THE CROATIAN ACADEMY OF SCIENCES AND ARTS

The Department of Biomedical Sciences in Rijeka

CROATIAN ACADEMY OF MEDICAL SCIENCES - Branch office Rijeka

UNIVERSITY OF RIJEKA - MEDICAL FACULTY

CLINICAL MEDICAL CENTER RIJEKA

THE CROATIAN MEDICAL ASSOCIATION – Branch office Rijeka

19th symposium

HORMONES AND CYTOKINES IN INFLAMMATION AND PREGNANCY

September 12th, 2017
9,00 am
University Campus Rijeka, University Departments, Lecture hall O-030, Radmile Matejčić 2, Rijeka

Organizers

THE CROATIAN ACADEMY OF SCIENCES AND ARTS
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Scientific and Organizing Committee

Daniel Rukavina, president
Julia Szekeres Bartho, Herman Haller, Gordan Gulan and Marin Dominović

Registration: 8,00 – 9,00 am

Free admission. Participants who want a certificate from the Croatian Medical Chamber need to register.
Parking is free and provided in the building of the Student Center Rijeka, Radmile Matejčić 5

Information

Željana Mikovčić, Department of Biomedical Sciences in Rijeka
Radmile Matejčić 2, Rijeka
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P R O G R A M
OPENING
(9,00 – 9,30)

Introduction

Daniel Rukavina, M.D., PhD. Professor Emeritus, Head, Department of Biomedical Sciences in Rijeka, Croatian Academy of Sciences and Arts, Rijeka, Croatia

Julia Szekeres Bartho, M.D., PhD., Professor, President of the European Society for Reproductive Immunology

Welcome adresses

Davor Štimac, M.D., PhD., Professor, Head, Clinical Hospital Center Rijeka, Rijeka, Croatia

Tomislav Rukavina, M.D., PhD., Professor, Dean of the Medical Faculty, University of Rijeka, Rijeka, Croatia

Snježana Prijić-Samaržija, PhD., Professor, Rector of the University of Rijeka, Rijeka, Croatia

9,30 – 11,30 h

I. MEDIATORS OF INFLAMMATION

Chairmen: Daniel Rukavina and Barbara Bottazzi

Barbara Bottazzi, M.D., PhD., Humanitas Clinical and Research Center, Rozzano, Italy
The long Pentraxin PTX3 in the regulation of inflammation

Bojan Polić, M.D., PhD, Medical Faculty, University of Rijeka, Rijeka, Croatia
The role of pro-inflammatory cytokines in progression of Diabetes mellitus type 2 caused by viral infections in obesity

Gordana Laškarin, M.D., PhD, Medical Faculty, University of Rijeka, Rijeka, Croatia
Inflammation mediated by heat shock proteins

Tamara Gulić, PhD., Medical Faculty, University of Rijeka, Rijeka, Croatia
M2-like Tumor-associated macrophages express Molecule 1, a new marker for macrophage polarization affecting monocytes motility

Coffee break: 11:30 – 12:00

12,00 – 15,30 h

II. INFLAMMATION AND PREGNANCY

Chairmen: Julia Szekeres Bartho and Marie-Pierre Piccinni

Julia Szekeres Bartho, M.D., PhD., Department of Medical Biology, Medical School, University of Pecs, Pecs, Hungary
Extracellular vesicles as means for communication between the mother and the embryo

Marie-Pierre Piccinni, M.D., PhD., University of Florence, Florence, Italy
T cell cytokines : foes or friends for pregnancy

Lunch with a panel of speakers: 13:00 – 13:45

Biserka Mulac Jeričević, M.D., PhD., Medical Faculty, University of Rijeka, Rijeka, Croatia
The Role of Progesterone in Orchestrating Embryo-Uterine Interactions

Chiara Agostinis, M.D., PhD., Institute for Maternal and Child Health, University of Trieste Trieste, Italy
Role of endothelium in the pathophysiology of pregnancy

Marin Dominović, PhD., Department of Biotechnology, University of Rijeka, Rijeka, Croatia
Maternal love: Decidual lymphocytes possess lower cytotoxic potential

Coffee break: 15:15 – 15:30

15,30 – 17,00 h

III. CLINICAL ASPECTS

Chairmen: Herman Haller and Gordana Laškarin

Emma Lucas, PhD., Warwick Medical School, Clinical Sciences Research Laboratories, University Hospital, Coventry, UK
Endometrial stem cell deficiency in recurrent pregnancy loss

Tea Štimac, M.D., PhD., Medical Faculty and Clinical Hospital Center Rijeka, Rijeka, Croatia
Progesteron and Pregnancy

Lana Glavan Gačanin, M.D., Medical Faculty and Clinical Hospital Center Rijeka, Rijeka, Croatia
Immunological processes at the maternal-embryonal interface of missed abortion and anembryonic pregnancy

The long Pentraxin PTX3 in the regulation of inflammation

Barbara Bottazzi[1], Kenji Daigo[1], Marina Sironi[1], Andrea Doni[1], Roberto Leone[1], Antonio Inforzato[1,2], Alberto Mantovani[1,3].

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[2]Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy
[3]Humanitas University, Rozzano, Milan, Italy

Pentraxins are essential molecules of the innate immunity. CRP is the prototype of the short pentraxin family and a main acute phase protein in humans. Long pentraxin 3 (PTX3) is a distant relative of CRP identified in the early 80'. Differently to CRP, produced systemically by the liver in response to IL-6, PTX3 is locally and rapidly produced by different cell types, most efficiently by phagocytes (macrophages; myeloid dendritic cells and neutrophils), in response to inflammatory signals and Toll-like receptor engagement. In addition lymphatic endothelial cells are new unexpected cellular sources of PTX3. Homology to CRP resides in the C-terminal pentraxin-like domain, while the long N-terminal domain present in PTX3 is unrelated to other known proteins. The secreted protein is made by eight identical disulphide bond-linked protomers, each containing a single N-glycosylation site fully occupied by complex type sialylated oligosaccharides that have been involved in a number of biological functions. PTX3 has emerged as an essential component of the humoral arm of innate immunity, acting as a functional ancestor of antibodies: it interacts with selected microbial molecules; it has opsonic activity via Fcgamma receptors; it activates and regulates the Complement cascade by interacting with C1q, Factor H and ficolins; it regulates inflammation by interacting with Complement and P selectin via its glycosidic moiety. In keeping with its regulatory role on inflammation, it emerged that PTX3 can limit cancer-related inflammation acting as an oncosuppressor. PTX3 has an essential role in resistance against selected pathogens such as Pseudomonas aeruginosa, Aspergillus fumigatus, uropathogenic E.coli, cytomegalovirus and selected strains of influenza virus. Preliminary data on a new antimicrobial mechanism will be discussed. Correlative genetic evidence is consistent with the view that PTX3, highly conserved between mouse and man, plays an essential role in resistance against selected microbes in humans. PTX3 is now a candidate new diagnostic for inflammatory conditions, better related to outcome than CRP. In summary, PTX3 is a fluid phase pattern recognition molecule which has served as a paradigm for humoral innate immunity, linking cellular and humoral effectors and having a regulatory function on inflammation.

The role of pro-inflammatory cytokines in progression of Diabetes Mellitus type 2 caused by viral infections in obesity

Marko Šestan[1], Sonja Valentić[1], Inga Kavazović[1], Đurđica Cekinović[2], Tamara Turk Wensveen[3], Ilija Brizić[1], Stipan Jonjić[1], Felix M. Wensveen[1] and Bojan Polić[1]

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[3]Department of Endocrinology, Clinical Hospital Center Rijeka, Rijeka, Croatia

Diabetes Mellitus type 2 (DM2) is a chronic progressive metabolic disease mostly associated with obesity. It is characterized by high blood glucose levels, insulin resistance (IR) and systemic chronic low-grade inflammation. Accumulation of fat causes metabolic disbalance in tissues and systemic low-grade inflammation which both lead to the gradual development of IR, glucose intolerance (GI) and DM2. However, it has been clinically observed that after a pretty stable prediabetic phase, DM2 develop often very rapidly and stays rather permanent despite of relatively high insulin levels. What is the trigger and mechanism of such an aggravation is still unknown.

Since obese people are often exposed to various infections which drive a strong systemic inflammatory response, we asked whether they are cause of the fast progression to DM2. First, we established a small prospective clinical study where we measured parameters important for glucose homeostasis in obese and non-obese patients exposed to acute viral respiratory infections. We found that the acute infections transiently increase fasting insulin levels in both, normal (BMI < 25) and overweight (BMI > 25) patients, where in latter this was more pronounced. Furthermore, we noticed that three months after the infection systemic IR was decreased in normal weight patients, while in overweight group it remained rather high.

To elaborate these findings in more detail we used diet induced obesity (DIO) mouse model which was exposed to mouse cytomegalovirus (MCMV) as a model for a common human infection. We infected mice 6 weeks upon start of high fat diet (HFD) when mice are exposed to certain metabolic disbalance, but still do not develop GI and IR. Infection induced strong IR 7 days after the infection of HFD and normal chow diet (NCD) mice. However, in contrast to the HFD group, GI was not observed in NCD mice. IR and GI remained high in HFD mice also 3 weeks after infection. To assess how MCMV infection causes increased IR we tested whether some of virally induced cytokines (IFNα/β, TNFα, IL-1β, IFNγ) are involved. We identified IFNγ as the major aggravating factor of IR. Furthermore, by conditional ablation of IFNgR1 in tissues important for glucose homeostasis we identified skeletal muscle cells as main target of IFNγ, while hepatocytes and adipocytes were not involved. Next, we found that IFNγ specifically downregulates expression of insulin receptor on muscle cells, but not on hepatocytes. The outcome of this mechanism is muscle specific IR which in turn causes prevention of muscle glucose uptake and increased insulin production by β-cells to compensate hyperglycemia. While in normal weight mice (and humans) it causes a transient effect, in obese subjects it results in permanent aggravation of glucose homeostasis.

Inflammation mediated by heat shock proteins

Gordana Laškarin[1,2], Tamara Gulić[1] and Daniel Rukavina[2]
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Heat shock proteins (hsps) are evolutionary highly conserved proteins which are expressed in various subcellular localizations, indicating their high biological significance. All of them are strongly cytoprotective for the host cell due to their chaperone functions. Hsps maintain "client" protein folding, translocation or degradation mediating in a signal transduction and cell survival, regardless of their structural differences and family affiliation due to the molecular mass (HSP 100, HSP 90, HSP 70, HSP 60 or small HSPs). In addition to temperature shock, hsps are induced in different human tissues after the "physiological" stress such as the influence of growth factors and hormones, cell and tissue differentiation, including mild pro-inflammatory events during tissue remodeling at the normal maternal-embryonal interface. Constitutive HSC70 and inducible HSP70 forms were detected in decidua basalis of normal early pregnancy, blighted ovum and missed abortion. They were always higher when compared to term placenta. Glycoprotein 96 was the lowest in decidua of missed abortion when compared to normal early pregnancy and term placenta. Pathological conditions, such as infection, tumorigenesis, various radiations and cytotoxic agents (chemotherapy), nutritional and oxidative stress, ischemia and autoimmune processes significantly enhance expression and secretion of hsps from damaged viable and necrotic, but not apoptotic cells. The extracellular hsp/peptide complexes emerged as "danger" signals which might elicit strong innate and acquired immune response on unconventional manner. Members of HSP70, HSP90 and Hsp27 bind to pattern recognition receptors including CD91 and Toll-like receptor-4 (TLR-4), expressed on antigen presenting dendritic cells and macrophages. In decidua of missed abortion labeling intensity of CD91 and TLR4 was lower than in decidua of normal pregnancy, blighted ovum and term placenta, suggesting less efficient gp96/peptide internalization. Internalized hsp-chaperoned peptides are presented in the context of MHC class I molecules (cross presentation) and MHC class II molecules to CD8+ or CD4+ T cells, respectively. Thus the expansion of cytotoxic hsp specific T cell clones was found in atherosclerotic plaque, autoimmune Behcet disease, infections and tumors mediating tissue damage. Large hsps might act as highly effective cancer vaccines when associated with cancer antigens. The internalization of hsp/peptide complexes in antigen presenting cells engage highly conserved pro-inflammatory and anti-apoptotic NF-kappa B signaling pathway with specific pattern of cytokine and chemokine secretion, and co-stimulatory or differentiation marker expression. However, the gp96, HSP90, HSP70 and Hsp27 act differentially, and each induces some, but not all molecules. Recently was shown that hsps/peptide complexes could also induce tolerogenic/regulatory effects depending on inflammation status of the surrounding tissues and probably concentration of hsp/peptide complexes. Thus the immune responses mediated by hsp/peptide complexes are not unique, particularly in anti-

gen processing, and presentation, in maturation program of antigen presenting cells and immune orientation.

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M2-like Tumor-associated macrophages express Molecule 1, a new marker for macrophage polarization affecting monocytes motility

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Tumor-associated macrophages (TAMs) are key orchestrators of the tumor microenvironment directly affecting many biological activities such as neoplastic cell growth, neoangiogenesis, and extracellular matrix remodelling in order to promote tumor growth. Levels of TAMs often correlated with bad prognosis and more recent studies have also highlighted a link between their abundance and the process of metastasis. Recently, it was shown by gene profiling that human Molecule 1 (Mol-1) is selectively expressed by M2 macrophages and by tumor-associated M2-like macrophages. This observation suggests that Mol-1 could represent a novel marker of macrophage polarization and could exert functional properties in tumor progression.

We focus our attention on the characterization of reagents to study Mol-1 biology. After immunization with the Mol-1, we selected a monoclonal antibody recognizing specifically human Mol-1. The antibody was used to purify by immunoaffinity chromatography recombinant human Mol-1 produced by transfected CHO cells. Initial efforts were aimed at defining the conditions ensuring protein stability over the time. Given that the alternative phenotype can be induced in macrophages by different stimuli, Mol-1 expression was validated upon several anti-inflammatory treatments. The anti human-Mol-1 mAb (IgG1) was then used in to detect the endogenous expression of Mol-1 in sections of paraffin embedded tumor tissues (lung, breast, colon and pancreatic cancer) as well as in normal tissue samples (liver, skin and decidua of first trimester). The expression was also confirmed by in situ hybridization. In the same specimens, Mol-1 expression was evaluated in tumour associated macrophages (TAMs) by double staining using the anti Mol-1 mAb and some of macrophages markers such as an anti-CD68 or CD206 or CD163 antibodies, respectively. In addition, the biological activity of purified rh Mol-1 was tested in migration and invasion assays with monocytes and cancer cells, using boyden chamber or transwells respectively.

Mol-1 was induced by M-CSF, IL-4, and IL-10 underlying the down-regulatory effect of proinflammatory stimuli (INF-$_\gamma$). mRNA and protein analysis clearly demonstrated

that it is specifically associated with the M2 polarization status of macrophages. This study indicates anheterogeneous pattern of Mol-1 expression in the tumor tissues. Mostly Mol-1 is found in tumor cells, a subset of stromal cells, fibroblasts and in tumor-associated macrophages. Interestingly, in the double-stained specimens, most CD68 or CD206 or CD163 positive macrophages were found to express Mol-1. Mol-1 can affect the motility of a different human cancer cell line, as well as migration of human monocytes and neutrophils, suggesting that it can promote monocyte/macrophage and neutrophils recruitment into tissues.
The observation that human Mol-1 is selectively expressed by M2 macrophages and TAMs suggest that could represent a novel marker of macrophage polarization. In addition, modulating monocyte migration, Mol-1 might promote and/or sustain a permissive microenvironment for cancer cell invasion and metastasis. Definitely, further efforts are required to evaluate the prognostic/diagnostic potency of this protein as a M2 marker.

Extracellular vesicles as means for communication between the mother and the embryo

Julia Szekeres-Bartho
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Extracellular vesicles (EVs) are phospholipid bilayer enclosed particles which are constitutively produced by both eukaryotic and prokaryotic cells. On the basis of their specific exofacial and intravesicular molecular pattern (DNA, RNA, protein), EVs play important role in intercellular communication, both in physiological and pathological processes. Although all cell types can produce any subpopulations of EVs, the different vesicles are induced by various stimuli.
Here we show that embryos produce extracellular vesicles. These can be demonstrated at the feto-maternal interface at day 5 murine pregnancy, as well as in the day 5 culture medium of embryos. Among others, embryo derived EVs contain PIBF, which affects the functioning of the maternal immune system both in vitro and in vivo.
In attempt to increase the chances of pregnancy in infertile women, many in vitro fertilization (IVF) centres transfer more than one embryos. This in turn also increases the hazard of twin pregnancies. Multiple pregnancies are among the most common causes of preterm birth, along with the increased risk for prematurity. Therefore, it would be of importance to select the embryo that is most likely to implant and to transfer that particular embryo only. This requires improved methods to identify the embryo that is most likely to implant.
We have developed such a test, based on determination of the DNA (PI+) containing extracellular vesicle (EV) count in day 5 embryo culture media. Ninety seven infertile women undergoing IVF were included in the study. More than one embryos were transferred to most patients. In 67 women, the transfer resulted in clinical pregnancy, whereas in 30 women the embryos failed to implant. In the 112 culture media of embryos from the “clinical pregnancy” group, the number of PI+ EVs was significantly lower than in those of 49 embryos, from the “implantation failure” group.

In 20 women, transfer of a single embryo resulted in a singleton pregnancy, or, transfer of two embryos in twin pregnancy. The culture media of 19 out of the 20 "confirmed competent" embryos contained a lower than the cut off level PI+EVs.
We conclude that the competent embryo can indeed be identified by low PI+EV counts, and based on this, we developed a non-invasive, simple, inexpensive, and quick test, which identifies the embryos that are most likely to implant in a receptive endometrium, with 0.796 sensitivity and 0.935 specificity.

T cells : Foes and friends for pregnancy

Marie-Pierre Piccinni
Department of Experimental and Clinical Medicine and DENOTHE Excellence Center, University of Florence, Florence, Italy

Trophoblast HLA-C antigens from paternal origins, which liken the trophoblast to a semiallograft, could be presented by the maternal APCs to the specific maternal CD4+ T helper cells, which could release various cytokines in response to these alloantigens. On the basis of the cytokines produced, these cells can be classified in Th1, Th2 and Th17 cells. Th2 cells together with regulatory CD4+ T cells, known to be involved in allograft tolerance, could be responsible, at least in part, for the success of pregnancy. Apparently, Th1 and Th17 cells, known to be responsible for acute allograft rejection, could be involved in miscarriage. However, Th17 cells are plastic. A part of human IL-17A-producing cells were found to also produce IFN-γ (they are named Th17/Th1) and Th17/Th1 exhibit plasticity towards Th1 cells in response to IL-12 or the prolonged exposure to IL-23. Nevertherless, the association of IL-17 and IL-4 production by CD4+ T helper cells (Th17/Th2 cells) has also been observed in particular in allergic disorders. An associated spontaneous production of both IL-17A, IL-17F and IL-4 is found in fresh decidua CD4+ T cells in successful pregnancy. A prevalence of Th17/Th2 cells (producing IL-17A, IL-17F, IL-22 and IL-4) is present in the decidua of successful pregnancy but the exclusive presence of Th17 (producing IL-17A, IL-17F, IL-22) and Th17/Th1 (producing IL-17A, IL-17F, IL-22 and IFN-γ) cells is present in the decidua of unexplained recurrent abortion. More importantly, Th17/Th2 cells are exclusively present at embryo implantation site, whereas Th17, Th17/Th1 and Th1 cells are exclusively present away from implantation site. Therefore, no pathogenic role of decidual Th17 cells on pregnancy, but rather a beneficial role when these cells also produce IL-4 (Th17/Th2 cells). HLA-G5 could be the factor of the uterine microenvironment responsible for the development of Th17/Th2 cells, which seem to be crucial for the successful embryo implantation.
Autoimmune disorders are characterized by tissue damage, caused by self-reactivity of different effectors mechanisms of the immune system, namely antibodies and T cells. Their occurrence may be associated with genetic and/or environmental predisposition and to some extent, have implications for fertility and obstetrics.
Th17- and Th1-type cells are aggressive and pathogenic in many autoimmune disorders and inflammatory diseases. The immunology of pregnancy underlies the role of Th2-type cytokines to maintain the tolerance of the mother towards the fetal semi-allograft. Thus, in pregnancy Th2, Th17/Th2 and Treg cells accumulate in the decidua

but may also be present in the mother's circulation and can regulate autoimmune responses influencing the progression of autoimmune and inflammatory diseases, are thus capable of regulating coincidental autoimmune responses to remission or worsening of the related Th1-/Th17- or Th2- autoimmune diseases, respectively. The postpartum exacerbation of some Th1/Th17 autoimmune disorders may reflect an imbalance in Th2- type cells and T reg cells, which is caused by the rapid fall in the numbers of these cells after delivery.
Pregnancy is able to influence the onset and progression of autoimmune and inflammatory diseases by influencing the T cell cytokine-mediated responses during the gestation period and the post-partum period.

The Role of Progesterone in Orchestrating Embryo-Uterine Interactions

Biserka Mulac Jeričević
Department of Physiology and Immunology, Medical Faculty, University of Rijeka, Rijeka Croatia

Infertility affects millions of women worldwide. A successful pregnancy requires a healthy uterus that is ready to receive and support an implanting embryo. Implantation site formation is an essential process during the establishment of pregnancy in mammals. It is initiated with the attachment of the blastocyst to a receptive uterine epithelium followed by its invasion into the stromal tissue. The uterus is dependent on the secretions of the ovarian hormones estrogen (E) and progesterone (P) which mediate their biological functions via their cognate receptors, the estrogen and progesterone receptors (ER and PR). In human, rodents and many other species, PR is present in two isoforms, PR-A and PR-B. These isoforms exhibit tissue-specific transcriptional activity. The transcriptional activity of PRs leads to the synthesis of cytokines, growth factors, lipid mediators, and genes that control the dynamics of uterine transition to a receptive state and the establishment of pregnancy. The progesterone receptor not only functions using classical nuclear receptor signaling, but also participates in non-genomic signaling at the cellular membrane. The complexity of P signaling is further enhanced by post-translational transcriptional regulation via kinases and transcription co-regulators. The window of uterine receptivity corresponds to the P-mediated downregulation of estrogen receptor (ER) activity in the uterine luminal epithelium (LE). Suppression of ER-mediated proliferative activity by PR is mandatory for implantation and the establishment of pregnancy. Genetically modified mouse models with total or conditional gene knockout mutations have become powerful tools for determining the functional roles of molecular factors involved in various aspects of implantation biology. These studies have uncovered molecular cues, which are produced under the influence E and P via paracrine exchange between the epithelial and stromal compartments of the uterus during peri-implantation. In this presentation current knowledge of the P regulated molecular events leading to successful implantation and decidualization will be presented.

Role of endothelium in the pathophysiology of pregnancy

Chiara Agostinis[1], Fleur Bossi[1], Oriano Radillo[1], Francesco Tedesco[2] and Roberta Bulla[2]

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Human endothelial cells (ECs) are multifunctional cells capable to secrete a variety of biologically active mediators. In disease states, ECs are activated by several mechanisms enhancing the expression of cell adhesion molecules and cytokines, in order to orchestrate the inflammatory response. ECs, although similar in function and morphology, represent an heterogeneous population of cells in terms of secretion of inflammatory mediators, modulation of adhesion molecules, leakiness and procoagulant activity.

In the early phase of human pregnancy, maternal spiral arteries undergo physiological transformation characterized by gradual loss of the normal muscoloelastic structure of the vessel wall and replacement by amorphous fibrinoid material, in which trophoblast cells are embedded. Invading trophoblast cells (endovascular trophoblast cells), penetrate the spiral arteries and migrate upward against blood flow, forming intravascular islands and mosaic vessels, as a result of partial replacement of the endothelium (Bulla et al., 2004). Decidual endothelial cells (DECs) are influenced by the special immunological and inflammatory environment established in decidua. DECs play a key role in controlling the traffic of leukocytes across the vessel wall, which needs to be tightly regulated in order to guarantee the success of pregnancy.

We have previously shown (Bulla et al., 2008) that DECs are the only endothelial cells able to express C1q under physiologic conditions. More recently, we have further characterized these cells, comparing their immunological phenotype to that of ECs isolated from other districts, including HUVEC, microvascular endothelial cells isolated from adult skin (ADMEC), and UtMEC, endothelial cells isolated from non pregnant uterus. DECs were found to be are hypo-responsive to LPS stimulation in terms of IL-6, CXCL8 and CCL2 production, to express low levels of TLR4 and to manifest strong constitutive activation of the non-canonical NF-κB pathway and low responsiveness of the canonical pathway to LPS. Furthermore, DECs display a different "arsenal" of adhesion molecules and cytokines in response to classical pro-inflammatory stimuli compared to ADMEC and HUVEC expressing constitutively the adhesion molecules ICAM2 and ICAM3, but failing to show increased expression of ICAM1. Also, these cells produce higher levels of the chemokines CXCL9/MIG and CXCL10/IP-10 in response to IFN-γ compared to other ECs, and promote selective migration of FOXP3 positive T cells. These findings are consistent with the local changes that occur during pregnancy aimed at controlling the inflammatory response at feto-maternal interface.

In an effort to find the microenvironmental decidual conditions (or factors) that promote the shift from UtMECs to the anti-inflammatory phenotype of DEC, we hypothesized that HGF, RANTES and P4R highly expressed in DECs may represent markers for the decidualization of these cells. To prove this, we incubated UtMECs with progesterone (P4), 17-β-estradiol and cAMP for two weeks and monitored the expres-

sion of these proteins. We observed a significant increase of HGF, RANTES and P4R expression by stimulated UtMECs , although we failed to observe C1q expression.
In conclusion, DECs manifest an anti-inflammatory and tolerogenic immune-phenotype (E2 phenotype?) and the identification of factors inducing this shift could be very useful to understand the pathogenetic mechanisms of pregnancy disorders such as idiopathic recurrent spontaneous abortion (RSA) or pre-eclampsia (PE).

Maternal love: Decidual lymphocytes possess lower cytotoxic potential

Dominović Marin[1], Laškarin Gordana[2,3], Rukavina Daniel[2]
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Decidualization of human endometrium is associated with massive recruitment of distinct CD56bright+ CD16- natural killer (NK) cells. The population of decidual lymphocytes (DLs) usually consists of more than 70% of NK cells. Granulysin (GNLY) is cytotoxic mediator of immune reactions, and is involved in the lysis of prokaryotic cells and apoptosis of eukaryotic cells. It is constitutively expressed in human NK cells, while in cytotoxic T cells is expressed only in the activated ones. GNLY has two forms with distinctive roles. The cytotoxic 9 kDa GNLY form is the result of cleaving the 15 kDa precursor GNLY form. While both GNLY forms are secreted from the cytoplasmic granules upon stimuli from the microenvironment, the 15 kDa GNLY form is also secreted constitutively via a calcium-independent secretory pathway. In the extracellular space, 15 kDa GNLY act as "alarmin" and enhances immunologic reactions by recruiting the immunocompetent cells to the site of inflammation. The distribution of GNLY+ cells in the stroma and around decidual glands and vessels resembles decidual CD56+ NK cells.
The expression of cytolytic mediator perforin (PER) is similar to GNLY expression in DLs, implying that these cytotoxic mediators are present in the same cells. PER and GNLY are both in over 85% of decidual CD56bright+ cells, the predominant immune cell population at the maternal-embryonic interface. The decidua contains the highest levels of PER and GNLY in any known physiological and pathological conditions.
In our study, we determined the dynamics of colocalization changes in DLs and suggested the importance of these changes for the functional maturation of DLs. We investigated the: (1) expression of GNLY forms in DLs and peripheral blood lymphocytes (PBLs), (2) colocalization level of both GNLY forms with PER and LAMP-1 (a marker of degranulation) in DLs and PBLs after activation, (3) influence of HLA-C and HLA-G molecules, and proinflammatory cytokines IL-15, IL-2. Decidual tissues and peripheral bloods were obtained from the Clinic of Gynecology and Obstetrics, Clinical Hospital Centre of Rijeka after informed consent. The expression of GNLY forms and PER was determined by using immunofluorescence and confocal microscopy.
Both GNLY forms are expressed in DLs. After activation 9 kDa, cytotoxic GNLY form and PER colocalize more with LAMP-1, as well as 9 kDa GNLY form with PER. In DLs HLA-C molecule significantly decreased the colocalization of 9 kDa GNLY with

PER, while HLA-G molecule increased the colocalization of immunoregulatory, 15 kDa GNLY with PER and PER with LAMP-1. IL-15, but not IL-2 in DLs decreased the colocalization of 9 kDa GNLY form with LAMP-1.
This investigation for the first time suggests the importance of GNLY forms and PER, as mediators of cytotoxic and cytolytic activity at the maternal-embryonic interface. Cytokine IL-15, HLA-C and HLA-G molecules of trophoblast cells mediates the important inhibitory influence on DLs functional maturation in terms of GNLY forms and PER colocalization and possibly contributing to the successful pregnancy outcome.
Acknowledgement: This work was supported by the grant from the University of Rijeka (No. 13.06.1.1.06) to professor Daniel Rukavina.

Endometrial stem cell deficiency in recurrent pregnancy loss

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In human endometrium, menstruation is followed by rapid estrogen-dependent growth, dependent on activation of endometrial progenitor cells. Upon ovulation, progesterone and rising cellular cAMP levels activate the transcription factor Forkhead box O1 (FOXO1) in endometrial stromal cells (EnSCs), leading to cell cycle exit and differentiation into decidual cells that control embryo implantation. Aberrant responsiveness to deciduogenic cues is associated with recurrent pregnancy loss (RPL), suggesting defects in cellular maturation. Previously, we demonstrated that RPL is associated with reduced clonogenic capacity in human endometrium in association with accelerated stromal senescence. Our findings indicated that stem cell deficiency and accelerated stromal senescence limit the differentiation capacity of the endometrium and predispose for pregnancy failure. Here we show that FOXO1 also causes acute senescence of a subpopulation of decidualizing EnSCs in an IL-8 dependent manner. Selective depletion or enrichment of this subpopulation revealed that decidual senescence drives the transient inflammatory response associated with endometrial receptivity. Further, senescent cells also prevent differentiation of endometrial mesenchymal stem cells in decidualizing cultures. As the cycle progresses, IL-15 activated uterine natural killer (uNK) cells selectively target and clear senescent decidual cells through granule exocytosis. Our findings reveal that decidual senescence governs endometrial remodeling at embryo implantation, and suggest a critical role for uNK cells in maintaining cellular homeostasis in cycling endometrium.

Progesteron and pregnancy

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Progesterone, also called "the pregnancy hormone" is steroid hormone crucial for gestational maintenance. Corpus luteum is the main producer of progesterone during first weeks of pregnancy, after 8 weeks of gestation the placenta gradually becomes

the main source of progesterone. Progesterone blood levels increase throughout pregnancy, peaking during last 4 weeks of gestation and decreasing after labour and placental delivery. This steroid hormone is mainly synthesised from maternal cholesterol, through a two-step reaction occurring in the syncytiotrophoblast mitochondria. Progesterone acts in several events throughout the gestational period, its main functions are exerted in the uterus.

Progesterone is essential before pregnancy and has crucial role in its maintenance based on different mechanisms such as: modulation of maternal immune response and suppression of inflammatory response, reduction of uterine contractility, improvement of utero-placental circulation and luteal phase support. Hence, the therapeutic application of progesterone during pregnancy is targeted to the prevention and treatment of threatened miscarriage, recurrent miscarriage and preterm birth.

For the maternal immune system, the fetus is recognized as a semi-allograft, so maternal immune response has a key role in pregnancy, particularly during implantation. Alteration of the complex local immune network in "feto-maternal interface" can result in failing of implantation. Progesterone participates in immunotolerance and it is interesting therapeutic agents for the modulation of the effects that pro-inflammatory and anti-inflammatory cytokines have on the fetus and placenta.

A defect of corpus luteum function is associated with implantation failure and miscarriage. Progesterone may promote the invasion of extra villous trophoblasts to the decidua by inhibiting apoptosis of extra villous trophoblasts.

Progesterone has been shown to exert a tocolytic effect on the myometrium. It has been demonstrated that adequate progesterone concentrations in myometrium are able to counteract prostaglandin and oxytocin stimulatory activity. Also, it is effective in the maintenance of uterine quiescence during some procedures (cervical cerclage or following abdominal surgery).

Preterm birth is the leading cause of perinatal mortality and morbidity. Its incidence has not declined (12% of all births) despite improvements in the perinatal care. Due to its long-term neuro-developmental sequelae, it is one of the major expenses on health and educational resources. The mechanism of parturition is the expression of anatomic, biochemical, physiologic and clinical events that occur in the mother and in the fetus. Preterm labour is the consequence of the pathologic activation of: myometrial contractility, cervical dilatation and rupture of the amniotic membranes. According to published guidelines progesterone administration has been demonstrated to be a safe and effective intervention in the reduction of the risk of recurrent preterm birth as well as primary prevention of preterm birth in women with asymptomatic cervical shortening in the midtrimester. Identification of patients and timely administration of progesterone will be expected to reduce the morbidity and mortality related to preterm birth.

Immunological processes at the maternal-embryonal interface of missed abortion and anembryonic pregnancy

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Blighted ovum (BO) and missed abortion (MA) represent the early pathological pregnancies with hindered development of embryoblast or with a dead embryo. The fertilisation products are lagging in the uterus for more than four weeks before the outward signs of pregnancy termination appeared. The possible immunological reason(s) of such events are currently uncompletely understood.

The aim: We investigated the expression of cytotoxic mediator granulysin (GNLY), the adapter Apoptotic protease activating factor 1 (Apaf-1) molecule, transcription factor NFkB at embryo-maternal interface and NKG2A receptor expression on NK cells in decidua basalis of early normal pregnancy (NP), BO and MA to gain a better insight into possible GNLY mediated apoptotic mechanism responsible for delayed termination of pregnancy.

Material and methods: We performed immunohistology (GNLY, Apaf-1, NFkB) and double immunofluorescence labeling of these markers with cytokeratin in paraffin embedded decidual tissue sections. The results were analysed by light or confocal mycroscopy and quantified using the Alphelys Spot Browser 2 integrated system. NKG2A expression was analized by flow cytometry in CD3-CD56+ labeled cells. RT-qPCR was performed to compare mRNA for GNLY in decidual mononuclear cells of NP and pathological pregnancies.

Results: mRNA for GNLY decreased in BO and increased in MA for four times. Nuclear patern of GNLY labeling was found in decidua of BA and MA when compared to cytoplasmic pattern of NP. Lower GNLY labeling intensity (H score) was found in the nucleai of trophoblast cells lining the glands and infiltrating decidua in BO and MA then in NP, while GNLY was similarly expressed in surrounding decidual tissue cells in all samples. The highest frequency of Apaf-1 was found in trophoblast cells of MA. The most abundant NFkB+ cells was found in decidua of BO, where NFkB was labeled in the cytoplasm and nuclei of cells. Decreased percentage of decidual NK cells was found in MA when compared to BO and NP. NK cells from MA express less NKG2A receptor then NP.

Conclusion: Less active GNLY mediated killing might be implicated in slower rejection of resiatant trophoblast cells in BO and MA. Decreased number of authentic decidual NK cells seems to determine low cytotoxicity against trophoblast cells in MA. In BO, trophoblast cells likely have higher survival potential due to increased NFkB expression in the vicinty of numerous NK cells.

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