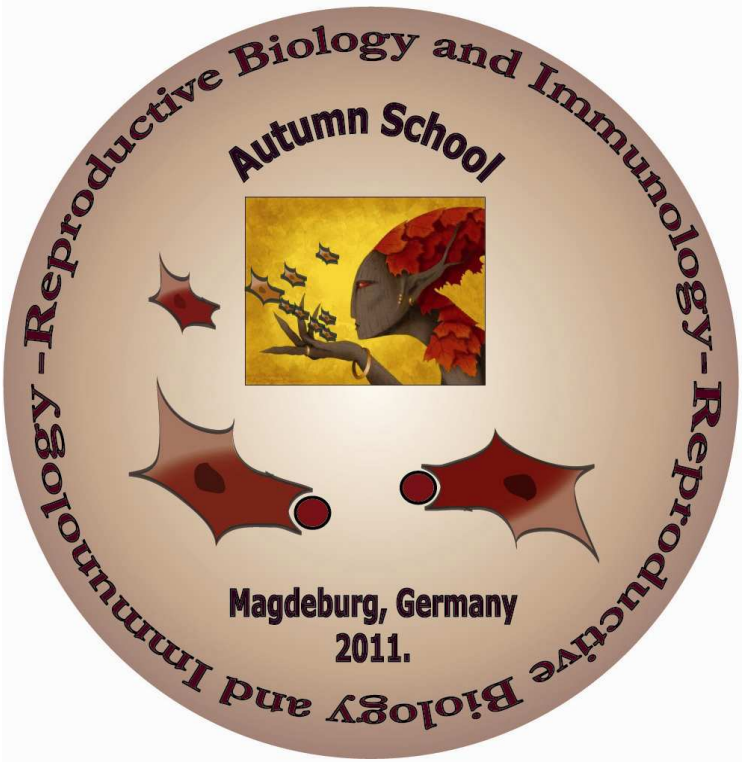
FIRST REPRODUCTIVE BIOLOGY AND IMMUNOLOGY AUTUMN SCHOOL



October 5th -8th 2011, Magdeburg, Germany 2011

Venue: **Herrenkrug Park Hotel**
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Scientific Organization

Prof. Dr. Ana Claudia Zenclussen & Dr. Federico Jensen
Experimental Obstetrics and Gynecology, Medical Faculty, Otto-von-Guericke-University, Magdeburg, Germany

Scientific Committee

Ana Claudia Zenclussen (Magdeburg, Germany), **Federico Jensen** (Magdeburg, Germany), **Gil Mor** (New Haven, USA), **Petra Arck** (Hamburg, Germany), **Anne Leber** (Magdeburg, Germany), **Monica Vazquez-Levin** (Buenos Aires, Argentina)

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DFG Deutsche Forschungsgemeinschaft

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We also acknowledge the financial support of the participants by

ISA "International Society of Andrology"

City of Magdeburg

CONFERENCE PROGRAM

Wednesday, October 5th 2011

15:00 Registration

17:30 Welcome Ceremony

Welcome notes from:

Prof. Dr. Ana Claudia Zenclussen (Organizer)
Dr. Federico Jensen (Organizer)
Dr. Klaus Puchta (City of Magdeburg)
Prof. Dr. Leßmann (Research Pro-Rector of the Otto-von-Guericke University, Magdeburg)

18:00 Keynote Lecture
Chair: Ana Claudia Zenclussen

Gil Mor, Yale Medical School, New Haven, USA: **"Pregnancy and inflammation: the good and the bad"**

19:00 Dinner and get together

Thursday, October 6th 2011

Morning Session I:
Chairs: Berend Isermann/Federico Jensen

8:00 **Signalling crosstalk of nuclear hormone receptors in the female reproductive tract**
Günter Vollmer, Institute for Zoology, Technical University, Dresden, Germany.

8:50 **Molecular determinants of sperm-oocyte interactions**
Monica Vazquez-Levin, National Research Council of Argentina, Buenos Aires, Argentina.

9:40 **Coffee break**

10:00 **Molecular and cellular mechanisms involved on the process of embryo implantation**
Udo Markert, Placenta laboratory, Friedrich-Schiller-Universität Jena, Germany.

10:50 **Placenta physiology and regulation**
Berthold Huppertz, Medical University, Graz, Austria.

Workshop I

11:40 **Cells at the fetal-maternal interface**
Gil Mor, Yale University School of Medicine, New Haven, USA.

12:30 **Lunch**

13:40 **Transfer to the laboratory for the practical course**

14:00 **Practical training I**

A) Isolation of immune cells from murine decidua. Sample preparation and analysis by flow cytometry. **Assistants:** Anne Leber, Nadja Linzke, Katja Woidacki, Tarek El-Mousleh, Ana Teles.

B) Isolation of murine spermatozoa and oocytes, sperm capacitation and in vitro sperm-oocyte interaction assays. **Assistants:** Mónica Vazquez-Levin, Nadia Edelsztein.

17:00 **Transfer to the Herrenkrug Park Hotel**

17:30 **Boat trip with sightseeing followed by an informal party at Mückenwirt Restaurant (www.mueckenwirt.de).**

Friday, 7th October 2011

Minicourses

8:00 **Minicourse I: How to plan, organize and conduct animal experiments.**

Oliver Zierau, Institute for Zoology, Technical University, Dresden, Germany.

8:30 **Minicourse II: The long way from the bench to the patients, going from basic research to clinical trials.**

Serban-Dan Costa, Department of Obstetrics and Gynecology, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany.

09:00 **Selected oral presentations**

Chairs: Guillermina Girardi/Berthold Huppertz

09:00 **(1) Notch pathway expression in the male mouse reproductive tract**

Murta D, Batista, Trindade A, Silva E, Henrique D, Duarte A, Lopes da Costa L

09:15 **(2) IL-17A concentration of seminal plasma and follicular fluid in infertile men and women with various clinical diagnoses**

Mohammad Ali Sabbaghi, Hessam Roustaei, Raheleh Aram, Mahla Fadavi Islam, Maryam Daneshvar, Raul Castano, Alireza Haghparast

09:30 **(3) Influence of transgenerational soy isoflavones exposition on physiological and endocrinological relevant parameters in female rats**
Thomas Brockmann, Annekathrin Keiler, Oliver Zierau, Günter Vollmer

09:45 **(4) The cytoskeletal protein LASP is expressed by trophoblast cells and chorioncarcinoma cells and modulates their proliferation and migration**
S.E. Segerer, L. Rieger, M. Kapp, J. Dietl and U. Kämmerer

10:00 **(5) Epidermal Growth Factor triggers STAT5 phosphorylation and enhances proliferation of trophoblastic cell lines.**
Ospina-Prieto S, Morales-Prieto DM, Markert UR

10:15 **(6) Anti-microbial peptide expression in the female reproductive tract in response to reproductive status and viral infection**
Karen Racicot, Ingrid Cardenas, Paulomi Aldo and Gil Mor

10:30 **(7) Origin of regulatory T cells during pregnancy**
Teles A, Leber A, Scharm M, Zenclussen A.C

10:45 **Coffee Break**

Workshop II

11:00 **Animals models for studying pathologies related to pregnancy**
Guillermina Girardi, The Queen's Medical Research Institute, University of Edinburgh, UK.

Morning session I

Chairs: Carlos Tadakoro/Franziska Schmerse

11:50 ***In vivo*** **imaging of the immune system**

Peter Reichardt, Institute of Molecular and Clinical Immunology, Otto von Guericke University, Magdeburg, Germany.

12:40 **Lunch**

Afternoon Session I

Chairs: Judith Bulmer/Stefan Fest

14:00 **Management of pre-term birth**

Serban-Dan Costa, Department of Obstetrics and Gynecology, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany.

14:50 ***In vitro*** **fertilization**

Jürgen Kleinstein, Department of Reproductive Medicine, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany.

15:40 **Coffee break**

Afternoon Session II

Chairs: Udo Markert/Nadja Linzke

16:00 **Human natural killer cells in uterine mucosa**

Judith Bulmer, Newcastle University, Medical School, Newcastle, UK.

16:50 **Uterine natural killer cells: new paradigms**

Anne Croy, Queen's University Kingston, Queens, Canada.

17:40 Neuroendocrine regulation of fetal-maternal interaction

Petra Arck, Experimental Feto-Maternal Medicine, Eppendorf University, Hamburg, Germany.

19:30 Poster Party (finger food, beer and wine)

Saturday 8th, October 2011

9:00 Practical training II (*in vivo* imaging of the uterus)

Dr. Carlos Tadokoro, Lab of Immune Regulation and Unit of Cell Imaging, Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Lecture Abstracts

Inflammation and Pregnancy: the good and the bad

Gil Mor

Department of Obstetrics, Gynecology & Reproductive Sciences. Reproductive Immunology Unit Yale University School of Medicine, New Haven CT

Placental immune response and its tropism for specific viruses and pathogens affects the outcome of the pregnant woman's susceptibility to and severity of certain infectious diseases. The generalization of pregnancy as a condition of general immune suppression or increased risk is misleading and prevents the determination of adequate guidelines for treating pregnant women during pandemics. There is a need to evaluate the interaction of each specific pathogen within the fetal/placental unit and its responses in order to design the adequate prophylaxis or therapy.

In addition, it is essential to evaluate the presence of maternal viral infections prenatally to prevent long-term adverse outcomes for the child and the mother.

Here, we will discuss new data associated with the specific role of inflammation during pregnancy, specifically during the process of implantation. In addition, a different inflammatory process might be initiated as a response of the placenta to viral infection which will have a significant effect on fetal development. We propose that in normal conditions the female reproductive tract prevents immune responses to bacterial products, which are a natural component of the tract, in order to avoid a chronic state of inflammation. Viral infection may reverse this state of homeostasis. If the infection occurs during pregnancy, the outcome of the pregnancy may be jeopardized. Our objective is to elaborate on the mechanisms mediating the changes associated with viral and bacterial infection during pregnancy.

Signalling crosstalk of nuclear hormone receptors in the female reproductive tract

Günter Vollmer

Technische Universität Dresden, Fachrichtung Biologie, Professur für Molekulare Zellphysiologie & Endokrinologie, Zellescher Weg 20b, 01217 Dresden, Germany

Nuclear hormone receptors are key players in the regulation of proliferation and differentiation of female reproductive tract organs. The aim of this lecture is to review key aspects of the molecular mode action of nuclear receptor signaling, primarily estrogen receptor (ER) dependent signaling pathways. Particularly emphasis will be given on the regulation of transcription and proliferation. In addition potential mechanisms of developmental origin of hormone dependent diseases and the prevention thereof will be discussed.

Mechanisms of transcriptional control comprise ER/estrogen-response-element (ERE) dependent signaling pathways, tethering mechanisms, estrogen independent pathways and membrane initiated steroid signaling, also referred to as rapid action. In this part of the lecture an overview will be given on the role of available pharmacological tools to study the above mentioned signaling pathways.

The section on regulation of proliferation in the reproductive tract will focus on two major issues: a) the contribution of ERE-independent mechanisms in the regulation of proliferation in the reproductive tract and b) the role of the tissue compartments epithelium and stroma in the control of proliferation. In this part of the presentation the crucial role of suitable transgenic animal models to unravel the molecular mechanisms of regulation of proliferation in the reproductive tract will be illustrated.

Finally, more and more evidence accumulates that developmental endocrine disruption will lead to a later disease onset and will even trigger multi- or transgenerational phenotypes of compromised reproduction. In addition, first pieces of evidence become available that nutritional prevention of disease by hormone-like substances contained in the diet may also have a in part a developmental origin. This part of the lecture will briefly review available information on epigenetic mechanisms involved in triggering late onset or even multigenerational effects on health outcomes following developmental exposure of an organism to endocrine active substances.

Molecular determinants of sperm-oocyte interactions

Mónica Vazquez-Levin

Instituto de Biología y Medicina Experimental National Research Council of Argentina, Buenos Aires, Argentina

Fertilization is a fundamental process that involves a highly coordinated sequence of interactions between the female and male gametes. In the last 30 years, many events of this process have been elucidated, but the molecular bases are not fully understood. Spermatozoa that have completed spermatogenesis in the testis undergo epididymal maturation, a process by which they acquire ability to move progressively and to interact with the egg vestments. Once ejaculated, spermatozoa are deposited in the female reproductive tract, where they interact with the oviductal

cells and their secretions. As result of these interactions, spermatozoa undergo a complex series of changes collectively known as sperm capacitation and acquire full competence to fertilize the egg. Recent studies indicate that capacitated spermatozoa follow guidance mechanisms towards the egg by means of thermotaxis and chemotaxis. Once they arrive to the oviductal *ampulla*, spermatozoa interact with cellular (*cumulus oophorus*) and acellular (*Zona Pellucida*; ZP) structures that surround the egg. These interactions trigger the sperm Acrosome Reaction (AR, or Acrosomal Exocytosis, AE), a unique exocytotic event that involves fusion of the sperm plasma and outer acrosomal

membranes and the release of the acrosomal granule content. Once acrosome-reactedspermatozoa reach the perivitelline space, they bind and fuse to the egg plasma membrane. Ultimately, the fertilizing spermatozoon is incorporated into the egg cytoplasm and the nucleus is decondensed.

Our group has developed several projects aimed at studying proteins and mechanisms involved in fertilization. We have 1) identified modulators of sperm function (i.e. incubation temperature, calcium ion concentration, and antisperm antibodies present in biological fluids upon sperm motility, capacitation and AE), 2) characterized proteins from spermatozoa (i.e. CaM Kinase IV, proacrosin/acrosin) and from female tract secretions (Grp78/BiP), 3) assessed their role in the development of sperm fertilizing ability and, in some cases, 4) evaluated their relationship with infertility. In recent years, our projects have been extended to study members of the cadherin superfamily: we have 1) characterized the expression of epithelial and neural cadherin in reproductive tissues and gametes and 2) shown evidence of their participation in fertilization-relatedevents. Our studies have utilized human, murine and bovine models.

Molecular and cellular mechanisms involved on the process of embryo implantation: from signalling molecules to micro-RNAs

Udo Markert

Placenta Lab, Department of Obstetrics, Friedrich-Schiller-University Jena, Germany

Trophoblast cells develop mannifold functions depending on progress of pregnancy, their localization and their environment. This environment is composed of a potpourri of maternal stroma and immune cells as well as of soluble factors which may be released locally or systemically and which include numerous hormones, cytokines and others. A variety of receptors on trophoblast cells receive extracellular signals and transform them into intracellular signals. Several cytokines of the interleukin-6 (IL-6) family are present in the placenta and trophoblast cells express gp130 which is the common receptor chain for these cytokines as well as receptor chaines specific for several members of the IL-6 family. The receptor activates various intracellular signalling pathways, such as the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT), the Mitogen Activated Protein Kinase (MAPK) or the mammalian Target Of Rapamycin (mTOR) pathways, which are able to crosstalk between each other. Leukemia Inhibitory Factor (LIF), IL-6, IL-11 and oncostatin are major members of the IL-6 family which activate the mentioned pathways. A crucial function seems to play STAT3, which exists in at least two isoforms and has two phosphorylation sites (tyr 705 and ser 727). Homo- and heterodimers translocate into the nucleus and induce transcription of several genes and functions. Activation of STAT3 correlates with proliferation, motility and invasiveness of trophoblast cells similarly to tumor cells. Post-

transcriptional gene silencing of STAT3 inhibts the functions of the above-mentioned cytokines. LIF and STAT3 also control a new class of regulatory molecules: microRNA. Expression of several microRNA which are involved in tumor invasion are regulated by LIF, which on the one hand underlines the role of LIF in governing trophoblast, but which on the other hand also demonstrates the involvement of miRNA in tuning placentation.

In vivo imaging of the immune system

M. Gunzer[1] and P. Reichardt[2]

1. Department of Molecular Immunology, University of Duisburg-Essen, Essen, Germany
2. Institute of Molecular and Clinical Immunology, Otto von Guericke University, Magdeburg, Germany

The ability for autonomous migration is considered a key feature of immune cells. As a prime example for this fact we have investigated the behavior of neutrophil granulocytes as well as dendritic cells and T cells in vivo by intravital 2-photon microscopy. The cells present with largely different patterns of migration. While neutrophils in the resting state in their place of origin, the bone marrow, do not show considerable motility, they do dramatically increase their migratory capabilities upon injection of G-CSF, a hematopoietic cytokine. We could demonstrate that this increased motility is based on the induction of CXCR2 ligands which leads to the recruitment of cells from the marrow niches into local blood vessels and the periphery. Dendritic cells in the marrow as well as in lymph nodes presented with little locomotion but very profound motility at the periphery, as shown by imaging them in the bone marrow and peripheral lymph nodes. A very close look at T cells in peripheral lymph nodes showed, that LFA-1, a key adhesion molecule for their recruitment from the peripheral blood into the node seems to be largely irrelevant, once the cells have reached the internal tissue site. Here, neither the migration speed nor the contact with local dendritic cells was distinguishable in LFA-1 wt versus ko cells. However, we were able to demonstrate that LFA-1 allows T cells to re-enter or mediate prolonged contact with lymphatic structures, a mechanism that possibly ensures efficient T cell activation. This behaviour was not detectable in any in vitro assay tested before. Collectively these data show the enormous dynamics of immune cells in vivo and demonstrate how intravital microscopy is able to detect large but also delicate differences in cellular behaviour.

Management of pre-term birth

Serban-Dan Costa

Department of Obstetrics and Gynecology, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany.

In-vitro Fertilization

Jürgen Kleinstein

Department of Reproductive Medicine, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany.

Since the pioneering days of Patrick Steptoe and Robert Edwards in 1978, the IVF Revolution has brought about many innovations. A lot of which is already a part of the Assisted Reproduction Technique – ART – history. And what has remained as the core businesses of infertility therapy are: IUI (Intrauterine Insemination), IVF-ET (In-Vitro Fertilization with Embryo Transfer) and ICSI (Intracytoplasmatic Sperm Injection). It is the state of the art of these treatments that will be presented at this meeting.

Intrauterine Insemination: Even despite its limitations, IUI has not lost its importance for infertility therapy because it is less cost intensive and less stressful than ART and still has a constant pregnancy rate of 11-13 %. Compared to the pregnancy rate for couples that have a good prognosis of natural spontaneous conception, the efficiency of IUI cycles can be demonstrated with the following numbers. IUI without ovarian stimulation is not very effective – it has a pregnancy rate of 5 % per cycle. Additional stimulation with Clomifen is not convincing. For gonadotropin stimulation, the pregnancy rate doubles. But, IVF is convincingly more successful with a pregnancy rate of 31 % per cycle. This has caused a change in treatment course: after only 3 cycles of IUI, the patient will be "fast-tracked" to IVF.

In-vitro Fertilization: There is nothing technically new about conventional IVF. It is much more about the oocytes. The only predictor of ovarian reserve is uniquely the Anti-Mullerian Hormone (AMH) level. It is a marker for ovarian reserve that is more consistent and it is not cycle-dependent. And it will be able to provide the basis for a prognosis earlier than other markers. The ultrasound parameter for ovarian reserve is the antral follicle count. AMH is a growth factor that is produced in the granulosa cells of the primordial and pre-antral follicle. Its main job is to protect the follicle from premature entry into the maturing process. It can best reflect the age-related loss of fertility, and it can do so much earlier than FSH. Intra and inter-cycle fluctuations are minor. Even with hormonal contraception, the AMH level can be reliably determined. The range is quite broad and it is between 1-4 ng/ml. PCO-S patients typically have a level 2-3 times the normal level. If the level is below 1 ng/ml – this is the critical limit for infertility treatments.

Intracytoplasmatic Sperm Injection: In most countries, the use of ICSI cycles is increasing, IVF cycles are remaining constant. This means that there is a growing problem in finding suitable sperm for assisted reproduction. The conventional ICSI methodology is not always sufficient. Here, the sperm is chosen according to pure morphological criteria and then injected. With the Sperm Slow Technique, there is an additional functional test built in. The binding of hyaluronic acid to receptors on the sperm´s head correlates with the integrity of the sperm. There were significantly better embryos with the best quality and development. Birefringence is also a functional test to identify sperm that have undergone the acrosome reaction. Birefringence develops when there are anisotropic structures in a cell, such as in sperm. Under these conditions, light will polarize and will be seen as faster and slower rays. With this method, a significant higher rate of implantation and pregnancy can be achieved.

Placenta physiology and regulation

Berthold Huppertz

Institute of Cell Biology, Histology and Embryology Medical University of Graz

The placenta is the fetal organ that provides the interchange between fetus and mother. A delivered placenta shows all features that have been necessary throughout pregnancy to assure adequate nutrition of the baby. The respective macroscopic structures include the chorionic and basal plate, the fetal membranes and the different types of chorionic villi where exchange between maternal and fetal blood takes place. The microscopic structures include the placental barrier separating the two blood systems. This barrier is mostly based on the epithelial cover of the placental villi, the villous trophoblast. Other microscopic structures comprise the placental vessel system, the placental macrophages (Hofbauer cells) as well as the extravillous trophoblasts that invade into the maternal uterine tissues to adapt these tissues to the needs of the growing fetus. This includes invasion into spiral arteries to open these vessels towards the placental intervillous space and to establish maternal blood flow towards the placenta and thus hemotrophic nutrition. They also invade into uterine glands to establish histiotrophic nutrition early in pregnancy.
Alterations of the extravillous trophoblast and its invasive properties may result in growth restriction of the baby (IUGR). Alterations of the villous trophoblast and its release of fragments into the maternal blood streams may cause an inflammatory response of the mother, ending in preeclampsia.

Human natural killer cells in uterine mucosa

Judith Bulmer

Reproductive and Vascular Biology Group, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK

The decidualised endometrium that lines the uterine cavity during pregnancy contains a large leucocyte population throughout pregnancy, comprising uterine natural killer (uNK) cells, macrophages and T lymphocytes, as well as small populations of dendritic cells and T regulatory cells; B lymphocytes are uncommon. Uterine NK cells differ from peripheral blood NK cells, being CD56bright CD16-. They are present in endometrium throughout the menstrual cycle but increase in number dramatically in the luteal phase of the cycle, at least in part by local proliferation. In the first trimester of pregnancy uNK cells account for up to 70% of the decidual leucocytes, but they decline in number in the second half of pregnancy. Although they possess perforin, granzyme and granulolysin in cytoplasmic granules, uNK cells show low cytotoxicity compared with peripheral blood NK cells. Uterine NK cells can produce a wide range of cytokines, including IFNγ, TNFα, TGFβ1, IL6 and IL8. This cytokine production may be important for maintaining a favourable cytokine balance locally within the uterus but may also play a role in control of trophoblast invasion: conditioned medium from cultures of uNK cells at 12-14 weeks (but not 8-10 weeks) gestational age stimulates trophoblast invasion. Evidence from mouse pregnancy has shown that uNK cells play a role in remodeling of uterine arteries in pregnancy. Uterine NK cells produce a range of angiogenic growth factors including VEGF-C, Ang1 and Ang2, which are able to cause separation of vascular smooth muscle cells in in vitro models of chorionic plate arteries and spiral arteries retrieved from non-pregnant myometrium. Cytokine production increases, while AGF production reduces, between 8-10 weeks and 12-14 weeks gestational age, suggesting that uNK

cells function may vary with gestation, with an early role in initial stages of spiral artery remodeling and a later role in stimulation of extravillous trophoblast invasion into uterine tissues. There are limited reports of uNK cells in pathological pregnancy. However, KIR receptors expressed by uNK cells can interact with HLA-C expressed by extraviilous trophoblast within placental bed and certain combinations of maternal KIR and fetal HLA-C are strongly associated with recurrent miscarriage, pre-eclampsia and fetal growth restriction. Despite many years of intensive research, the in vivo role of uNK cells in human pregnancy remains uncertain.

Uterine Natural Killer Cells: New paradigms

Anne Croy and Zhilin Chen

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON Canada K7L 3N6.

A common finding associated with human pregnancy complications is incomplete spiral arterial modification. This pathology is observed by midpregnancy in mice lacking lymphocytes (genotype Rag2-/-/Il2rg-/-) and can be corrected by pre-mating transplantation of bone marrow capable of differentiating uterine Natural Killer (uNK) cells. Secretion of interferon gamma by uNK cells is important for this unique vascular remodeling process although only a subset of uNK cells produce this cytokine. We sought the primary outcomes from incomplete spiral arterial remodeling and identified these as alterations to maternal and fetal hearts (J. Zhang et al, 2011 Am. J. Ob. Gyn) rather than hypoxia of the placenta and/or fetus (E. Leno-Durán et al, 2010 Placenta) or hypertension (S. Burke et al, 2010, Hypertension). To address early post-implantation relationships between trophoblast, the decidual vasculature and leukocytes, we combined whole mount in situ staining with flow cytometry. We visualized a leukocyte influx into early implantation sites and found only 50% of these CD45+ cells were uNK cells. Four vascular zones with vessels of distinct appearance were present during pre-placental stages with vascular density greatest in decidua basalis, the region enriched in CD45+ cells. Many CD45+ cells, including some uNK cells were CD31 (PECAM-1) reactive. Preliminary studies of pregnancies in alymphoid recipients of bone marrow from donors with ubiquitious expression of green fluorescent protein suggest that circulating endothelial progenitor cells are not incorporated into early implantation site vessels. The roles of immune cells in promotion of a distinct vasculature within implantation sites merit further study. Supported by NSERC, CIHR and the Canada Research Chairs Program.

Neuroendocrine regulation of fetal-maternal interaction

Petra Arck

Department of Obstetrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Fetal development is largely dependent on the mother. However, pregnancy maintenance and consequently fetal development are highly vulnerable and sensitive to disruption, triggered by the

macro- or microenvironment. High stress perception is becoming the 'epidemic' of the 21st century, as identified by a recent study carried out by the World Health Organisation in Northern European countries. Also, women giving birth to their first child are now in average 10 years older than 3-4 decades ago. Higher maternal age or high maternal stress perception affects the maternal endocrine and immune adaptation required for an uncomplicated pregnancy. Both, maternal age and stress perception could be linked to low levels of progesterone during pregnancy, associated with a high risk for spontaneous abortion or negative repercussions on the child's health later in life. It has been reported that prenatal stress also increases the risk for the child to develop chronic immune diseases, such allergies and asthma. The markers and mediators along which prenatal environmental challenges increase the offspring's risk for chronic immune diseases remain largely elusive. In basic science models and prospectively designed birth cohorts, we observed that prenatal stress challenge, mirrored by e.g. a decrease of maternal progesterone, impairs fetal immune ontogeny. Such impaired immune ontogeny carried over into postnatal life, rendering the child more prone to develop chronic immune diseases. Further, the data arising from our research endeavours pursued to date revealed a pivotal role of maternal progesterone during pregnancy in preventing allergic diseases in the offspring later in life. We envision that identification of relevant stress-sensitive biomarkers may eventually allow detection of pregnant women at risk to give birth to immune disease-prone offspring. The creation of therapeutic interventions designed to prevent negative consequences of prenatal stress would then be within reach.

Workshops

I: Cells at the fetal-maternal interface

Gil Mor

Yale University School of Medicine, New Haven, USA

II: Animals models for studying pathologies related to pregnancy

Guillermina Girardi

The Queen's Medical Research Institute, University of Edinburgh, UK.

Sometimes things do not go as planned in pregnancy and some complications arise. Miscarriage is the most common type of pregnancy loss and occurs anywhere from 10-25% of all pregnancies. Preterm delivery (PTD) is observed in about 12% of all pregnancies and contributes to both acute and long-term neonatal morbidity. Preeclampsia (PE) is another pregnancy-specific disorder characterized by maternal vascular disease and poor fetal outcomes. The global incidence of PE has been estimated at 5-14% of all pregnancies and it is a leading cause of maternal and fetal mortality and morbidity.

Despite considerable research, the cause/s of these pregnancy complications remain/s unclear, and there is no effective treatment. The study of pregnancy-related disorders in women is of critical importance, however studies in humans have obvious limitations that prevent investigation of many pathophysiological mechanisms and that often limit the ability to establish cause-and-effect relationships in pregnant women. Thus, development of animal models that recapitulate these

pregnancy complications may help to expand our understanding and may hold great potential for the design and implementation of effective treatment.

Here we will discuss three different animal models of pregnancy complications and how these models helped us identified mediators/effectors of disease and possible targets for therapy. 1) Because 30% of women who miscarry have elevated levels of antiphospholipid antibodies (aPL), we will describe a mouse model of fetal loss induced by aPL. In this model tissue factor (TF) modulates neutrophil activation, oxidative stress and fetal death. 2) We will also cover pregnancy loss associated to immune causes but not mediated by antibodies in the CBA/J x DBA/2 model. Interestingly, this is not only a model of recurrent miscarriage but also a model of PE. In this mouse model we also found that TF is an important mediator in placental injury and endothelial dysfunction and statins that inhibits the synthesis of TF prevent the onset of PE in these mice. 3) The last pregnancy disorder that we will discuss is PTD. We recently developed a mouse model of PTD induced by intravaginal administration of LPS. This model resembles most clinical scenarios in that localized inflammation occurs without systemic maternal illness. Using this model, we found that complement activation plays a crucial role in the cervical remodeling that leads to PTD. Complement component C5a activates metalloproteinases that digest collagen increasing the cervix distensibility and leading to PTD. Interestingly, these 3 animal models highlight the role of inflammation in the pathogenesis of pregnancy-related complications.

Research using different animal models might provide important knowledge of the mediators and effectors of pregnancy complications. If the results are then confirmed in humans they can eventually lead to the development of more effective diagnostic methods and clinical treatments.

Minicourses

I: How to plan, organize and conduct animal experiments.

Oliver Zierau

Molecular Cell Physiology & Endocrinology, Institute for Zoology, Technical University, Dresden, Zellescher Weg 20, 01062 Dresden, Germany

An animal experiment (AE) is by definition an operation on a living animal for experimental purposes; which potentially results in harm, pain or distress for the animal. Aim of this minicourse is to give a general introduction into rational planning, organization and execution of animal experiments. Therefore it is necessary to know what is actually an AE, what are alternatives to AEs, how far is the law involved in animal testing in the EU and what has to be taken into consideration when planning an AE. It will be presented on the basis of practical examples on how an AE should be ideally planned. Specifically, variable factors in an AE, like the choice of the animal species or strain, the source of animals, influence of diet and general handling will be discussed. Besides this other often underestimated factors like the bias or the design of the environment, the microbiological status, the general applicability, practical randomization as well as cases for realistic refinement and reduction of AE will be elucidated. A focus will be set on ways to increase the signal/noise ratio and thereby getting better results and calculating the right dosage for an AE, which is then equivalent to the one used in the human situation.

In a second part we will try as a group to (re-)plan an already performed animal experiment and then compare it to the original design and to criticize the chosen approach.
Finally we will concentrate on the validity of an experiment under differing circumstances. This evaluation will be focusing on the question whether your data are applicable to other strains, the other sex, in other environment, with other diets etc.

II: The long way from the bench to the patients, going from basic research to clinical trials.

Serban-Dan Costa
Department of Obstetrics and Gynecology, Medical Faculty, Otto-von-Guericke University, Magdeburg, Germany.

Practical courses

Practical Training I:

a) Isolation of immune cells from murine decidua - sample preparation and analysis by flow cytometry.

Anne Leber, Nadja Linzke, Katja Woidacki, Tarek El-Mousleh, Ana Teles
Experimental Obstetrics and Gynaecology, Medical Faculty, Otto-von-Guericke-University, Magdeburg, Germany.

During normal pregnancy the maternal immune system has to tolerate the foreign antigens expressed by the fetus. Several immune cell subtypes have been shown to contribute to fetal tolerance by modulating immune responses directly at the fetal-maternal interface. Thus, characterisation of those immune cells will help to understand the mechanisms supporting fetal acceptance.
The aim of the practical course is to provide insights in techniques and possibilities of immune cell isolation from murine decidua followed by the analysis of different immune cell populations by flow cytometry.
In small groups the students will learn the taxidermy of a female mouse including the retraction of the uterus and the identification of the decidua basalis, placenta and the embryo. During the practical training the dissociation of the tissue and the immune cell enrichment using density gradient centrifugation will be shown and exercised. Immune cells from the decidua basalis will be stained for T cell, mast cell and uterine natural killer cell subtypes and analysed by flow cytometry. Finally the data obtained by flow cytometry will be evaluated.

b) Isolation of murine spermatozoa and oocytes, sperm capacitation and in vitro sperm-oocyte interaction assays.

Mónica Vazquez-Levin, Nadia Edelsztein

Laboratory of Studies on cell-cell interaction in reproduction and cancer models.
Instituto de Biologia y Medicina Experimental IBYME-CONICET

Fertilization is a fundamental process that involves a highly coordinated sequence of interactions between the female and male gametes, giving rise to a diploid zygote. During this process, spermatozoa that have successfully completed spermatogenesis, epididymal maturation, and transport through the female reproductive tract, first bind to the extracellular matrix that surrounds the egg, called the Zona Pellucida (ZP). Sperm binding to ZP glycoproteins triggers sperm Acrosomal Exocytosis (AE), involving fusion of the sperm plasma and outer acrosomal membranes and the release of the content from the acrosomal granule; these components, in conjunction with the hyperactivated vigorous motility, help sperm penetration through the ZP. The spermatozoon reaches the perivitelline space, binds and fuses to the egg plasma membrane (oolemma); the sperm head enters the egg cytoplasm (ooplasm), and the sperm nucleus undergoes decondensation. Ultimately, sperm entrance triggers mechanisms to block polyzoospermy.
During this practical course we will teach the student how to obtain female as well as male gametes and how to handle them. Additionally, we will *in vitro* induce the sperm capacitation and perform a sperm-oocyte interaction assay.

Practical training II (*in vivo* imaging of the uterus)

Dr. Carlos Tadokoro

Lab of Immune Regulation and Unit of Cell Imaging, Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Two-photon Microscopy (TPM) allows image acquisition in deep areas inside tissues/organs. In combination with the development of new stereotactic tools and surgical procedures it becomes a powerful technique to identify "niches" inside organs and to document cellular "behaviors" in live animals. Intravital imaging acquisition is the alternative that better resembles the real cellular behavior inside the organ, but it is both more laborious and demanding in terms of equipment than ex vivo imaging acquisition. In this demonstration I will show a surgical procedure and new "stereotactic" organ holder to allow the in vivo observation of cells inside the uterus. In brief, animals will be anesthetized with ketamine/xylazine (alternatively, with isofluorane), one of the uterus side will be exposed, and a small aluminum foil clip will allow its immobilization inside the "stereotactic" organ holder. We will add botulinum toxin on top of the preparation to stop involuntary muscle movement and time-serial image acquisitions performed in a Zeiss LSM 7 multiphoton microscope.

Selected abstracts for oral presentation

1. Notch pathway expression in the male mouse reproductive tract

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Introduction The male reproductive tract is continuously undergoing cellular proliferation and differentiation. Two of the key cellular events that take place in this system are the production of sperm cells in the testis and their maturation in the epididymis. The molecular factors that drive these events are still poorly understood.

The Notch signaling pathway is a central regulator in several physiological phenomena and comprises four receptors (Notch1, 2, 3, 4) and five ligands (Jagged1, 2, Delta-like 1, 3, 4).

Methods of study We analyzed the male reproductive tract expression profiles of Notch1, 2 and 3, Jagged1 and Delta-like1 and 4 (Dll4) through immunohistochemistry. Four CD1 wild type male mice were humanely sacrificed at 4 and 15 days, ten weeks and 5 months of age, and their reproductive tracts were collected and processed for immunostaining.

Results and conclusions The expression patterns of these receptors and ligands change along the mice genital tract and life time. The main features are the presence of Dll4 in only some seminiferous tubes at 15 days and in almost all germline cells in the adult, being the other member's expression pattern more cell type specific. The interstitial cells are also stained by anti-Dll4 but their staining varies during the mice life time. Dll4, Notch2 and 3 show a specific expression profile along the epididymis.

These results propose a role for Notch signaling. The expression in the male germline indicates a possible function in sperm differentiation, while in the epididymis point out to a involvement in sperm maturation.

2. IL-17A concentration of seminal plasma and follicular fluid in infertile men and women with various clinical diagnoses

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5 Veterenary medicine cell and molecular research group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran.

Introduction: Seminal plasma and follicular fluid (FF) cytokine analysis are valuable tools for diagnosis of infertility and validity of therapeutic approaches for improving the chance of conception. Despite the initial discovery over a decade ago, the IL-17 family has not received much attention in the case of infertility.

Methods of study: We have analyzed the level of IL-17A in seminal plasma, follicular fluid and blood of infertile patients with different clinical diagnosis by Enzyme Linked Immunosorbent Assay (ELISA).

Results: The results show that the level of IL-17A was significantly different in blood and seminal plasma of astenozoospermia compared to the control group. Furthermore, the level of this cytokine was significantly lower in unexplained infertile and polycystic ovary syndrome (PCOS) patients than the control group. In endometriosis, PCOS, tubal factor and unexplained infertile patients, the level of IL-17A was also significantly lower than the control group. In addition, we analyzed the quality parameters of sperm and oocyte and found a correlation between alterations in quality factors and cytokine measurements.

Conclusion: The significant differences in the level of IL-17A in seminal plasma and follicular fluid observed in this study could have a future involvement in understanding the immunopathogenesis, development of more effective diagnosis tools and therapeutic treatments for human reproductive disorders.

Keywords: Human IL-17A, Blood, Follicular Fluid, Seminal Plasma, Enzyme Linked Immunosorbent Assay (ELISA)

3. Influence of transgenerational soy isoflavones exposition on physiological and endocrinological relevant parameters in female rats

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Introduction: Isoflavone phytoestrogens are advertised for treatment of different complaints e.g. of postmenopausal women, while their potential hazards cannot completely be excluded to date. The aim of the present study was the examination of the role of soy isoflavones (sISO) in the development of rats.

Methods: Female rats were exposed to either sISO-free or sISO-rich diet. Therefore Wistar rats were allocated to two breeding groups (6 female and 2 male) receiving either sISO-enriched (35% soy) or sISO-free diet. Bodyweight and anogenital distance of the female offspring were determined in weekly intervals up to postnatal day (pnd) 80. At pnd 80 the uterine and ovarian wet weights were determined, mRNA expression was analyzed as well as the bone mineral density (BMD) of the tibial metaphysis was determined by peripheral quantitative computer tomography (pQCT).

Results: We observed a significant higher bodyweight in the sISO rich diet group starting from pnd 12. The anogenital distance was significantly increased compared to the sISO free diet after pnd 26. In addition, no significant differences in the uterine and ovarian wet weights were observed. Beside this sISO also influenced the bone as we observed a significant difference in bone mass and bone density in both the trabecular and cortical bone. The bone mineral content and the BMD of the group receiving the phytoestrogen-enriched diet was significant higher.

Conclusion: In conclusion we observed that a lifelong sISO diet exposure, starting in utero had a significant effect on different parameters such as bodyweight, anogenital distance and bone mineral density in female rats.

4. The cytoskeletal protein LASP is expressed by trophoblast cells and chorioncarcinoma cells and modulates their proliferation and migration

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Introduction: The LIM and SH3 Protein 1 (LASP-1) has recently been reported to play a pivotal role in the actin cytoskeleton organization of metastatic breast cancer cells. Thereby, studies revealed that an overexpression of LASP-1 correlated with an increased rate of breast cancer metastasis. In contrast, silencing of LASP-1 resulted in a reduction of the migration and proliferation of breast cancer cells. During early pregnancy, a controlled invasion of trophoblast cells into the decidualized endometrium is observed. So far, it is not known whether LASP-1 could play a role in this delicate migration process of trophoblast cells.

Methods: First-trimester decidual tissue was obtained from healthy women undergoing legal therapeutic abortion of a normal pregnancy (7-8 weeks of gestation). Positive selection of trophoblast cells was performed by labelling with anti c-erb B2 antibody. Expression of LASP-1 in isolated trophoblast cells and cells of chorioncarcinoma lineages (JEG, JAR, BEVO) was revealed by Western Blot. In the following, chorioncarcinoma cells were transfected with specific LASP-si-RNA. Those transfected chorioncarcinoma cells were then tested concerning their proliferation and migration rate using WST-8 testing and the matrigel migration assay.

Results: The cytoskeletal protein LASP-1 is expressed by trophoblast cells and chorioncarcinoma cells (JEG, JAR, BEVO). Transfection of the chorioncarcinoma cells with LASP-specific si-RNA resulted in a significant downregulation of LASP-1 expression. Those transfected chorioncarcinoma cells also exhibited a reduced proliferative and migratory activity.

Conclusion: This is the first study describing LASP-1 expression in trophoblast cells and chorioncarcinoma cells. Detecting a reduced migratory and proliferative activity after LASP silencing, we propose that LASP-1 could play a pivotal role during placentation.

5. Epidermal Growth Factor triggers STAT5 phosphorylation and enhances proliferation of trophoblastic cell lines.

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Introduction: Epidermal Growth Factor (EGF) is able to influence positively or negatively a variety of fundamental cell properties including proliferation and invasion/migration. Its role in fetal growth regulation and its relation with JAK/STAT pathway are still only partially understood. STAT5 is one intracellular protein downstream tyrosine-kinase receptors, which stimulates proliferation and cell cycle progression. The aim of this study was to analyze STAT5 activation mediated by EGF and the changes in the proliferative rate and invasion when STAT5 expression is abrogated.

Methods of study: JAR and HTR-8/SVneo were stimulated with EGF at diverseconcentrations and times. Phosphorylation of STAT5 was determined by Western blotting. Proliferation of JAR in

presence or absence of EGF was measured by MTT assay. SiRNA technique was used to silence STAT5B, changes in the proliferation and invasion of JAR and HTR-8 cells were followed up after 48h and 24h respectively.

Results: EGF is the major inducer of STAT5 phosphorylation in JAR and HTR8 cells. The effect starts up to 20 ng/ml and within 15 minutes of stimulus. Expression of STAT5 was silenced in ~70% as confirmed by western blotting. EGF increases proliferation of both cell lines and STAT5 silencing resulted in a slightly decrease. In JAR, invasion was decreased by STAT5 inhibition but EGF addition had no further effects.

Conclusion: EGF activates p-STAT5 and induces proliferation in JAR and HTR8 cells. Silencing of STAT5 results in decrease of their proliferation and invasion suggesting a role of STAT5 in the regulation of EGF-mediated behavior in trophoblast cells.

6. Anti-microbial peptide expression in the female reproductive tract in response to reproductive status and viral infection

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Introduction: Approximately 30-40% of preterm births are due to maternal infection and the resulting immune response. Viral infection contributes to these loses, though the mechanism of viral-induced preterm labor and its affect on the mothers immune status remain unclear. Immune cells, plus non-immune tissues female reproductive tract (FRT) epithelia and the placenta, produce factors that protect the mother and fetus from infection. These factors include natural antimicrobial peptides (AMPs) such as defensins and SLPI. We hypothesize that AMP expression is affected by reproductive status and viral infection, altering the immune response to bacterial challenge.

Methods of Study: We used our recently developed model of viral infection and qPCR to quantify AMPs. To further elucidate affects of hormones on uterine infection and AMP expression, we used a human endometrial stroma cell line (HESC) and HESCs decidualized using hormone treatment.

Results/conclusions: Mice infected with herpes virus MHV68 are sensitized to LPS leading to preterm birth and a pro-inflammatory response. We speculate that this could be due to a change in expression of AMPs. Therefore, we quantified defensins in the FRT during the cycle compared to three timepoints during pregnancy and during viral infection. Multiple defensins were constitutively expressed while some were differentially expressed dependant on status. Moreover, MHV68 infection changed expression of most defensins during pregnancy. Interestingly, decidua were infected, but no virus was found in non-pregnant uteri. Therefore, we infected HESCs and decidualized HESCs and found the kinetics of viral infection and expression of AMPs were affected by decidualization. These data suggest hormones and viral infection affect AMP expression in the FRT, and future studies will determine if AMPs contribute to sensitization of virally infected mothers to bacteria.

7. Origin of regulatory T cells during pregnancy

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Introduction: To establish and maintain fetal tolerance, the maternal immune system developed regulatory mechanisms. Foxp3^{+} regulatory T cells (Treg) exhibit a protective effect towards paternal alloantigens. The mechanisms of origin and recruitment of these cells at the fetal-maternal interface are not completely known.

Methods: We employed animals in which Foxp3^{+} cells can be depleted by application of diphtheria toxin. By using a model of adoptive cell transfer in Rag$^{-/-}$ animals and by exploring the expression of Helios to differentiate thymic-derived from peripheral induced Tregs, we aimed to contribute to the understanding of the origin of Treg during pregnancy. To study the trafficking of Treg during pregnancy, we analyzed different chemokines and their receptors.

Results: Treg depletion prior to and during very early pregnancy results in an impaired pregnancy, while their depletion at later time points does not interfere with gestation. Pregnancy-induced Foxp3^{+} Treg are mostly peripherally de novo generated although naturally occurring thymic Treg also contribute to fetal tolerance at later pregnancy stages.

Although CCL21 was up-regulated in uterine tissue of pregnant mice and CCL25 increased in the placenta, the gestation of knock out mice for their receptors CCR9 and CCR7 was not compromised in these animals.

Conclusion: Our results suggest that the maternal immune system evolved a strategy to promote Treg expansion during pregnancy and that trafficking of these cells to the fetal-maternal interface at different pregnancy stages is not dependent on single chemokine signals. Understanding these mechanisms is of great importance for the design of therapies to help patients with immunologically disturbed pregnancy.

Selected abstracts for poster presentation

1. Is ovarian stimulation affecting endometrial NK cell response during implantation window?

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Introduction.

Controlled ovarian stimulation (COS) during FIV-ET could affect embryo implantation. In addition, we have previously demonstrated that elevated counts of cytotoxic uNK and inadequate expression of IL-6 are associated to implantation failure. Our aim is to compare the impact of two differents COS protocols on immunological endometrial quality in fertile women.

Methods of study.

Endometrial biopsy from 10 fertile women were obtained 5-8 days post-ovulation during three consecutive cycles: 1st stimulated with GnRH agonist + recombinant FSH, 2nd natural cycle (NC) and 3rd GnRH agonist + hMG. CD9+CD56+CD16+ subset was determined by flow cytometry and

mRNA expression of IFN-γ, IL-6 and gp130 were determined by RT-qPCR. The effect of hCG was determined by in vitro culture of NK92 cell line.

Results.

Both COS protocols increased estradiol (E2) levels and CD9+CD56+CD16+ count ($p<0.05$) compared with NC, but only hMG group have shown a significant positive correlation ($r=0.8487$). Moreover, only hMG significantly increased IFN-γ, IL-6 and gp130 levels ($p<0.05$), and have shown a significant positive correlation between IL-6 and E2 ($r=0.8715$), the number of CD9+CD56+CD16+ ($r=0.7863$) and IFN-γ ($r=09827$). Moreover, in vitro experiments have shown that hCG up-regulate gp130 expression in NK92 cell line.

Conclusions.

Ovarian stimulation has a deleterious effect on endometrium by increasing cytotoxic NK cell counts, whereas only hMG seems to be related to a higher local activity of these cells. Moreover, hCG (a differential component of hMG) potentially affects the ability of NK cells to respond to IL-6 by increasing the expression of gp130 receptor.

2. Pro-Coagulant capacity of syncytiotrophoblastic microparticles (STBMs)

Göhner, C, Bonnke, C, Brückmann, A, Enke, U, Seyfarth, L, Schleussner, E, Markert, UR, Sossdorf, M, Lösche, W, Fitzgerald, JS

Introduction:

A characteristic of the severe pregnancy pathology, preeclampsia (PE), is endothelial dysfunction as seen through vasoconstiction and platelet activation. The release of syncytiotrophoblastic microparticles (STBM) is associated especially with severe, as opposed to mild, forms of PE. Classic STBM research has been geared to investigating their effects on the endothelial compartment, however, their thrombogenic potential is not well characterized.

Aim of the study was to investigate the pro-coagulant activity of STBMs.

Methods: STBMs were derived from placenta perfusates and, after staining with FITC-labeled annexin-V that binds to negatively charged phospholipids, quantified by flowcytometry. Pro-coagulant activity was determined on immobilized STBMs as prothrombinase activity in a plasma-free system or as the velocity of fibrin formation after addition of STBMs to normal plasma. ADP-induced aggregation of blood platelets in platelet-rich plasma (PRP) was measured in absence and presence of STBMs using PAP-4 aggregometer (mölab GmbH, Hilden).

Results: STBMs expose negatively-charged phospholipids at their surface which can be used for flowcytometric quantification. Due to these phospholipids, STBMs exert a significant pro-coagulant activity indicated by their prothrombinase activity as well as by the accelerated fibrin formation. STBMs also significantly increase the rate of ADP-induces platelet aggregation.

Conclusion: STBMs have a pro-coagulant activity as well as a stimulating effect on platelet aggregation. Both effects may contribute the impaired microcirculation in PE. The prothrombotic effects of STBMs are at least partially related to the exposure of negatively charged phospholipids. However, also other factors such as exposure of tissue factor and receptors for interaction with platelets may contribute. Further research is underway to validate this hypothesis.´

3. Cervical remodeling/ripening at term and preterm delivery: The same mechanism initiated by different mediators and different effector cells.

Juan M Gonzalez, Roberto Romero and Guillermina Girardi.

Perinatology Research Branch, NICHD/NIH, USA, The Queen's Medical Research Institute, University of Edinburgh, UK.

Background. Premature cervical remodeling/ripening is believed to contribute to preterm delivery (PTD), the leading cause of perinatal morbidity and mortality. Despite considerable research, the causes of term and PTD remain unclear, and there is no effective treatment for PTD. Using a mouse model of localized inflammation-induced-PTD which resembles most of the clinical scenarios, we previously demonstrated that complement activation plays a causative role in cervical remodeling that leads to PTD.

Methodology/Principal findings. Here we found that complement activation is not required for the physiological process of term delivery. Neither increased C3 cervical deposition nor increased C3a and C5a serum levels were observed at term (antepartum levels of C5a (ng/ml): 91±10 in PTD vs 18±3 in term delivery). In addition, cervical macrophages infiltration was found in PTD in contrast to term delivery were no leukocytes were found. Despite the different role of complement and different cellular effector cells, PTD and term delivery share a common downstream pathway characterized by increased metalloproteinases (MMPs) release and increased collagen degradation. However, different sources of MMPs were identified. Macrophages are the source of MMPs in PTD while cervical fibroblasts and columnar epithelial cells synthesize MMPs at term delivery. A dramatic diminution in serum progesterone levels precedes parturition at term (63 ng/ml antepartum vs 26 ng/ml intrapartum, $p<0.001$) but not in PTD (75 ng/ml antepartum vs 78 ng/ml intrapartum, suggesting that progesterone withdrawal initiates cervical remodeling at term. On the other hand, MMPs release in PTD is triggered by C5a.

Conclusion and significance. In conclusion, inflammation-induced preterm and term cervical remodeling occur through the same mechanism but they are initiated by different mediators and effector cells. That complement activation is required for PTD but not for the physiological process that leads to term labour, suggests that complement is a potential specific biomarker and selective target to prevent PTD and thus avert neonatal mortality and morbidity.

4. Protein profile in sera from women with pregnancy losses associated with Antiphospholipid Syndrome

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Funding support: : Grants from School of Medicine - University of Antioquia and Colciencias 1115-493-26157-Colombia and from the German Ministry of Education and Research, the German Science Foundation, Germany.

Problem

The search for biomarkers in the diagnosis of Antiphospholipid Syndrome (APS) is necessary and currently there is no enough information in proteomics of women with pregnancy losses associated to APS. The aim of this work was study the protein expression profiles associated with this syndrome.to identify potential disease protein biomarkers using SELDI-TOF.

Methods of study

Eighteen women with pregnancy losses were included in the study: Ten were positive for aCL by ELISA and 8 were negative. Protein profile from serum was determined by SELDI-TOF over an anion exchange Q10 chip . The analysis was carried out using Ciphergen ProteinChip Software. Looking for the protein identification, a search in several databases using the m/z value obtained for each differential expressed peak was performed.

Results:

Levels of 14 proteins (below 30 kDa) were found diferentially expressed in APS-positive and negative samples. The most of the proteins have a ROC area value close to 1, which indicate that its use like biomarkers could differentiate properly between women with or without this APS.

Conclusions:

The SELDI-TOF analysis provides a good discrimination of the peptides and proteins in a complex mixture like the human serum and allow reporting a protein profile of each group. However doesn´t allow its identification because the search with just the m/z value is difficult. These results encourage us to the protein identification using 2D-SDS-PAGE or MS/MS with the aim to propose new and more stable biomarkers for the early diagnosis of APS.

5. Aspirin Triggered-Lipoxin A4 reduces inflammation induced by preeclamptic plasma

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Financial Support: Research Committee-Universidad de Antioquia and Administrative Department of Science, Technology and Innovation-Colciencias, Colombia (Grants # 91196 and 1115-408-20531, respectively). National Institutes of Health grants, USA

Introduction: Preeclampsia is a disorder of pregnancy, characterized by hypertension and proteinuria after 20 weeks of gestation, and is a leading cause of maternal and fetal morbidity and mortality. Here, we evaluated the inflammatory and oxidative responses in plasma from preeclamptic women, and determined in vitro the role of aspirin triggered-lipoxin A4 (ATL) on the modulation of these responses.

Methods of study: Plasma from preeclamptic, normotensive pregnant, and non-pregnant women were analysed for factors involved in regulating angiogenesis, inflammation and lipid peroxidation. Primary human umbilical vein endothelial cells were incubated with plasma from preeclamptic women, and the adhesion of human polymorphonuclear neutrophils (PMN) (incubated with or without ATL) to endothelial cells exposed to preeclampsia plasma was evaluated.

Results: Preeclampsia was associated with an overall anti-angiogenic, oxidative and pro-inflammatory systemic environment, which might have a central role in the inflammatory response,

as well as in the increased human PMN- endothelial cell adhesion found in preeclamptic patients. Of interest, this cell adhesion was reduced when human PMN were incubated with ATL prior to addition to endothelial monolayers.

Conclusions: Despite advances in the understanding of preeclampsia, therapeutic approaches to the treatment of this disease are limited. For this reason, pharmacological approaches to counteract and/or dampen the anti-angiogenic, oxidative and pro-inflamamtory states in a single medicine such as ATL could be promising.

6. Comparative effects of Leukemia Inhibtory Factor on trophoblast cell lines signalling and function

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Introduction: Well balanced functions and differentiation of trophoblast cells are essential for inception and maintenance of successful pregnancy. Trophoblast cells perform invasion similar to tumors, but in a well regulated physiological manner and dysregulation may lead to severe pathologies. Leukemia inhibitory factor (LIF) induces trophoblast invasiveness via signal transducers and activators of transcription 3(STAT3), but activation mechanisms seem to differ in different cell lines and are not yet completely investigated. Therefore, the aim of our study is to analyze and compare the role of Extracellular Regulated Kinase (ERK)/STAT in trophoblast and choriocarcinoma cells with different invasive capacities.

Methods of study: The immortalized human trophoblast cell line HTR-8/svneo, the choriocarcinoma cell line JEG-3, and the hybrids of JEG-3 derivates and 1st and 3rd trimester trophoblast cells ACH-3P and AC1-M59, were treated with or without LIF. The activation and expression of STAT3 and extracellular regulated kinase 1/2 (ERK1/2) were measured by gel electrophoresis and Western blotting and quantified by use of a chemiluminescence gel documentation system. The effect on cell proliferation and invasiveness were determined by a MTS(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) colorimetric assay and a Matrigel invasion assay.

Results: LIF stimulated the phosphorylation of STAT3 and ERK1/2 in all analyzed cell lines, but with different intensities. LIF also enhanced invasiveness of ACH-3P cells and JEG-3 cells. In contrast, proliferation was not affected by LIF.

Conclusions: These findings demonstrate that the LIF-ERK1/2-STAT3 axis exists in different trophoblastic cell lines, but its activation may lead to different functions, which may be due to specific properties of each cell line.

7. Expression of FXYD5 in human and mouse reproductive tissues and spermatozoa. Co-localization studies with Epithelial Cadherin and Actin

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Introduction: FXYD5/Dysadherin is a transmembrane glycoprotein expressed mainly in human tumors and in several normal murine tissues. A role as a negative modulator of Epithelial-Cadherin (Ecad) has been suggested, at least in part, by modulating the actin cytoskeleton. Presence of Ecad

and actin and participation of the former in human and murine sperm-oocyte interaction have been described by our group. **Objective:** To characterize FXYD5 expression in male reproductive tract tissues and its presence/localization in spermatozoa. Colocalization studies with Ecad and actin were also done. Methods:Bioinformatics, RT-PCR, Western-Immunoblotting and immunohisto/cytochemical analyses of testis/epididymis/spermatozoa both in human and murine cells and tissues. **Results:** 1) The FXYD5 mRNA was detected in human testis, epididymis and spermatozoa, 2) A specific signal for FXYD5 was observed in round spermatids of human testicular sections, 3) FXDY5 was immunolocalized in the acrosomal region and flagelum of testicular and ejaculated human spermatozoa, 4) A distinctive 91 KDa protein form was detected in human testicular and sperm protein extracts, 5) FXYD5 co-localized with Ecad and actin in the acrosomal region, 6) A bioinformatic analysis revealed a 45-50% homology between the human and murine protein sequences, 7) Expression of the mouse FXYD5 transcript was also found in the testis and epididymis, 8) The protein was immunodetected in the testis and in cauda epididymal spermatoza. **Conclusions:** Our studies first describe the expression of FXYD5 in the male reproductive tract and in spermatozoa. Colocalization of FXYD5 with Ecad and actin leads us to postulate its role as a modulator of sperm function.

8. Trophoblast fusion and the role of Phospholipid Scramblase 1

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INTRODUCTION: During villous trophoblast fusion, regular cell membrane asymmetry is transiently abolished as phosphatidylserine is externalized to the outer membrane leaflet. One of the enzymes involved in this rearrangement is phospholipid scramblase 1 (PLSCR1).

METHODS: Gene expression profiling was performed in primary trophoblasts from human first trimester and term placenta as well as in the trophoblast-derived cell line BeWo by microarray. BeWo cells were stimulated with forskolin to syncytialize while DMSO treated cells served as controls. PLSCR1 expression was analyzed by quantitative real time PCR, Immunostaining and Western Blot in BeWo cells and placental tissue, respectively.

RESULTS: Microarray analysis revealed that PLSCR1 showed the strongest expression among the candidate enzymes. While PLSCR1 protein was abundantly expressed in syncytiotrophoblast, macrophages and endothelial cells, cytotrophoblasts showed only weak staining in first trimester and term placenta. In contrast, in BeWo cells PLSCR1 was not co-localized with β-hCG positive syncytia. No significant changes in PLSCR1 mRNA and protein expression were observed between forskolin treated and control cells.

CONCLUSION: Localization of PLSCR1 in the villous trophoblast compartment suggests a putative role in regulation of trophoblast membrane architecture. However, so far no obvious changes in protein expression during BeWo differentiation process could be detected. Work is in progress to identify the level of involvement of PLSCR1 in trophoblast fusion.

9. Pregnancy-associated Tregs and experimental Malaria

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Introduction: Pregnancy associated malaria (PAM) is the major public health concern in malaria endemic areas, causing around 100,000 infant death per year. It is characterized by a sequestration of malaria parasites inside the placenta, where parasite proteins like the VAR2CSA and its placental ligand Condroithin Sulfate A (CSA) seems to be important. An animal model to study PAM was recently described and it demonstrated that infected-pregnant females had a higher percentage of abortion and reduced litter size when compared with non-infected-pregnant sisters. **Methods:** Using this model, key elements of the immune system response during this specific pathological condition have been studied. These studies are then compared with the data collected in endemic areas to better understand the disease pathology. We are interested in one aspect of this disease. We want to evaluate if regulatory T cells (Tregs) developed during PAM would have an impact in the pregnancy itself and/or the malaria symptoms. **Results:** Despite we just started our project, it seems that PAM induced an increase in Tregs inside the brain, suppressing CD4+ T cell activation, and partially protecting the mother against cerebral malaria. **Conclusion:** Further studies will confirm our findings and also evaluate the impact of Tregs in pregnancy and healthy of survival offspring of malaria-infected mothers.

10. Human Chorionic Gonadotropin favors expansion and function of regulatory T cells during murine pregnancy

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Introduction

Regulatory T cells (Treg) have been shown to encourage the maternal immune system to tolerate the fetus. Although Treg increase systemically as well as locally at the fetal-maternal interface at very early pregnancy stages, the factors promoting their expansion and function are still under investigation. Recently we showed in vitro, that the pregnancy hormone Chorionic Gonadotropin (hCG) attracts human Treg efficiently to the fetal-maternal interface contributing to their expansion locally. Here, we aimed to investigate whether the application of hCG can prevent abortion in an abortion-prone mouse model by supporting expansion and suppressive function of Treg during pregnancy.

Methods of study

DBA/2J-mated CBA/J females known to present high abortion rates were treated either with hCG or PBS on different pregnancy days. The number of Treg and the abortion rate were determined. As immature DCs were described to contribute to Treg expansion the effect of hCG on DC maturation was examined. Additionally, suppressive activity of Treg isolated from hCG or PBS treated abortion-prone animals on effector T cells was investigated in vitro in a mixed leukocyte reaction.

Results

By applying hCG in abortion-prone females we were able to observe a Treg expansion systemically, which further prevented fetal rejection. We demonstrated that hCG maintains DCs in an immature state favouring Treg expansion. Moreover, hCG-treatment was able to increase Treg suppressive activity on effector T cells in vitro.

Conclusions

Our results suggest an important role for hCG in fetal protection by supporting expansion and function of Treg directly at the fetal-maternal interface.

11. Regulatory B cells expand during normal pregnancy in mice and seem to be essential for the acquisition and maintenance of fetal tolerance

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Introduction

B cells are classically known as effectors cells of the adaptive immune system. Recently, a subset of B cells producing IL-10 and referred as "regulatory B10 B cells" has been described and characterized. B10 cells are contained within a phenotypically unique CD19+CD1dhiCD5+ subset in the spleen. This regulatory B cell subset is antigen specific and significantly inhibits T cell activation and inflammatory responses through the production of IL-10. The participation of this new tolerance-associated subset in the acquisition of tolerance towards the fetus during pregnancy remains to be explored.

Objective

The main goal of this work was to evaluate the participation of CD19+CD1dhiCD5+ regulatory B10 cells in the process of immune tolerance acquisition during pregnancy.

Methods

CD19+CD1dhiCD5+ cell levels were analyzed in the spleen of pregnant and non-pregnant CBA/J females by flow cytometry. We next isolated antigen-specific (H2d) B10 cells from BALB-c mated pregnant mothers (~ 0 % of abortions) and transferred then into DBA/2J-mated CBA/J females, know to have ~ 30% of abortions. The adoptive cell transfer occurred between days 0-2 of pregnancy. Females were sacrificed on day 14 of pregnancy and the abortion rate was recorded.

Results

Splenic CD19+CD1dhiCD5+ regulatory B10 cells expand from 7.37 ± 1.33 % to 15.43 ± 2.5 % with the onset of allogenic pregnancy with BALB/c but remained unchanged in abortion-prone combinations. The transfer of regulatory IL-10 producing B cells (isolated from BALB-c pregnant CBA/J females) into abortion-prone DBA/2J-mated pregnant CBA/J females completely prevented immunological rejection, resulting in normal pregnancies. This was associated with an increase of CD4+foxp3+ regulatory T cells in lymph nodes draining the uterus.

Conclusion

IL-10 producing regulatory B cells are augmented in normal pregnancy and can be used as an effective therapeutic tool in a model of disturbed tolerance.

12. Some insights in the function of GPR30 in the biology of mamma carcinoma

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Introduction

The G-protein coupled receptor, GPR30, was recently found to be a new membrane-bound estrogen receptor involved in rapid non-genomic signaling events of estrogen. GPR30 is known to mediate proliferative effect on breast-, endometrium- and ovarian cancer cells. Furthermore, it is to some extent connected to higher aggressiveness of cancer and to formation of metastasis. Our aim was to investigate whether GPR30 is able to activate the chemokine receptor CXCR4 known to be involved in formation of metastasis. Due to decreased expression of GPR30 in breast cancer with worst prognosis we further aimed to analyze the mechanism of its deactivation by combined treatment with methyltransferase inhibitor 5'-azadeoxycytodine (Aza) and the specific GPR30 ligand G-1.

Methods of study

To study the effect of GPR30 on CXCR4 we stimulated GPR30 positive MCF-7 and SKBR-3 cells with G-1. CXCR4 expression was analyzed by RT-PCR, western blot and flow cytometry. A MTT proliferation assay was performed to investigate the impact of Aza and G-1 on cell proliferation of MCF-7 and SKBR-3 cells.

Results

In our set of experiments concentration and cellular distribution of the CXCR4-receptor expression remained unchanged after G-1 stimulation of MCF-7 and SKBR-3 cells. G-1 was able to inhibit the MCF-7 cell growth. The growth of SKBR-3 cells was inhibited only by high concentrations of G-1. In addition Aza treatment was be able to increase the expression of GPR30 in both cell types.

Conclusions

Our results suggest that formation of metastasis by GPR30 is not mediated by CXCR4 induction. GPR30 promoter is more likely hypermethylated.

13. Exploring the therapeutical potential of low doses carbon monoxide in pregnancy complications

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Introduction: Heme Oxygenase-1 (HO-1), the enzyme responsible for the degradation of free heme, has been shown to play an essential role on pregnancy outcome and its ablation is related to abnormal placentation with intrauterine fetal growth restriction (IUGR) and subsequent intrauterine fetal death as we recently showed. Among different metabolic end products of HO-1 on the heme-degradation pathway, CO has been found to have the most impact in mimicking the protective effects of HO-1. Thus, we hypothesized that CO might have the potential to prevent intrauterine growth restriction. **Methods:** For exploring this, we established the optimal doses and application

frames in a clinically relevant mouse model of IUGR. We next investigated the pathways activated upon CO application in vivo.

Results: 50 ppm resulted to be the ideal dose of CO necessary to prevent growth restriction being the optimal time frame day 3-8 of pregnancy. CO enabled a proper placentation which lead to higher fetal and placental weights without showing any pathologic effects. CO breathing suppressed inflammatory Th1 and Th17 responses, while diminishing placenta apoptosis.

Conclusion: Our results confirm the protective role of the HO-1/CO axis and point it as an emerging therapeutic possibility which is worth to further explore.

14. uNKs control formation of spiral arteries, placentation and fetal development via HO-1

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Introduction: Uterine Natural Killer cells (uNKs) represent the major immune cell population at implantation sites and are reportedly necessary for a proper trophoblast invasion. It is however unknown which pregnancy-specific signals lead to their expansion and maturation. We have recently confirmed the vital role of heme oxygenase-1 (HO-1) in placentation and intrauterine fetal survival.

Aim and methods: Here, we aimed to investigate whether the genetic ablation of HO-1 has an impact on the number of uNKs invading the uterus, which would impact the development of spiral arteries at the feto-placental unit. We took advantage of a murine model consisting of mice partially or totally lacking HO-1. *Hmox1*$^{+/+}$, *Hmox1*$^{+/-}$ or *Hmox1*$^{-/-}$ implantation sites were analyzed for the numbers of uNKs by DBA lectin staining. The spiral artery development was characterized by the determination of artery wall thickness and the spiral artery wall:lumen ratio. The area of the whole placenta, embryo cavity and feto-placental unit was measured with ImageJ.

Results: We observed that *Hmox1*$^{+/-}$ or *Hmox1*$^{-/-}$ implantations presented diminished number of uNKs and showed shallow spiral artery development as well as augmented wall:lumen ratio as compared to *Hmox1*$^{+/+}$ sites. Besides, heterozygote or homozygote fetuses for HO-1 were smaller than wild type ones at day 10 of pregnancy, presenting defective placentation as well. These features very much resemble intrauterine growth retardation.

Conclusion: Our data strongly suggest the enzyme HO-1 as one relevant mechanism underlying the positive effect of uNKs on formation of spiral arteries, placentation and fetal development.

15. HO-1 regulation in placenta during premature brith

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Introduction

Prematurity is a serious medical problem associated with augmented infant mortality and high costs. One major cause of prematurity is infection, which provokes pre-term contractions and

delivery of an immature baby. Trophoblast cells produce modulators that ensure the immune balance at the fetal-maternal interface. Disturbing factors of this balance like bacterial infections can lead to miscarriage or pre-term delivery. The enzyme heme oxygenase 1 (HO-1) is known to be important for placentation and fetal growth on the one side, and to have well-characterized anti-inflammatory properties on the other hand. Here we aimed to investigate the effect of infections, mimicked experimentally by the application of lipopolysaccharid (LPS) at trophoblast level, focusing on HO-1 regulation and cytokine production.

Methods of study

Human first trimester trophoblast cells were isolated from placentas of legally terminated pregnancies or spontaneous miscarriages. The cells were stimulated with 10µg/ml LPS for 0, 2, 4, 8, 12, 24, 48 hours and then examined for HO-1 protein levels and cytokine secretion.

Results

We observed lower basal HO-1 levels in trophoblasts isolated from miscarriages as compared to legally terminated, otherwise normally progressing pregnancies. The addition of LPS provoked an augmentation in the levels of HO-1 in trophoblasts isolated from legally interrupted pregnancies, accompanied by high levels of IL-6. In cells originally from miscarriages, the levels of HO-1, which were already low, were not significantly modified by the addition of LPS, while pro-inflammatory cytokines, especially IL-6, were very high.

Conclusion

Our results suggest that HO-1 acts as a pregnancy-protective molecule in trophoblast cells with the aim of protecting the fetus from infection-induced inflammation.

16. A role for mast cells in Treg-induced pregnancy tolerance?

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Introduction: Mast cells are well-characterized in the context of allergic disorders. Recent break-through studies revealed an unsuspected novel role for MCs in the Treg-dependent allograft tolerance by secreting interleukin-9. It had also been previously shown that genes predominantly expressed by MCs were over-expressed in Treg cultures and in tolerated allografts. Interestingly, in this new concept, MCs emerge as effector and not as initiator cells. Because of certain comparabilities between tissue allografts and the semiallogenic fetus, we wondered whether mast cells would be involved in Treg-induced pregnancy tolerance.

Methods: We employed a well-characterized murine combination for spontaneous abortion (DBA/2J-mated CBA/J females) to identify and quantify MC and their related molecules at the fetal-maternal interface. BALB/c-mated CBA/J females served as controls for normal pregnancy. A third group was included, which consisted of animals rescued from abortion after transfer of antigen-specific Treg. Localization of MCs at the fetal-maternal interface was verified by Giemsa and Alcian Blue staining, their quantification was done by flow cytometry (CD117) while the mRNA expression of mast cell protease (Mcpt)-1 and Mcpt-5 and other MC-relevant genes was analyzed in placental and decidual tissues by real-time RT-PCR.

Results: Interestingly, Treg treatment augmented not only the numbers of CD117+ mast cells but also the mRNA levels of mast cell proteases in placenta and decidua to the levels observed in NP mice. In our model, stem cell factor SCF and IL-3 but not IL-9 seem to be responsible for MC-expansion upon Treg transfer. **Conclusion:** Our results indicate that as already observed in transplantation-associated tolerance, MCs might contribute to the Treg-induced tolerance at the fetal-maternal interface.

17. Human Chorionic Gonadotropin supports the conversion of human naïve T cells into regulatory T cells

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Introduction

The acceptance of the semiallogeneic fetus during pregnancy has been shown to be achieved partially by CD4+CD25+ regulatory T cells (Tregs). Recently, we were able to show that the pregnancy-hormone human chorionic gonadotropin (hCG) attracts human Treg efficiently to trophoblast cells in vitro. Here, we aimed to analyze whether hCG is also involved in the conversion of naïve CD4+CD25- T cells into Tregs.

Methods of study

CD4+CD25- T cells were isolated from blood of pregnant women by magnetic cell sorting and co-cultured with either hCG-producing choriocarcinoma cells (JEG-3) or non hCG-producing cell lines (HaCat, HTR8, SWAN71). CD4+CD25- T cells cultured with medium served as controls. In additional experiments, CD4+CD25- T cells were cultured in the presence of different doses of recombinant hCG (rhCG). Conversion of naïve cells into Tregs was assessed by measuring CD25, CTLA-4 and Foxp3 expression after 24h, 48h and 72h of culture by flow cytometry.

Results

Co-culture of CD4+CD25- T cells with hCG-producing JEG-3 cells resulted in a statistically significant increase in the expression of Foxp3, CD25, and CTLA-4 after 72h as compared to the results obtained when co-culturing these cells with the non hCG-producing HaCat, HTR8 or SWAN71 cells or CD4+CD25- T cells alone. The addition of rhCG to the cell culture resulted in a dose-dependent increase in Foxp3 expression after 24h.

Conclusion

Our results strongly suggest a key role for hCG in Treg expansion during pregnancy as it is able to convert naïve T cells into Tregs.

18. Inflammatory effects of LL37 on the lower female reproductive tract

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Introduction: LL37 is a small protein produced by epithelial surfaces with broad spectrum antimicrobial and immunomodulatory activities. I have recently identified hCAP18/LL37 as a component of cervicovaginal secretions of pregnant women, but little is known about its function

within the female reproductive tract. Our objective was to investigate the function of LL37 in the inflammatory response of the lower genital tract.

Methods: Three immortalized cell lines derived from endocervical (END), ectocervical (ECT) and vaginal (VK2) epithelium were cultured in vitro with synthetic LL37. The role of MAPK pathway was investigated using specific inhibitors, PD 96059 (ERK inhibitor), JNK II (JNK inhibitor) and SB 203580 (p38 inhibitor). Production of inflammatory cytokines IL-8 and IL-6 was determined by enzyme-linked immunosorbent assay of cell culture media. Data was analyzed by analysis of variance and expressed as median.

Results: IL-8 was secreted by all three cell lines, but IL-6 was only secreted by END and ECT cells. LL37 increased production of IL-8 in a dose and time dependent manner in all cell lines (END 2693 versus 213.3 $p< 0.001$; ECT 798 versus 143; VK2 239 versus 23.6) and IL-6 in END and ECT cells (145 versus 9.083 and 226.99 versus 86.556 respectively). The ERK inhibitor PD 96059 and the p38 inhibitor SB 203580 partially inhibited the effect of LL37 on IL-8 in END and ECT cells. However in VK2 cells MAPK inhibitors had no effect.

Conclusion: LL37 has an inflammatory effect on cells of the lower female reproductive tract. This appears to be mediated by different pathways in cells derived from different tissue.

19. Repression of the genome organizer SATB1 in regulatory T cells is required for suppressive function and inhibition of effector T-cell differentiation

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Introduction: Regulatory T cells (T_{reg} cells) are an integral part of dominant tolerance and a major player in immune homeostasis. In several mouse models it has been shown that this unique regulatory identity of T_{reg} cells is an endowment of the transcription factor Foxp3. SATB1 as a global chromatin organizer and a transcription factor is significantly down-regulated in $Foxp3^+$ T_{reg} cells. In this study the regulation of SATB1 by Foxp3 in T_{reg} cells has been deciphered. Foxp3 as a transcriptional regulator targets SATB1 and represses its expression directly at the SATB1 locus. Histone modifications in addition, SATB1expression are indirectly regulated at the post-transcriptional level via the FOXP3-dependent induction of microRNAs binding to the 3´UTR of SATB1. Loss of Foxp3 function either by knock down or in genetic models leads to significant up-regulation of SATB1 and subsequent pro-inflammatory cytokine production. Functionally, overexpression of SATB1 in human T_{reg} cells abrogates their suppressive function and reprograms them towards T-effector phenotype. Expanded murine T_{reg} cells overexpressing SATB1 after lentiviral transduction show impaired suppressive function after adoptive transfer in $RAG2^{-/-}$ animals. Taken together, these data strongly suggest that inhibition of SATB1-mediated T-cell specific modulation of global chromatin remodeling is necessary for the full function of T_{reg} cells.

20. Function and regulation of Death-associated protein kinase (DAPK) in human pregnancy

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Introduction: DAPK is a ubiquitous expressed Ca2+/Calmodulin regulated serine/threonine kinase that contributes to pro-apoptotic signalling after cytokine exposure. The role of DAPK in human placenta is currently unknown. Our study aimed to investigate whether DAPK is expressed in placental tissues and to gain insights in its function.

Methods: We analyzed the mRNA and protein expression of DAPK in tissues from normal and pathological pregnancies, i.e. spontaneous abortion and pre-eclampsia. In isolated trophoblasts from first trimester placentas we analyzed the expression of DAPK and phosphorylated DAPK as well as the expression of possible interaction partners of this enzyme. Knock down of DAPK via siRNA in JEG-3 cells was used to clarify the importance for DAPK in trophoblast cell growth.

Results: We observed decreased DAPK expression in placenta samples as well as in isolated trophoblasts from preeclampsia patients in comparison to normal term placentas. The expression-levels of ERK1/2, SAPK/JNK and their phosphorylated forms suggest that their co-expression in trophoblasts and DAPK-mediated phosphorylation is important for trophoblast physiology. Knock down of DAPK provoked a marked diminution in trophoblast viability and proliferation.

Conclusion: We propose DAPK as an important player for trophoblast proliferation.

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