Reproductive Immunology – an Update

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Summary

Pregnancy is one of the most challenging experiences for the immune system. It entails the confrontation and cooperation of maternal cells with allogeneic (sperm) and semi-allogeneic (fetal) cells and factors. On the one hand, it must actively acquire a specific tolerance towards the foreign cells and organism (the fetus) to avoid harming reactions, while recognizing the very same to support and control their development and growth. On the other hand, the immune system simultaneously may not reduce its capability to defend mother and fetus from microorganisms and pathogens. Almost all branches of the immune system are claimed to react and adapt in order to fulfill these complex duties. In this review, current knowledge concerning the most important cellular and soluble immunological components in the decidua is presented. Special regards are made to decidual NK, T and dendritic cells as well as to trophoblast cells, representing the fetal counterpart of most bilateral interactions. Furthermore, the role and functions of soluble factors, including HLA-G, PIBF, IDO and a variety of cytokines, are described.

Schlüsselwörter

Reproduktionsimmunologie · Deziduale NK-Zellen ·
Trophoblastzellen · HLA-G

Zusammenfassung

Die Schwangerschaft stellt eine der größten Herausforderungen für das Immunsystem dar. Sie beinhaltet die Konfrontation, aber auch die Kooperation von mütterlichen Zellen mit alloenen (Spermien) und semi-alloenen (fetalen) Zellen und Faktoren. Einerseits muss das Immunsystem aktiv eine Toleranz gegenüber den fremden Zellen und dem fremden Organismus ausbilden, während es gleichzeitig dieselben in ihrer Entwicklung und ihrem Wachstum unterstützen und kontrollieren muss. Andererseits darf das Immunsystem während der Schwangerschaft nicht seine Fähigkeit einbüßen, Mutter und Fetus gegen Mikroorganismen und Pathogenen zu verteidigen. Fast alle Bereiche des Immunsystems sind gefordert zu reagieren und sich anzupassen, um diese komplexen Aufgaben zu erfüllen. In diesem Übersichtsartikel wird das momentane Wissen über die wichtigsten zellulären und löslichen immunologischen Komponenten in der Dezidua präsentiert. Spezielle Schwerpunkte bilden dabei sowohl die dezidualen NK-, T- und dendritischen Zellen als auch die Trophoblastzellen, die den fetalen Partner der meisten bilateralen Interaktionen repräsentieren. Darüber hinaus werden die Rolle und Funktionen von löslichen Faktoren, einschließlich HLA-G, PIBF, IDO und einer Reihe von Zytokinen, beschrieben.

Introduction

Reproduction is a basic event of life and for this reason it is often taken for granted. In truth, reproduction overcomes common biological and physical rules known to establish life. The immunology of reproduction, and especially the tolerance of two genetically distinct organisms and their fruitful symbiosis is one of the most imposing of these paradoxes.

Beginning from sperm invasion and not ending with the delivery of the baby and its placenta, the maternal immune system is confronted with huge masses of paternally derived, and thus, foreign antigens. These foreign particles are usually interpreted as potential aggressors, but the maternal reaction is not the expected immediate and acute rejection, but instead an intimate cooperation with the invader. This includes mutual aid between the mucosal immune system of the female reproductive tract and foreign paternal antigen containing material as observable during sperm passage, conception as well as migration and implantation of the blastocyst. Thereafter, the major immunological proceedings take place in the developing placenta throughout the entire pregnancy. The placenta is the site with the most profound contact between fetal cells – mainly trophoblast cells – and maternal immune cells. Within the placenta, three main borders are distinguished:

- i) The chorionic villi are covered by syncytiotrophoblast, which are in continuous contact with maternal blood, including the complete spectrum of immune cells therein.
- ii) Cytotrophoblast cells invade the decidua, an organ saturated with maternal immune cells, implicating a long-lasting propinquity and exchange of signals.
- iii) Highly invasive cytotrophoblast cells overrun maternal decidual blood vessels, completely substitute the endothelium, and compel vasculogenesis. Similar to syncytiotrophoblast cells, they are in continuous contact with maternal blood.

Further contact of fetal antigens with the maternal immune system exist within the maternal body outside the placenta: Up to 3 g trophoblastic particles per day enter the maternal circulation and reach other organs. The major fraction is caught in the lung capillaries where particles are digested by macrophages. Other fetal cells and antigens – mainly from the blood – can also pass to small (as a common event) or large degrees (fetomaternal transfusion syndrome) into the maternal organism during pregnancy or delivery. Some of those cells survive there for several years and are suspected to be involved in the development of autoimmune diseases in later life. Finally, the developing fetal immune system is continuously exposed to maternal antigens, and maternal cells may enter the fetus and survive.

Reproductive immunology involves all facets of immunology during the unique circumstances surrounding pregnancy. Due to this enormous complexity, only selected aspects can be briefly explained in this review. We intend to focus on the most investigated and established aspects relevant to disor-

ders and those with potential for clinical diagnostics and therapies.

Cellular Aspects

The human decidua is composed of a unique pattern of cells: maternal decidual stroma cells, maternal immune cells, and fetal trophoblast cells. This constitution is not static – the course of pregnancy requires incessant adaptation since the placenta is one of the fastest growing tissues within the human body. During the first trimester of pregnancy, 70% of immunocompetent decidual cells are CD45⁺ leukocytes. The total percentage of CD45⁺ leukocytes on all endometrial cells is elevated compared to their proportion during the menstrual cycle (10–25% of all endometrial cells) [1–3]. The majority of these lymphocytes are localized in large lymphoid cell clusters, near endometrial glands, or as intraepithelial lymphocytes in glandular epithelium. Most of these cells are NK cells (46%), including CD56⁺ TCR- $\gamma\delta$ ⁺ cells, followed by macrophages (19%) and T cells (8%), which are mainly TCR- $\alpha\beta$ ⁺ CD4⁺ cells, and TCR- $\alpha\beta$ ⁺ CD8⁺ cells [4, 5]. The portion of B cells and mast cells is around 1% [6].

Most classes of immune cells are indispensable for successful pregnancy, as shown in various knockout animal models, but their function and mode of action is modified compared with leukocytes from peripheral blood, other tissues or inflammation areas.

NK Cells

Decidual NK cells are morphologically and functionally distinct from peripheral blood NK cells. Reasons include selected homing, but also the influence of the cellular and extracellular environment in the decidua [7]. Decidual NK cells do not form a single clearly definable population, but several different subsets the composition of which varies throughout pregnancy. The major decidual NK cell subset express largely CD56 (CD56^{bright}), and is CD16[–] [8] and CD160[–]. A minor decidual NK cell subpopulation is CD56^{dim} and CD160⁺ [9]. In contrast to CD56^{bright} peripheral blood NK cells which are small and agranular, most CD56^{bright} decidual NK cells are large granular lymphocytes [10]. Various receptors are expressed on decidual NK cells to interact with HLA-G (see below), such as Ig-like transcript 2 (ILT2) [11, 12], the killer cell Ig-like receptor KIR2DL4 [13, 14], and CD160 [15]. ILT2 and KIR2DL4 have been exclusively detected on decidual NK cells while CD160 is also on some T cells and endothelial cells [9, 16–18]. CD160 is expressed mainly on CD56^{dim} cells, the minor population of first trimester decidual NK cells [19, 20]. The effects of HLA-G binding to CD160 are not yet well analyzed, but HLA-C induces cytotoxicity via the CD160 receptor [9, 21]. It may be argued that HLA-G exerts a blocking func-

tion and competes with HLA-C. ILT2 is only expressed on 20–25% of decidual NK cells and exerts inhibitory functions [11, 12, 22]. However, in T cells ILT2 mediates both stimulating and blocking functions [23]. KIR2DL4 also has a dual role: in resting peripheral blood NK cells it has no cytotoxic potential, but in activated cells it can induce cytotoxicity [24]. Since it is a rare event to obtain physiological human placentae of mid-pregnancy and animal models are not completely comparable, the exact expression profiles of these mentioned receptors in decidual NK cells throughout the course of pregnancy and at term are not yet known.

In a recent study, we reported that soluble HLA-G (sHLA-G) reduces cytotoxicity of IL-2-stimulated decidual NK cells by reducing the expression of one of its most important intracellular signal mediators, the signal transducer and activator of transcription 3 (STAT3) [25].

T Cells

About 60% of the T cells in the early human decidua express TCR- $\gamma\delta$. They are CD4⁺ CD8[−], and about half of the TCR- $\gamma\delta$ ⁺ cells express the memory/activation marker CD45RO. At least 50% of the TCR- $\alpha\beta$ ⁺ cells are CD8⁺. The surface density of the TCR/CD3 complex is reduced in decidual T cells [3]. Decidual T cells do not respond to stimulation by alloantigens or mitogenic anti-CD3 monoclonal antibodies but respond to the same extent as peripheral blood lymphocytes to mitogenic lectins and PMA/ionomycin. Local selective down-regulation of surface expression of the TCR/CD3 complex and of activation involving this complex might be one of the mechanisms by which a maternal immunologic reaction against the semi-allogeneic fetus is prevented [4].

Human decidual lymphocytes from early, normal pregnancy express activation markers (CD45RO, Kp43, and/or HML-1) and MHC class II antigens (HLA-DR, HLA-DP, and/or HLA-DQ) [4]. In decidual CD3⁺ CD4⁺ CD8⁺ cells the level of CD45RO⁺ is higher and that of CD45RA⁺ lower as compared with peripheral blood lymphocytes. The relatively high percentage of intradecidual T cells expressing CD45RO suggest decidual accumulation of antigen-committed memory cells [26].

Decidual lymphocytes display cytoplasmic processes, microvilli and characteristic cytoplasmic granules, and have intimate contact with neighboring cells. Two main morphotypes of $\gamma\delta$ -T cells can be distinguished. One has single microvilli, membrane-bound granules, and nuclear inclusions. The other has many microvilli, nonmembrane-bound granules, and cytoplasmic multivesicular bodies. The activated cells may guard against infections and undue trophoblast invasion and/or be involved in modulating the local maternal immune system toward unresponsiveness against the semi-allogeneic fetus [4]. 14% of the decidual CD4⁺ T cells have the CD4⁺ CD25⁺ phenotype. The decidual CD4⁺ CD25⁺ T cells express high

frequencies of intracellular CTLA-4 (CTLA-4i). The majority of CD4⁺ CD25⁺ CTLA-4i⁺ cells are also positive for GITR and OX40, typical markers for human regulatory T (Treg) cells. Also the frequency of CD4⁺ CD25⁺ T cells in peripheral blood from pregnant women is increased during the first and second trimester of gestation when compared to nonpregnant controls [27].

The potentially harming cytotoxic CD8⁺ lymphocytes in the decidua seem to be efficiently controlled by HLA molecules. In activated CD8⁺ T lymphocytes, but also in CD8⁺ NK cells which lack the TCR, both classical and nonclassical sHLA class I molecules trigger apoptosis [28–30]. The binding of sHLA-A, -B, -C and -G1 molecules to CD8 leads to Fas ligand (FasL) upregulation, soluble FasL (sFasL) secretion and CD8⁺ cell apoptosis by Fas/sFasL interaction [29, 31, 32]. In a preliminary investigation, we tested the expression of CD8 on isolated CD56⁺ cells: 72.7% did not express CD8, 9.3% expressed CD8^{bright} at the same intensity as CD8⁺ T cells, and 18% expressed CD8^{dim}, with approximately 10% intensity of the CD8^{bright} population [25].

Dendritic Cells

In the endometrium of early pregnancy, an unusual accumulation of a multiplicity of immunocompetent cells can be detected. Among these are maternal antigen-presenting cells (APC) composed of CD14⁺ macrophages [33] and dendritic cells. The latter have the ability of both preventing immune responses as immature dendritic cells exhibiting the DC-SIGN (dendritic cell-specific ICAM grabbing nonintegrin) phenotype [34, 35] or acting as immunostimulatory cells after differentiation to mature CD83⁺ dendritic cells [36].

Several mechanisms have been proposed which drive decidual APC to tolerance-inducing cells.

One factor possibly initiating immunoinhibitory effects on dendritic cells is HLA-G. This unusual monomorphic MHC class I molecule is produced by invading fetal cytotrophoblast cells (see below) [reviewed in 37]. Recent studies revealed that HLA-G is able to modulate APC functions by inducing the production of immunosuppressive cytokines [37] and to suppress their T cell stimulatory capacity [38]. CD14⁺ monocytes and dendritic cells can directly be influenced by HLA-G via the inhibitory receptor ILT4 [39] which was detected on decidual monocytes by RT-PCR [40].

From the very start of pregnancy, a marked rise of the steroid hormone level is observed in peripheral maternal circulation [41]. Thereby, these hormones are able to affect APC in a different manner. Estrogen and human chorionic gonadotropin (β -HCG), highly elevated at different terms of pregnancy, are known to support the maturation process of dendritic cells by upregulating costimulatory molecules such as CD40, CD80 and CD86 [42, 43]. However, only estrogen altered the cytokine expression profile of dendritic cells by promoting the

production of tolerance-inducing cytokines such as IL-6 [44]. Analysis of the effects of progesterone, the predominant hormone produced by the corpus luteum and later by the placenta, revealed that progesterone modulates cytokine production of dendritic cells (significant upregulation of IL-10) towards tolerogenic cytokines [45].

In contrast glucocorticoids were found to act as immunosuppressive agent in various ways. They inhibit the maturation process of dendritic cells, support the production of IL-10, and decrease the ability of dendritic cells to stimulate T cell responses [46, 47].

Apart from these hormonal substances, there is evidence that trophoblast and decidual stromal cells as well as uterine NK cells produce various immunomodulatory cytokines such as IL-10 [48]. IL-10 is described to inhibit full maturation of dendritic cells and results in a dendritic cell phenotype that induces tolerance by silencing T cells [49]. In line with these findings, diminished IL-10 levels were observed to be associated with pathological pregnancies [50].

Finally, decidua provides a multitude of soluble and cellular factors, which ensure an adequate microenvironment for generation of tolerance-inducing dendritic cells which may be the key players for the acceptance of the fetal allograft by the maternal immune system.

Trophoblast Cells

During attachment to the uterine mucosa, the blastocyst is composed of a 5-cell inner cell mass and surrounded by a 53-cell trophoblast hull, the trophoctoderm, which is destined to differentiate into the placenta. Normally, epithelial cells do not allow adhesion of other cells to their apical surface, but the apical plasma membranes of trophoblast and uterine epithelium adhere to each other, making this a unique circumstance [51, 52]. The expression of adhesion molecules, such as integrins, selectins and the immunoglobulin superfamily, seems to be responsible for the acquisition of a receptive state. A cyclic adaptation of the endometrial integrin profile during the menstrual phase reflects its role for implantation [53]. After a very short period of syncytiotrophoblast invasiveness, the cytotrophoblast, which are considered trophoblast stem cells, replenish trophoblast of the invasive phenotype while the syncytiotrophoblast panel the chorionic villi and intervillous space and take over a more endocrinologic task [54]. Integrins of the decidual matrix around the trophoblast cells and their capacity to produce a variety of matrix metalloproteinases (MMP) determine the direction of invasion [55].

Trophoblast cells of the implanting blastocyst produce β -hCG which in turn converts the corpus luteum of the menstrual cycle to the progesterone-producing corpus luteum of pregnancy that promotes decidualization, protects gravidity as well as supports the development of the embryo. Progesterone is involved in immunosuppression at the fetomaternal interface

and thereby protects the fetus further from unwanted immunological responses.

Trophoblast cells display invasive characteristics comparable to malignancies with the difference that trophoblast invasion occurs in a completely physiological setting [56–59]. Disorders of trophoblast invasion can result in pregnancy-related pathologies, e.g. preeclampsia. Furthermore, trophoblast cells are able to mask or protect themselves from the maternal immune system and thus escape potentially detrimental immunological responses stemming from the mother. Cancerous tumor cells abuse and mimic these characteristics to the disadvantage of the affected ‘host.’ These two characteristics make trophoblast cells exceptional in comparison to other physiological cells in that they may pose as a unique model in investigating the process of invasion and its control.

Numerous studies indicate that the invasiveness of trophoblast cells (and tumor cells) is regulated on the intracellular level. A balance between invasion-promoting and -inhibiting intracellular factors is controlled partly by trophoblast cells themselves to regulate their invasive growth in space and time (usually limited to the first trimester and the decidua). This may be seen in the situation of trophoblast cells originating from extrauterine or xenogeneic gravidities since these cells are just as capable of invasion as the trophoblast of regular pregnancies [60, 61]. The intracellular balance is furthermore regulated by an extracellular network of cytokines, growth factors and cell surface receptors.

The cytokines are partly produced by trophoblast cells themselves or by other surrounding cells. They are able to control trophoblast behavior by either modulating proliferation and migration or inducing trophoblast cells to differentiate into an (non-)invasive phenotype. All of these cytokines use various intracellular signal-mediating pathways. Some of these mechanisms have been explored by several groups, but we are yet far from understanding the whole network. The signaling pathways of some cytokines, particularly in respect to possible crosstalk, are incompletely understood [62]. Especially, STAT3 and one of its major antagonists, suppressor of cytokine signaling 3 (SOCS3) seem to be main players of the mentioned intracellular signaling balance [63].

Soluble Factors

Soluble HLA Molecules

sHLA-G1 is one out of seven HLA-G isoforms which belong to the MHC-class Ib. The HLA-G and sHLA-G1 genomes are identical up to a stop codon in intron 4 [64, 65]. The different sequence in sHLA-G1 contains a 21 amino acid short tail at the $\alpha 3$ domain, which is unable to fix the protein within the cell membrane. The $\alpha 1$, $\alpha 2$ and $\alpha 3$ domains are similar to the HLA-G domains. Both proteins are associated with a $\beta 2$ microglobulin [66, 67].

sHLA-G1 was primarily found to be expressed by proliferating villous and extravillous cytotrophoblast cells and fetal endothelium. Subsequently, sHLA-G1 was detected in thymocytes where it supports T cell selection and viral defense [68]. sHLA-G1 was also found in malignancies which seem to mimic physiological processes of pregnancy: It is insinuated that during malignancy, inflammation, and allogeneic reactions reversal of methylation-mediated repression may directly induce HLA-G cell-surface expression, supporting the idea that HLA-G might be activated by such a mechanism [69]. Melanoma patients have elevated sHLA-G1 serum levels [70]. Clinical studies revealed significantly increased sHLA-G plasma levels in patients suffering from malignant melanoma, glioma, breast and ovarian cancer [71].

Trophoblast cells lack expression of classical HLA-A and -B alleles but express some HLA-C, and non-classical HLA-G, -E and -F. The lack of expression of classical HLA class I molecules partly prevents cytolytic activity of T cells toward trophoblast cells.[72] The HLA-G molecule modulates the effector function of maternal NK cells via interaction KIR2DL4, ILT2 and possibly CD160 [13, 14, 37, 73].

In the human placenta two sHLA-G isoforms have been detected: sHLA-G1 (also called G5) and sHLA-G2 (G6). Several subpopulations of trophoblast cells express sHLA-G1. sHLA-G2 has been exclusively found on extravillous cytotrophoblast cells, but mRNA encoding sHLA-G2 was detected also in placental villous cytotrophoblast cells [74]. The major isoform of sHLA-G circulating in maternal blood is sHLA-G2 [75]. Major functions of sHLA-G seem to be induction of apoptosis of activated CD8+ T cells, downregulation of proliferation of CD4+ T helper (Th) cells and reduction of cytotoxicity of NK cells [25, 28, 76–78].

The mode of interaction and precise functions of sHLA-G on NK cells, especially those from the human decidua, are still widely unclear.

The physiological role of sHLA-G seems to be the preparation of maternal NK cells for successful embryo implantation [79, 80], and defects in sHLA-G production may lead to clinical problems such as placental abruptions [81].

Progesterone-Induced Blocking Factor

In the presence of progesterone peripheral lymphocytes from healthy pregnant women produce a mediator protein named the progesterone-induced blocking factor (PIBF) [82]. PIBF has been shown to exert an immunomodulatory function both in vitro and in vivo [83]. In mice PIBF contributes to the maintenance of pregnancy. PIBF isolated from culture supernatants of progesterone-treated mouse pregnancy lymphocytes protects fetuses from resorption induced either by anti-progesterone or by high NK activity [84, 85]. On the other hand, treatment of pregnant mice with neutralizing antibodies against the murine PIBF causes resorption of mouse embryos [86].

The main mechanism of action of PIBF during pregnancy is the induction of Th2-dominant cytokine response [87]. The secreted PIBF facilitates the production of IL-3, IL-4 and IL-10, while it suppresses Th1 cytokines such as IL-12 and IFN- γ both in vitro and in vivo [87, 88]. Neutralization of PIBF by specific antibodies results in a shift towards Th1 in vivo, which is also a characteristic of failed pregnancies [88, 89]. The cytokine effects of PIBF are manifested via the Jak/STAT signal transduction pathway. Upon PIBF binding, the GPI-anchored PIBF receptor forms a heterodimer with the α -chain of the IL-4 receptor and induces STAT6 activation, at the same time inhibits the phosphorylation of STAT4. Silencing of STAT6 by siRNA reduces the cytokine effects [90].

The effect of PIBF on humoral immune responses includes the induction of asymmetric antibody production [91, 92]. This population of antibodies, owing to the presence of a mannose-rich oligosaccharide residue on one of the Fab arms of the molecule, does not precipitate; however, these antibodies might have a blocking effect. The percent of asymmetric IgG was significantly higher in supernatants of hybridoma cells cultured in the presence of PIBF than in those cultured in the absence of PIBF, and there was a positive relationship between asymmetric antibody content of pregnancy sera and PIBF expression on lymphocytes from the same women. Furthermore, blocking of progesterone receptors by RU 486 or neutralizing endogenous PIBF activity by specific anti-PIBF antibodies significantly reduced the production of asymmetric antibodies in pregnant mice [92].

The above-mentioned biological effects of PIBF suggest that it might contribute to the maintenance of a normal pregnancy. Therefore, alterations of PIBF concentrations in biological fluids might indicate the well-being of the fetus and the prognosis of pregnancy.

PIBF concentration measured by ELISA in urine samples from pregnant women reflects certain pathological events and is related to the outcome of pregnancy [93]. The concentration of PIBF correlates with the positive or negative outcome of pregnancy. Furthermore, premature pregnancy termination is predictable by lower than normal pregnancy PIBF values, suggesting that this method predicts problems of immunological origin and can contribute to the diagnosis of pre-term pregnancy termination due to immune pathology.

Indoleamine 2.3-Dioxygenase

Indoleamine 2.3-dioxygenase (IDO) is an enzyme which catalyzes the initial step of the oxidative metabolism of tryptophan in macrophages [94].

It has been shown that IFN- γ and further signals from activated T cells induce macrophage expression of IDO, thus inhibiting T cell proliferation and activation in vitro by rapid consumption of tryptophan [95]. When the CTLA-4 receptor on Treg cells binds to B7 (CD80/86) surface molecules, the IDO

activity of dendritic cells and monocytes is enhanced by the induction of IFN- γ production. During normal pregnancy, IDO expression is upregulated on both peripheral blood and decidual dendritic cells and on monocytes, whereas in spontaneous abortion both IDO expression on dendritic cells and monocytes after IFN- γ or CTLA-4 treatment are decreased [96]. Pregnant mice treated with the pharmacological IDO inhibitor 1-methyl-Trp display a significantly reduced number of allogeneic concepti. In contrast, the mean number of syngeneic concepti was not affected by the inhibitor [97]. In summary, IDO expression by human syncytiotrophoblast and dendritic cells may contribute significantly to the local immunotolerance at the fetomaternal interface by supporting T cell anergy.

Cytokines, Growth Factors

A variety of cytokines and growth factors influence invasive properties of extravillous trophoblast and surrounding cells. Among those, epidermal growth factor (EGF), insulin-like growth factor II (IGF-II), transforming growth factor β (TGF- β), granulocyte-macrophage colony-stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), hepatocyte growth factor (HGF) as well as IL-1, IL-6, IL-10, IL-11 and IL-15 have been intensively studied in human implantation. For this review we have selected four factors for a brief description. We recommend several reviews for more detailed information [for review see 159–163, 165]. Also leptin contributes to regulation of trophoblast functions (especially invasion). Leptin-deficient mice are infertile, pointing to paracrine mechanisms operating during implantation and placentation (leptin further reviewed in [98]).

One of the most important endometrial cytokines may be TGF- β . It interacts with macrophage-secreted IL-10 and PGE₂, which synergistically exert a strong immunosuppression in the endometrium [for review see 161]. In general, in the endometrium and deciduas, Th2 cytokines are predominant over Th1 cytokines [164]. Cytokines described in the endometrium so far are produced mostly by uterine NK cells and monocytes/macrophages [for review see 160], but also by T cells and trophoblast cells themselves.

Hepatocyte Growth Factor

First of all, the morphogenic HGF has been described in mediating motility in tumors, making this growth factor a potential candidate in the regulation of trophoblast invasion. Indeed, HGF dose-dependently increases the invasive potential of trophoblast cells in vitro [99]. HGF is mainly expressed by placental stromal cells while the receptor is located on the trophoblast [100] and is of fundamental importance during the development of the placenta for the mesenchymal induction of trophoblast growth and differentiation [101].

In the mouse model, HGF deficiency leads to lethal placental failure due to a complete lack in the development of

labyrinthine trophoblast [102, 103]. In humans, reduced levels of HGF production was found in the placentae of preeclamptic women thus denoting HGF as an important player in the pathomechanism of this disease [99]. Finally, in placental tissues of malformed fetuses an altered expression of HGF has been observed [104].

IL-6 and IL-11

As IL-6 is positively correlated with increasing metastatic potential of some cancer cells [105, 106], IL-6 is an attractive cytokine to investigate in terms of invasive growth in reproduction.

IL-6 mRNA and protein is maximally expressed at the time of implantation [107, 108]. Simultaneously, its receptor is present on endometrial epithelial cells and fetal tissues (trophoblast and blastocyst), as well as during placentation [107, 109, 110]. Trophoblast cells are presumed to be the major source of IL-6 found in the amniotic fluids of early pregnancy. Here, it is positively correlated to gestational age [111]. Invasive cytotrophoblast cells express high levels of IL-6 [112], correlating with high activity of MMP-2 and MMP-9, both important proteins in invasion and implantation [113]. Furthermore, IL-6 increases expression of integrins associated with embryo attachment [112].

Supplementation of IL-6 to human choriocarcinoma cultures failed to stimulate cell growth, but knocking out of IL-6 mRNA resulted in inhibiting cell growth [114]. This observation may be due to autocrine mechanisms.

IL-11 is another cytokine of the 'IL-6 cytokine family'. Female mice with a null mutation of the IL-11 receptor α -chain are infertile because of defective decidualization [115]. The same receptor subunit has been detected on developing decidual cells. IL-11 expression is at a maximum at the time of decidualization in the human gravid uterus.

Leukemia Inhibitory Factor

Several interesting observations indicate that LIF is essential to pregnancy in general and implantation in particular [116, 117]. First of all, both human placental and endometrial tissues generate LIF to such a degree that considerable concentrations may be detected at the fetomaternal interface, with highest concentrations measured during the implantation window [118]. Furthermore, trophoblast cells are able to respond to LIF as they express the LIF receptor [110]. LIF increases HLA-G expression on human choriocarcinoma cells [119] and enhances the proliferation and invasion of these cells as well as in trophoblast [63]. Indeed, knocking down STAT3, the main intracellular transducer of the LIF signal, resulted in loss of LIF-mediated invasion in both cell types [120]. All of this points to the fact that LIF is essential to invasion, and thus, implantation. In fact, knockout experiments with pregnant mice uncovered that LIF-deficient mice are infertile, though not sterile, as fertility could be restored through intrauterine LIF infusion [121]. The blastocysts of LIF receptor knockout

mice implant, but die within 24 h of birth due to impaired placenta function [122].

On the other side of the spectrum, NK cells are a major source of decidual LIF and are said to be thus involved in negatively regulating trophoblast invasion [123]. Perhaps for this reason, it is not surprising that LIF is involved in a negative feedback mechanism by suppressing its intracellular signaling pathway (see below) [124].

In clinical terms, both too low and too high levels of LIF in uterine flushings have been suggested to have negative predictive value in implantation success [125, 126]. Taking these observations as a whole, it may be said that LIF facilitates implantation and regulates crucial trophoblast functions [127].

Granulocyte-Macrophage Colony-Stimulating Factor

Much evidence backs up the idea that GM-CSF at least functionally supports the development of the placenta. This is probably accomplished through the induction of the differentiation and secretory activity of human and mouse cytotrophoblast cells as well as through DNA proliferation, in vitro [128, 129]. GM-CSF is produced in dependence of estrogen by uterine epithelial cells in mice, sheep and humans [130–132]. Due to an impedance in placental function, fetal growth and viability are seriously endangered in mice whose mothers are deficient in GM-CSF. These effects are augmented when the conceptus is deficient of the cytokine as well, suggesting that GM-CSF, regardless of origin, is required for optimal placental function [133]. As a matter of fact, the administration of miniscule amounts of exogenous GM-CSF dramatically influences the outcome of murine pregnancies [134, 135].

Although their functions are not identical, but synergistic, the here described factors share one important characteristic: They use the IL-6 receptor type followed by intracellular signaling via the janus kinase STAT cytokine signal-transducing pathway. Moreover, they all mainly use the same factor, STAT3, which promotes migration, proliferation and invasion of extravillous trophoblast cells. STAT3, on the other hand, is controlled by its major antagonist SOCS3, which can be induced by STAT3 activation itself as well as by several parallel signaling pathways.

The balance of STAT3 and SOCS3 may be one of the most important regulators in the invasiveness of trophoblast cells.

Asymmetric Antibodies

During gestation, a temporary maternal tolerance against paternal antigens is induced by different mechanisms. However, mothers produce antibodies directed against fetoplacental antigens of paternal origin. Different authors have published the existence of blocking antibodies which are supposed to block paternal fetoplacental antigens. Between them, asymmetric antibodies represent 10–15% of the total IgG population in normal sera, but this proportion increases up to 50%

during pregnancy and decreases after delivery within 20–30 days [136]. This type of antibodies has been reported in several mammalian species [137–140].

Asymmetric IgG paratopes have dissimilar affinity, one of them is a high-affinity combination site, and the other has 100 times lower affinity to the antigen [141]. As a consequence, since an adequate antibody-antigen complex is not formed, they fail to initiate the host biological mechanism that leads to the destruction of the aggressive agent. This univalent function is due to steric hindrance present in one paratope by a high-mannose carbohydrate moiety attached to the Fd fragment of the Fab region [142]. Considering this, it is possible to isolate asymmetric IgG molecules from the total IgG present in normal or immune sera using concanavalin A / sepharose B chromatography [143]. Normal bivalent antibodies and asymmetric antibodies are synthesized by the same cellular clone, so the biological activity of a serum sample would depend on the relationship between symmetric and asymmetric IgG molecules. In term placenta, asymmetric antibodies represent 70% of total IgG fixed to paternal antigens [136].

Taking into account that during gestation there is a delicate balance between inflammatory and anti-inflammatory cytokines, and that an exacerbated Th1 response could lead to fetal losses and considering that Th2 cytokines leads to an increase in antibody production, an anti-inflammatory cytokine regulation of the asymmetrical antibody synthesis was proposed. Among Th2 molecules, IL-6 is recently implicated in the regulation of the transition from Th1 to Th2 prevalence and from innate to acquired immunological response. Since we demonstrated that placental IL-6 increases asymmetric antibody ratio in vitro [144] and that inoculation of this cytokine in high abortion rate females during pregnancy corrected IL-6 deficiency, resulting in increased asymmetric antibody proportion and preventing abortion [145], we postulate that this cytokine could be involved in the modulation of humoral response during pregnancy.

Psychoneuroimmunology and Reproduction

Psychosocial stress has long been suspected as a possible cause of infertility, spontaneous abortion, late pregnancy complications, and impaired fetal development, as indicated by a wealth of epidemiological studies [146, 147]. Over the past decade, such insights have been advanced by endeavors in basic research aiming to identify pathways of stress-triggered pregnancy complications by employing a mouse model [148–150].

To date, it is widely accepted that psychological stress causes an activation of the hypothalamus-pituitary-adrenal (HPA) axis. This results in the upregulation of prototypic stress hormones such as corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) or prolactin (PRL) [151, 152]. Via these stress-related hormones, supplementary

mediators of stress response implementation such as glucocorticoids are activated [153]. Such a stress-triggered endocrine skew greatly modifies immune responses towards the selective suppression of the Th1-mediated cellular immunity [152]. Since this is the prerequisite to ensure fetal tolerance, should stress perception not be auspicious to sustain pregnancy maintenance? As Albert Einstein pointed out 'everything should be made as simple as possible, but not simpler', there are clearly more aspects to take into consideration to explain the effect of stress on adverse pregnancy outcome. For example, besides the suppression of the Th1 response, CRH also exerts potent pro-inflammatory actions [154]. Furthermore, stress activates the sympathetic nervous system and results in increased secretion of catecholamines [153]. Interestingly, pioneering research in immunology revealed that the immune system is regulated via the sympathetic nervous system/catecholamines locally and systemically [155]. Lymphocytes which express adrenergic receptors may be stimulated by catecholamines [151]. Subsequently, a stress-induced lymphocytosis develops, accompanied by increased lymphocyte trafficking and secretion of Th1 cytokines [153, 156].

Moreover, the neurotrophin nerve growth factor (NGF) is widely acknowledged as an essential mediator of stress response pathways [157] and acts as a potent immunomodulator, facilitating leukocyte migration through vascular endothelium.

Recently published data revealed that stress-triggered upregulation of the frequency of abortion is accompanied by increased expression of uterine NGF in a murine model and may be abrogated by NGF neutralization [158].

Besides neurohormones and neurotrophins, progesterone – the hormone of pregnancy – is involved in the complex interdependency of stress response execution. Here, stress has been reported to cause a decrease of progesterone in mice, subsequently challenging mechanisms of fetal tolerance such as decreased levels of PIBF and Th2 cytokines [149]. Strikingly, stress-triggered fetal rejections may be therapeutically approached by application of a progesterone derivative which restores levels of PIBF and Th2 cytokines [150].

Taking the immense intricacy of nervous, endocrine and immune crosstalk into consideration, it is evident that neither the successful reproductive outcome nor pregnancy failure is due to a single entity but the consequence of a multifaceted imbalance which may be triggered by stress perception.

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