The Immunology of a Successful Pregnancy

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Wednesday, 16 October 2013
Cineworld: The O2, London, SE10 0DX, UK

This event will challenge scientists and clinicians interested in the field of reproductive immunology to evaluate many of the 'classical concepts' associated with pregnancy immunology. This event aims to define new approaches to allow a better understanding of immunity during pregnancy that will benefit mothers and foetuses in different clinical scenarios.

This event has **CPD accreditation** and is part of the 2013 Pregnancy Summit- www.PregnancySummit2013.com

Meeting Chair: *Dr Rupsha Fraser*, Reproductive and Cardiovascular Disease Research Group, Division of Biomedical Sciences St George's, University of London, UK

Who Should Attend
Biotech and Pharma Industry Managers: CEOs, Chief Scientists, Group Heads, Senior and Junior Scientists, Research working in the field of immunology and pregnancy
Academic and Research Institutes: Group and Lab Heads, Postdoctoral Scientists and Research Students working in the field of immunology and pregnancy
Clinicians: Anyone working in the field of pregnancy and diagnosing pregnancy-related illnesses and pregnancy outcome

The deadline for abstract submissions for oral and poster presentation has now passed.

Talk times include 5 – 10 minutes for questions

9:00 – 9:45 **Registration**

9:45 – 10:00 **Introduction by the Chair:** *Dr Rupsha Fraser*, Reproductive and Cardiovascular Disease Research Group, Division of Biomedical Sciences St George's, University of London, UK

10:00 – 10:30 **The Role of Decidual Natural Killer Cells and Macrophages in Early Pregnancy**
Dr Rupsha Fraser, Reproductive and Cardiovascular Disease Research Group, Division of Biomedical Sciences St George's, University of London, UK
A successful pregnancy is dependent on efficient placentation and remodelling of maternal uterine vessels (spiral arteries) to allow sufficient oxygen and nutrients to be delivered to the developing fetus. Decidual natural killer (dNK) cells and macrophages (dMϕs) accumulate around spiral arteries in early pregnancy and are present during uterine spiral artery remodelling. We have modelled the cellular interactions at the maternal-fetal interface., providing the first demonstration of a functional role for dNK cells in influencing vascular cells, and a potential mechanism contributing to impaired vessel remodelling in pregnancies with a higher uterine artery resistance is presented. We also present the phenotypes of dMϕs that may be present during these events, their roles in the first trimester of pregnancy, as well as the effects of dNK-derived factors on dMϕ polarization, and spiral artery remodelling.

10:30 – 11:00 **Vaccination during pregnancy: which, who, why when?**
Professor Dilly OC Anumba, MBBS FWACS FRCOG MD LL.M (Medical Law), Professor of Obstetrics and Gynaecology, Honorary Consultant Obstetrician and Fetomaternal Medicine Subspecialist, The University of Sheffield, UK
This talk will summarise the immunological basis of vaccinations during pregnancy, highlighting routine and indicated vaccines, and the rationale for their administration. The evidence base for the recommenedd vaccines during pregnancy in the UK will be outlined and areas of uncertainty discussed including an outline of contraindicated vaccines .

11:00 – 11:30 **Speakers' photo then mid-morning break and poster exhibition and trade show**
Please try to visit all the exhibition stands during your day at this event. Not only do our sponsors enable Euroscicon to keep the registration fees competitive, but they are also here specifically to talk to you.

11:30 – 12:00 **Maternal immunity and the pathogenesis of Chlamydia abortus: peripheral immunity vs placenta.**
Mr Sean Wattegedera, Research Scientist, Moredun Research Institute, UK
The health and welfare of ruminants is key to providing safe and sustainable food for human consumption. Numerous pathogens cause reproductive loses in most sheep rearing countries worldwide and Chlamydia abortus is the most common cause of diagnosed ovine abortion in the UK. Animals can be infected prior to pregnancy and pathogenesis of disease appears to be intimately linked with the progression of pregnancy. To improve on our control measures, it is important to better understand the maternal and fetal immune responses during pregnancy. I will present data from aspects of our research covering these areas.

12:00 – 12:30 **Oral Presentations:**

12:00 – 12:15 **Oocyte Donation Pregnancies are Associated with a Higher Incidence of HLA Alloantibodies**
Lisa E.E.L.O. Lashley, Marie-Louise P. van der Hoorn, Geert Haasnoot, Dave Roelen, Frans H.J. Claas **.** *Leiden, The Netherlands*

12:15 – 12:30 **Maternal NK cells regulate fetal growth and placental efficiency in the mouse.**
Selma Boulenouar, Amanda Sferruzzi-Perri, Hong Wa Yung, Louise Gaynor, Steve Charnock-Jones, Abby Fowden, Graham Burton and Francesco Colucci
Department of Obstetrics & Gynaecology, University of Cambridge, UK

12:30 – 13:30 **Lunch, poster exhibition and trade show**
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13:30 – 14:30 **Discussion session**
This discussion session is an informal question and answer session. This is an ideal opportunity to get advice and opinion from experts in this area. This session is not for questions about specific talks, which can be asked after the speakers session, but for discussing either general topics or specific issues.

There are three ways you can ask questions:
1. *Before the session* you can submit your question to Euroscicon staff at the registration desk,
2. *Before and during the session* you can submit a question or comments, by email, which will be provided on the day of the event
3. *During the session* you can put your hand up and join in

14:30 – 15:00 **Anti-inflammatories as a strategy for the prevention of inflammation induced preterm labour**
Dr Lynne Sykes, NIHR Academic Clinical Fellow, Imperial College London, UK
Successful pregnancy is dependent on a carefully balanced modification of the maternal immune response so as to tolerate the semi-allogeneic fetus whilst maintaining protection from harmful pathogens. During term labour, a physiological activation of the immune system occurs which leads to uterine contractility,fetal membrane rupture and cervical remodelling. However, premature activation of the immune response, often in association with infection, is the most common identifiable cause of preterm labour. Inflammation is also associated with adverse neonatal outcomes, independent of prematurity. This talk will explore the role of inflammation in term and preterm labour, and the potential for anti-inflammatories in the prevention of inflammation/infection induced preterm labour.

15:00 – 15:30 **Afternoon Tea, last poster session and trade show**

15:30 – 16:00 **The immonumodulating effect of seminal fluid on maternal T cells**
Tess Meuleman M.D, PhD, Leiden University of Medical Centre, The Netherlands
Seminal fluid may play a role in priming the maternal immune system before implantation and therefore helping to create a tolerogeneic environment at the implantation site leading to normal pregnancy. To investigate the influence of seminal fluid on maternal peripheral blood mononuclear cells we performed mixed lymphocyte cultures. Seminal fluid is capable of inducing profliferation of T cells and it seems these cells have a more regulatory phenotype. Failure of this immunoregulatory function or less exposure to semen may be the underlying cause in abnormal implantation and leading to complicated pregnancies.

16:00 – 16:30 **The placenta behaves as a parasitic endocrine organ**
Professor Philip Lowry, Emeritus Professor, University of Reading, UK
The poorly implanted placenta secretes neurokinin B to correct the associated ischemia, but high concentrations then stimulate all three neurokinin receptors in the mother's circulation, probably causing many of the symptoms of preeclampsia. Subsequently it has been found that the placenta post-translationally modifies its neurokinin B — along with pro-corticotropin releasing factor, pro-activin, pro-follistatin and pro-hemokinin—with a moiety containing phosphocholine, a group originally found on certain secreted parasitic proteins that endowed them with immune-inhibiting properties. Thus the placenta may be utilising the same survival mechanism as some parasites, attenuating immune surveillance by the mother, and thus avoiding rejection.

16:30 - 17:30 **Chairman's Summary and Close of Meeting**

Keywords: Pregnancy, spiral artery remodelling, decidual natural killer cell, decidual macrophages, pre-eclampsia, IL-1 superfamily, soluble ST2, inflammation, cytokines, Seminal fluid, immunomodulation, pregnancy, Tregs, implantation, Host-pathogen interactions, cytokines, Chlamydia, placenta, Neurokinin B, Phosphocholine, CRF, Melanotropin, mmune activation, NF-kB, preterm labour

Registration Website: www.regonline.co.uk/ImmPreg2013

NOTES ABOUT THIS EUROSCICON EVENT
For your convenience we would like to bring your attention to the following

- You will be issued with a FULL delegate list within 14 days of the event, which will include the email addresses of the delegates (we are sorry that there is this delay in emailing the list, but we need to make sure that it takes into account any late arrivals). You will not be included in this list if you have opted out and you can do this by logging into your registration details. This list will not be sold or ever give out to third parties. Only people attending or sponsoring the event have access to the list
- There may be an independent meeting report published within a few months of this event. If this is published we will send you an email to let you know the reference details
- Notepads and pens are available from the Euroscicon reception desk
- We cannot give out the slides from our speaker's presentations as they are deleted immediately after each event. If you require a particular set of slides please approach the speaker
- Please remember that EuroSciCon is a small independent company with no subsidies from society memberships or academic rates for venues. We try to be as reasonably priced as possible and our delegate rates are substantially lower than comparable commercial meeting organisations
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- We may take pictures during the meeting. These pictures will be used to promote our events and placed on our various websites and the closed Euroscicon group on Facebook. If you do not want your photograph distributed please let one of the Euroscicon staff know.

About the Chair

Rupsha Fraser's first degree was in Biotechnology at the University of Edinburgh. She subsequently became interested in reproductive biology when doing a Masters at the University of Leeds, where her research was looking into the immunogenetics of pre-eclampsia. She went on to carry out a PhD at St George's, University of London, investigating the role of decidual natural killer cells in pregnancy, comparing pregnancies that have been defined as normal healthy pregnancies or those that are at high risk of pre-eclampsia development. Since completing her doctoral studies, Rupsha has been working on a postdoctoral project investigating decidual macrophages in early human pregnancy.

About the Speakers

Dilly Anumba is Professor of Obstetrics and Gynaecology UoS, and Honorary Consultant Obstetrician/Gynaecologist Sheffield Teaching Hospitals NHS Foundation Trust. Holding subspecialist accreditation in Maternal and Fetal Medicine Dilly runs clinics in Prenatal Fetal Imaging, Diagnosis and Therapy, as well as High Risk Pregnancy Care and Prematurity. He is Lead Obstetrician for the care of Infections during Pregnancy and leads research programmes that include the investigation of new techniques to predict premature birth, the contribution of vaginal microbes to inflammation-induced premature birth, and the impact of social exclusion on reproductive outcomes. He serves on the NICE Medical Technologies Advisory Committee (MTAC) and holds several professional committee and board appointments nationally and internationally.

Sean Wattegedera joined Moredun Research Institute in 1999 following an undergraduate degree in Biological Sciences (Hons) at Lancaster University. In 2004, Sean became a Research Scientist and has an interest in cytokine biology and host immunity to Chlamydia spp.. His research focus is primarily involved in the development and application of immunological reagents to investigate disease pathogenesis in both sheep and cattle. He is involved in Scottish Government-funded research to identify host-pathogen interactions and the immunological correlates for the zoonotic pathogen Chlamydia abortus in sheep. In 2012, he became a Researcher Co-investigator on the BBSRC Industrial Partnership Award grant 'The route to the identification of immune correlates of protection in ruminants' in conjunction with the Industrial Partner AbD Serotec (A Bio-Rad Company).

Lynne Sykes qualified from St Bartholomew's and the Royal London Medical School in 2003, and is a London trainee in Obstetrics and Gynaecology. Between 2008 and 2011 she undertook a Wellbeing of Women research training fellowship during which she undertook a PhD in preterm labour under the supervision of Professor Phillip Bennett and Mr TG Teoh at Queen Charlotte's Hospital. She is currently an Academic Clinical Fellow at Imperial College London, researching the potential for anti-inflammatories for the prevention of preterm labour.

Tess Meuleman is working as a prenatal docter at the obstetrics and gynaecology department at the LUMC combining this with her PhD. Next year she will start with her residency for gyneacologist.

In 1973, **Phil Lowry** moved to St Bartholomew's Hospital to head the Pituitary Hormone Laboratory and later the Protein Hormone Unit. In 1986 he took the chair of Biochemistry & Physiology at the University of Reading and in 1988 was appointed Head of Animal and Microbial Sciences. After a term as Dean of Science, he spent a short sabbatical at the Salk Institute. He served on an MRC Board and was a Council member of The Society for Endocrinology and Trustee of the British Society of Neuroendocrinology. He has published over 200 papers (15 in Nature) attracting 13,000+ citations.

POSTER PRESENTATIONS

OOCYTE DONATION PREGNANCIES ARE ASSOCIATED WITH A HIGHER INCIDENCE OF HLA ALLOANTIBODIES

Lisa E.E.L.O. Lashley, Marie-Louise P. van der Hoorn, Geert Haasnoot, Dave Roelen and Frans H.J. Claas
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Background: Foetuses in pregnancies conceived after oocyte donation (OD) have a higher level of antigenic dissimilarity with the mother compared to the semi-allogeneic fetus after natural conception. We questioned whether this leads to a higher level of HLA antibody formation in OD pregnancies. Method: Uncomplicated pregnancies after oocyte donation (n=43) were compared with naturally conceived pregnancies, either spontaneously (n=48) or by in vitro fertilization (n=19). We calculated the number of HLA antigen mismatches and epitope mismatches (HLA Matchmaker). Maternal sera were screened with enzyme-linked immunosorbent assay. Child-specific antibody production was determined using complement dependent cytotoxicity against a panel of HLA typed blood donors and, in case of highly sensitized individuals, by Luminex single antigen beads. Results: A significant higher level of epitope mismatches was observed for pregnancies after OD. Furthermore, we found a significantly higher incidence of HLA antibody production in women conceiving after OD (56% $p<0.0001$). In a multivariate backward- stepwise model analysis the remaining variables showing significant influence on antibody formation were epitope- and HLA mismatching. Antibody formation was positively correlated with the number of fetomaternal antigen (Spearman's rho 0.84, $p< 0.0001$) and epitope mismatches (Spearman's rho 0.87, $p< 0.0001$). Conclusion: We demonstrated that women conceiving after oocyte donation have a higher risk of developing child-specific HLA antibodies. The higher the number of immunogenetic differences, the more antibodies directed against the human leukocyte antigens of the fetus are formed. Despite the stronger antibody response, oocyte donation was associated with an uncomplicated pregnancy in the cases included in this study.

THE IMMUNOMODULATING EFFECT OF SEMEN ON MATERNAL T CELLS

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INTRODUCTION. Semen may play an important role in the creation of a tolerogeneic environment at the implantation site leading to normal pregnancy. Semen contains a variety of immunoregulatory factors of which the exact function at the implantation site remains unclear. The aim of this study was to investigate the influence of seminal plasma on maternal peripheral blood mononuclear cells (mPBMCs).
METHOD. mPBMCs were stimulated with semen with different sperm quality in a mixed lymphocyte culture with and without stimulator PBMCs. Stimulators had one HLA-DR mismatch with the responder to simulate implantation where mother meets her semi allogeneic fetus. At day 4 of the mixed lymphocyte culture supernatants were harvested and tested on cytokine concentrations with the luminex method. In addition mRNA analysis of mPBMCs was performed in the absence and presence of semen. RESULTS. Proliferation differed when semen was added to responders and stimulators depending on the quality of semen. Strikingly, already without addition of stimulator cells, responder cells showed a significant increase in proliferation under influence of semen (p =0.031). Additional mRNA analysis showed a significant upregulation of CD25 in T-cells under influence of semen (p<000.1). In addition, IL-10 was significantly upregulated ($p<0.001$). In contrast, IFN-y and TGF-B were decreased when semen was added ($p<0.001$, $p=0.004$, respectively).
CONCLUSION. These data shows that semen is immune active and has a direct effect on maternal T cells. Upregulation of both CD25 and IL-10 may be associated with a more regulatory phenotype of the immune response. Failure of this immunoregulatory function or less exposure to semen may be the underlying cause in abnormal implantation and leading to complicated pregnancies.

TO AVOID IMMUNE SURVEILLANCE, THE PLACENTA MODIFIES ITS SECRETORY PROTEINS WITH PHOSPHOCHOLINE.

P J Lowry

The University of Reading UK RG6 6AH.

During pregnancy the placenta secretes the tachykinin, neurokinin B, into the mother's circulation at concentrations that activate its preferred receptor (NKR3) in the mother, causing vasoconstriction of hepatic portal and mesenteric blood vessels and tachycardia, sometimes resulting in hypertension. In an attempt to correct the ischemia seen in pre-eclampsia at the placental/uterine interface, the placenta responds by increasing secretion of this tachykinin, which then progressively stimulates all three neurokinin receptors (NKR3, NK R2 and then NKR1) in the mother's circulation, thus explaining the many symptoms seen in this disease. The situation is even more complex: neurokinin B, along with other polypeptides synthesised by the placenta (pro-opiomelanocortin, pro-corticotropin releasing factor, pro-activin, pro-follistatin and pro-hemokinin), have all subsequently been found to be post-translationally modified with a moiety containing phosphocholine.It is known that, when attached to certain secreted parasitic proteins or to bacterial proteins, the phosphocholine group endows those organisms with immune-inhibiting properties, ensuring the survival of the organism in the host. Hence the attachment of phosphocholine to peptides secreted by the placenta would suggest that the placenta may be utilising the same survival mechanism, attenuating immune surveillance by the mother, and thus avoiding rejection. This presentation will discuss the observations that have led to these proposals and will suggest how this knowledge could be used in the management of other conditions: (a) most women with rheumatoid arthritis go into complete remission during pregnancy and it is possible that drugs with phosphocholine moieties may help manage this disease in the non-pregnant state; (b) engineering stem cells to express phosphocholinated proteins may make xeno-grafting possible; (c) although the enzyme responsible for phosphocholination of proteins appears to be expressed only in the placenta and testis, it is possible that some cancers use phosphocholination of proteins to evade immune detection, so that the ability to block the enzyme may lead to the detection and destruction of the cancer by the immune system.

MATERNAL NK CELLS REGULATE FETAL GROWTH AND PLACENTAL EFFICIENCY IN THE MOUSE.

Selma Boulenouar, Amanda Sferruzzi-Perri, Hong Wa Yung, Louise Gaynor, Steve Charnock-Jones, Abby Fowden, Graham Burton and Francesco Colucci

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INTRODUCTION. A consistent feature of decidualisation is the influx of a distinct lineage of uterine NK cells (uNK). Although the function of uNK cells is still uncertain, evidence strongly suggests that uNK cells participate in normal placental development to accommodate the demands of the developing fetus. METHODS. We assessed the contribution of NK, B and T cells to fetal and placental growth in crosses of KO female mice (Rag2-/-, Rag2-/-Il2rg-/- and E4BP4-/-, all on C57BL/6 background) with WT males. Offspring of these crosses and their placentae are heterozygous for the mutation, thus any potential abnormality is linked to the maternal genotype. We analyzed fetal and placental growth by measuring fetal and placental weights near term at gestation day 18.5 (murine gestation is 19.5 days in the C57BL/6 strain). uNK cells were assessed by flow cytometry and immunohistochemistry. Spiral arterial size, decidual density, trophoblast thickness and mesometrial lymphoid aggregate of pregnancy (MLAp) distribution were measured at midgestation (gestation day 9.5) by immunohistochemistry. We quantified placental function by determining the placental capacity for substrate transfer. Placental structural analysis was performed using the Computer Assisted Stereological Toolbox program on paraffin and resin embedded samples. RESULTS. NK-cell deficient females (Rag2-/-Il2rg-/- and E4BP4-/-) exhibit impaired placental efficiency, as indicated by the significantly reduced fetal/placental ratio generated by smaller fetuses and larger placentae. Structural and morphological analysis of these placentae at term showed enlarged labyrinthine and junctional zones, increased maternal blood space and the formation of precipitates in trophoblastic cells. Placental permeability for hydrophilic molecules (mannitol) by passive transfer is significantly reduced in alymphoid Rag2-/-Il2rg-/- but unaltered in E4BP4-/- mice. E4BP4-/- mice however do exhibit defective spiral artery remodelling, which corroborates previous published data from Rag2-/-Il2rg-/- mice. Other structural abnormalities observed at midgestation in E4BP4-/- mice include increased trophoblast thickness and decreased MLAp size, with a concomitant significant reduction in MLAp uNK cell frequency. CONCLUSIONS. Our results support the hypothesis that NK cells are necessary and sufficient for normal placental efficiency and fetal growth. Morphological, structural and transfer analyses of placentae are highly useful experimental techniques for determining the functional significance of defined NK cell subsets and other lymphocytes to reproductive outcome.

MENSTRUAL BLOOD CLOSELY RESEMBLES THE UTERINE IMMUNE MICRO-ENVIRONMENT AND IS CLEARLY DISTINCT FROM PERIPHERAL BLOOD

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The uterine immune cell population is important for successful pregnancy. Recently, we have developed a non-invasive technique to obtain and study uterine immunological cells by collecting menstrual fluid, limiting the need for biopsies. To validate this technique, 5 healthy non-pregnant females (mean age 31,5±5) with regular menstruation, collected menstrual fluid using a vaginal cup during 3 days in six 12-hour shifts for 5 cycles. Peripheral blood samples were taken for comparison. Mononuclear cells from menstrual fluid (MMC) and peripheral blood (PBMC) were isolated by using Ficoll density centrifugation and were shown to be viable. MMC and PBMC samples were characterized for the different lymphocyte subsets by flowcytometry, with emphasis on NK cells and T cells. Next, the functional capacity of MMC derived NK cells was determined by measuring intracellular production of IFN-g, granzyme B and perforin after culture in the presence of IL-2 and IL-15. MMC samples contained the typical composition of mononuclear cells expected from endometrial tissue, were phenotypically similar to the reported phenotype for biopsy derived endometrial cells, and distinct from PBMC, supporting their endometrial origin. Increased percentages NK cells and decreased percentages T cells were found as compared to PBMC from the same female. The MMC derived NK cells were pre-dominantly $CD56^{bright}/CD16^{-}$, in contrast to the primarily $CD56^{low}/CD16^{+}$ peripheral blood NK cells. MMC derived NK cells expressed CD103, indicating their mucosal origin. In addition the pattern of natural cytotoxicity receptor (NCR) expression on MMC derived NK cells was comparable to endometrial biopsy derived NK cells. Compared to PBMC the NKp30 expression was decreased, while the percentage of NKp44 positive cells was increased in MMC samples. CXCR3 and CXCR4 was hardly expressed by MMC derived NK cells, indicating that these cells are not from PBMC origin. NK cells from MMC samples were functional as shown by their capacity to produce IFN-γ, granzyme B and perforin, upon stimulation with IL-2 and IL-15. MMC derived T cells revealed an increased expression of CD103, CD69 and CXCR4 compared to PBMC derived T cells. Importantly, MMC collection using a menstrual cup proved highly reliable and reproducible between women and between cycles. In conclusion, menstrual fluid can be used as source for endometrial cells and this now opens up new research areas and opportunities to study endometrial cells.