is associated with reduced levels of progesterone, both during the menstrual cycle, as well as during pregnancy. Since NETosis is altered in SLE, it is possible that the negative feedback loop hindering NETs formation provided by progesterone contributes to the preeclampsia-like symptoms frequently observed in pregnant women affected by SLE.
These facets are being examined in ongoing studies, together with the Prof. P. Hasler, Aarau

Selected Publications

Gupta A, Giaglis S, Hasler P, Hahn S. (2014) Efficient neutrophil extracellular trap induction requires mobilization of both intracellular and extracellular calcium pools and is modulated by cyclosporine A. Plos One May 12;9(5):e97088
Chowdhury CS, Giaglis S, Walker UA, Buser A, Hahn S, Hasler P. (2014) Enhanced neutrophil extracellular trap generation in rheumatoid arthritis: analysis of underlying signal transduction pathways and potential diagnostic utility. Arthritis Res Ther 16
Wu M, Ries JJ, Proietti E, Vogt D, Hahn S, Hoesli I. (2016) Development of Late-Onset Preeclampsia in Association with Road Densities as a Proxy for Traffic-Related Air Pollution. Fetal Diagn Ther 39:21–27
Giaglis S, Stoikou M, Grimolizzi F, Subramanian B Y, van Breda SV, Hoesli I, Lapaire O, Hasler P, Than NG, Hahn S. (2016) Neutrophil migration into the placenta: Good, bad or deadly? Cell Adh Migr:1–18
Giaglis S, Stoikou M, Sur Chowdhury C, Schäfer G, Grimolizzi F, *et al.* (2016) Multimodal regulation of NET formation in pregnancy: progesterone antagonizes the pro-NETotic effect of estrogen and G-CSF. Frontiers in Immunology 7

Connection to Clinical Practice

Prof. Dr. med Irene Hösli,
Prof. Dr. med. Olav Lapaire
University Women's Hospital Basel

Is NETosis altered in pregnancies complicated by the "Great Obstetrical Syndromes"?
We have previously detected aberrant NETs formation in pregnancies affected by preeclampsia (PE). The key aetological feature driving the development of PE is defect in placentation, specifically a failure in the transformation of maternal spiral arteries from a high pulsatile system to a relaxed low-pressure system. This placental aberrancy in more evident in cases with early onset PE, than those developing PE close to term.
Recent studies have indicated that such placental defects are not restricted only to PE, but are also evident in intra-uterine growth restriction (IUGR), and as suggested by new reports, may even occur in preterm labour (PTL).
Consequently, defects in spiral artery modification may be common aetiological factors in the "great obstetrical syndromes" (GOS) of PE, IUGR and PTL. What is unresolved is how defective placentation contributes to such disparate pathologies.
Our query is to determined whether these defects in placentation evident in the GOS are analogous, or whether only those in early onset PE trigger an overt maternal inflammatory response. As a readout for our study, we will examine the activity of circulatory maternal neutrophils, particularly their ability to undergo NETosis, as we have observed this to be a reliable marker for inflammation. Hence, our study may shed new light onto similarities or differences between the GOS disorders, and also contribute into the development of new screening markers.
Together with Prof. Gabor Than (Budapest) we are investigating the role of PP13, a placentally derived galectin, in modulating neutrophil activity in PE and other GOS.