

Introduction

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The conditions that permit the human fetus to evade rejection by its mother's immune system are still not fully elucidated. Half of fetally derived antigens are of paternal origin; therefore, if classical rules of transplantation immunity applied to pregnancy, the fetus ought to be rejected. Anti-fetal, anti-placental and anti-paternal antibodies are detectable in sera of women with successful pregnancies [1], clearly showing that though maternal recognition of fetal antigens takes place, it does not compromise pregnancy.

Lymphocytes incubated with placentas from normal pregnancies release Th2 cytokines, whereas cells from aborted placentas induce production of Th1 cytokines [2]. Thus, the maternal immune system might not only recognize pregnancy, but react in a differential way resulting in either success or failure. Activation of the immune system seems to be necessary for a normal pregnancy outcome. Non-specific immune-stimulation of the pregnant females reduces the originally high resorption rates in an abortion prone murine strain combination [3] and the same effect is achieved by immunization of the mothers with paternal strain type spleen cells [4]. Taken together these data suggest that immunological recognition of pregnancy is needed for the success of gestation.

The maternal immune system is exposed to paternal alloantigens throughout gestation. An inadequate response to these antigens might result in infertility, or pregnancy failure. Successful gestation implies a complex interaction between the mother and the fetus.

The sperm already represents an antigenic challenge for the female immune system. Anti-sperm

antibodies are present in more than 10% of women with unexplained infertility, [5–7], and these antibodies have been shown to inhibit *in vitro* fertilization of viable human oocytes by human spermatozoa [8–12].

Seminal plasma contains paternal type I and type II MHC antigens and high concentrations of transforming growth factor- β (TGF β). The latter induces regulatory T cells or Th17 cells [13]. In mice, exposure to seminal fluid at mating induces tolerance to paternal alloantigens and an accumulation of Treg cells in the uterine draining lymph nodes, which may facilitate implantation [14].

IMPLANTATION

Implantation occurs between 6 to 12 days after ovulation. Several conditions need to be satisfied for a successful implantation. The "implantation window" is a well defined period, when the conditions are optimal and the endometrium is ready to receive the embryo. Several factors including hormones, growth factors, cytokines, chemokines, adhesion molecules, extracellular matrix components, and matrix-degrading enzymes are involved in creating the appropriate environment for implantation.

Progesterone regulated genes are crucial for the establishment and maintenance of pregnancy. A member of homeobox gene family; Hox-A10 plays a role in formation of the receptive endometrium, among others- by mediating progesterone-stimulated proliferation of uterine stromal cells [15]. In the peri-implantation endometrium of Hox-A10 deficient mice Natural Killer (NK) cell differentiation is inhibited [16] and polyclonal T cell proliferation can be observed [15].

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To be able to implant, the blastocyst first sheds the zona pellucida. This is followed by apposition, adhesion and implantation of the blastocyst. From this point on, embryo derived signals also contribute the proliferation of the stroma. The initial implantation steps are characterised by the rather extensive use of inflammatory molecules. Complement related pathways are involved in the regulation of angiogenesis as well as in expression of adhesion molecules, matrix metalloproteinases (MMPs) and serine proteases. The invading trophoblast anchors the embryo and also establishes an immunological interface between the mother and the fetus. The placenta formed from the decidua and outer layers of the embryo, connects the developing fetus to the uterine wall to allow nutrient uptake, waste elimination, and gas exchange via the mothers' blood supply.

UTERINE NK CELLS

In the peri-implantation period there is extensive infiltration of cells of uterine Natural Killer (uNK) lineage which will ultimately represent up to 50–70% of the cellular content of the basal decidual stroma. Decidual NK cells represent a phenotypically and functionally distinct cell population. While approximately 90% of human peripheral NK cells express a low density of CD56 (CD56^{dim}) and high levels of the FCγRIII (CD16), the majority of decidual NK cells express a high density of CD56 (CD56^{bright}) and no CD16 [17]. The temporal and spatial distribution of these cells suggests that one of their functions might be the control of placentation. By producing the chemokines CXCL8 and CXCL10, decidual NK cells regulate trophoblast invasion [18].

The genes selectively over expressed in decidual NK cells suggest that these cells might contribute to the generation of an immunosuppressive environment at the maternal fetal interface [19]. Via their intensive cytokine production, these cells play a crucial role in local angiogenesis, particularly, by controlling the development of a special functional arterial system, the spiral arterioles. Decidual NK cells secrete angiogenic factors [19] and induce vascular growth in the decidua [20]. NK cell deficient mice [21] have an abnormally small placenta and low birth weight fetuses [22].

The rapid increase of NK cell counts in the early decidua may be caused by proliferation of the resident population, recruitment of CD56^{bright} lymphocytes from the circulation, or both. Progesterone plays a role in uterine homing of NK cells by promoting NK

cell interactions with the endothelium [23], and by progesterone-induced specific endometrial production of chemokines [24, 25]. Uterine NK cells increase in number during estrus and proestrus in rodents and during the luteal phase in women and are enriched at sites where fetal trophoblast infiltrates the deciduas in early pregnancy [23].

Decidual NK cells fulfil dual tasks. During normal conditions they contribute to creating a favourable environment for placentation, but at the same time they are equipped with cytotoxic potential to fight intrauterine infections. High levels of perforin and granzymes A and B are expressed by decidual NK cells [19, 26], suggesting that decidual NK may have cytotoxic potential.

NK cells express activating and inhibitory receptors. NK cytotoxic activity results from the balance of inhibitory and activating signals, following the interaction of NK cell activating and inhibitory receptors with ligands expressed on the surface of the target cell [27–29]. Increased expression of HLA-G, the ligand for NK inhibitory and activating receptors, controls NK activity in the presence of progesterone.

It will be shown in this issue that uNK cells of women predisposed to miscarriage may have a higher activating potential and that both inhibitory and activating killer immunoglobulin like receptors (KIRs) on uNK cells are important for the maintenance of pregnancy.

TROPHOBLAST INVASION

Trophoblast invasion is a prerequisite of successful pregnancy. Invasive trophoblast anchors the placenta to the uterus, modulates implantation as well as secretion of hormones and cytokines and most importantly plays a crucial role in remodelling of maternal spiral arteries. During early human pregnancy extravillous cytotrophoblasts invade these vessels and replace the endothelial cells. This renders the spiral arteries expandable and of low resistance, which is required to increase blood flow to the placenta, in order to provide sufficient oxygen and nutrient supply for the developing fetus.

Failure of the latter process can result in pre-eclampsia [30], fetal growth restriction, and miscarriage. The invading trophoblast also gets in contact with the decidual lymphocyte populations, thereby establishing an immunological interface between the fetal and maternal compartments. Trophoblast invasion is transient, with spatial and temporal control

[31]. It involves attachment of the trophoblast cells to the extracellular matrix (ECM) components, degradation of the ECM and migration through the eroded connective tissue.

Several enzymes, hormones, cytokines, growth factors and extracellular matrix glycoproteins have been reported to play a role in both trophoblast and tumor invasion. Progesterone is one of the factors to control trophoblast invasiveness either directly [32] or via progesterone-induced blocking factor (PIBF) by reducing the secretion of MMP9 from trophoblasts.

Extracellular stimuli activate different signalling pathways, e.g. the MAPK-, focal adhesion kinase (FAK), the phosphoinositide 3-kinase (PI3K) – Akt, STAT or the Wnt pathway that control trophoblast invasion proliferation, differentiation, migration and apoptosis.

CYTOKINES

In the post implantation period, the invading trophoblast starts to express Class I MHC antigens. Class I antigens, render the fetus allogeneic, and are presented to specific maternal CD4+ T helper cells, which release various cytokines in response to these alloantigens. Th1, Th2 and Th17 cells are involved either in allograft rejection (Th1 and Th17 cells) or in allograft tolerance (Th2 cells). In the peri-implantation period both Th1 and Th2 type cytokines are expressed locally in a stage-specific manner [33] and certain Th1 cytokines (e.g. LIF, IL-11 and IFN- γ) seem to be crucial for implantation of the mouse blastocyst [34–37]. A mild local inflammatory reaction is required for loosening the endometrial tissue thus promoting implantation, however, in later stages of pregnancy the dominance of Th1 cytokines seems to be detrimental. It has been suggested that significantly increased Th1 cytokine expression might represent the underlying phenomenon leading to reproductive failure [38, 39].

NEUROENDOCRINE-IMMUNE INTERACTIONS

The successful establishment and maintenance of pregnancy is influenced by environmental factors. Epidemiological studies suggest the role of stress in miscarriage and pre-term delivery.

Progesterone is critical for the establishment and the maintenance of pregnancy, both by its endocrine- and immunological effects. The genomic actions of proges-

terone are mediated by the intracellular progesterone receptors; A and B [40, 41]. A protein called PIBF, by inducing a Th2 dominant cytokine production mediates the immunological effects of progesterone [42, 43]. Progesterone plays a role in uterine homing of NK cells [23] and up-regulates HLA-G gene expression [44], the ligand for various NK receptors. At high concentrations progesterone is a potent inducer of Th2-type cytokines as well as of LIF and M-CSF production by T cells.

High levels of glucocorticoids inhibit pituitary luteinizing hormone and ovarian estrogen and progesterone secretion. Exposure to stress induces abortion in mice via a significant reduction in progesterone levels, accompanied by reduced serum levels of PIBF and diminished expression of progesterone receptors at the feto-maternal interface. These effects are corrected by progesterone supplementation.

IN SUMMARY

There is a complex network of interacting molecules and systems that contribute to successful implantation and gestation. To provide a suitable microenvironment for the implanting embryo and the developing fetus, a coordinate action of the precisely tuned cytokine balance, appropriate function of decidual NK cells, controlled trophoblast invasion and an appropriate coordination of the neuroendocrine and immune systems are needed. However, there are still ample open questions, e.g., which are the signals that allow certain embryos, but not others- to implant in an otherwise receptive endometrium? What is the origin of decidual NK cells? How do embryo-derive signals affect their function?

Recently a European network of laboratories – named EMBIC – sponsored by the European Union, undertook to investigate these problems. Some aspects will be presented in this issue.

REFERENCES

- [1] Billington WD. Transfer of antigens and antibodies between mother and fetus. In: Immunological Obstetrics (C.B. Coulam, W.P. Faulk and J.McIntyre eds.) W.W. Norton & Co. New York, 1992; pp. 290-304.
- [2] Tangri S, Raghupathy R. Expression of cytokines in placentas of mice undergoing immunologically mediated spontaneous fetal resorptions. *Biol Reprod.* 1993; 49(4): 850.
- [3] Toder V, Strassburger D, Irlin I, Carp H, Pecht M, Trainin N. Nonspecific immunopotentiators and pregnancy loss: Complete Freund Adjuvant reverses high fetal resorption rate in CBA/J \times DBA/2 mouse combination. *Am J Reprod Immunol.* 1990; 24(3): 63.

- [4] Chaouat G, Kolb JP, Kiger N, Stanislawsky M, Wegmann TG. Immunological concomitants of vaccination against abortion in mice. *J Immunol.* 1985; 134(3): 1594.
- [5] Menge AC, Medley NE, Mangione CM, Dietrich WJ. The incidence and influence of antisperm antibodies in infertile human couples on sperm-cervical mucus interactions and subsequent fertility. *Fertil Steril.* 1982; 38(4): 439.
- [6] Cantuaria AA. Sperm immobilizing antibodies in the serum and cervicovaginal secretions of infertile and normal women. *Br J Obstet Gynaecol.* 1977; 84(11): 865.
- [7] Clarke GN, Stojanoff A, Cauchi MN, McBain JC, Speirs AL, Johnston WI. Detection of antispermatozoal antibodies of IgA class in cervical mucus. *Am J Reprod Immunol.* 1984; 5(2): 61.
- [8] Clarke GN, Lopata A, Johnston WI. Effect of sperm antibodies in females on human *in vitro* fertilization. *Fertil Steril.* 1986; 46(3): 435.
- [9] Clarke GN, Hyne RV, du Plessis Y, Johnston WI. Sperm antibodies and human *in vitro* fertilization. *Fertil Steril.* 1988; 49(6): 1018.
- [10] Yeh WR, Acosta AA, Seltman HJ, Doncel G. Impact of immunoglobulin isotype and sperm surface location of anti-sperm antibodies on fertilization *in vitro* in the human. *Fertil Steril.* 1995; 63(6): 1287.
- [11] Ford WC, Williams KM, McLaughlin EA, Harrison S, Ray B, Hull MG. The indirect immunobead test for seminal antisperm antibodies and fertilization rates at *in-vitro* fertilization. *Hum Reprod.* 1996; 11(7): 1418.
- [12] de Almeida M, Gazagne I, Jeulin C, Herry M, Belaisch-Allart J, Frydman R, et al. *In-vitro* processing of sperm with autoantibodies and *in-vitro* fertilization results. *Hum Reprod.* 1989; 4(1): 49.
- [13] Maitra U, Davis S, Reilly CM, Li L. Differential regulation of Foxp3 and IL-17 expression in CD4 T helper cells by IRAK-1. *J Immunol.* 2009; 182(9): 5763.
- [14] Robertson SA, Guerin LR, Bromfield JJ, Branson KM, Ahlström AC, Care AS. Seminal fluid drives expansion of the CD4+ CD25+ T regulatory cell pool and induces tolerance to paternal alloantigens in mice. *Biol Reprod.* 2009; 80(5): 1036.
- [15] Yao MW, Lim H, Schust DJ, Choe SE, Farago A, Ding Y, et al. Gene expression profiling reveals progesterone-mediated cell cycle and immunoregulatory roles of Hoxa-10 in the preimplantation uterus. *Mol Endocrinol.* 2003; 17(4): 610.
- [16] Rahman MA, Li M, Li P, Wang H, Dey SK, Da SK. Hoxa-10 deficiency alters region-specific gene expression and perturbs differentiation of natural killer cells during decidualization. *Dev Biol.* 2006; 290(1): 105.
- [17] Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends in Immunology.* 2001; 22(11): 633.
- [18] Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Nathanson-Yaron S, et al. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med.* 2006; 12(9): 1065.
- [19] Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *Journal of Experimental Medicine.* 2003; 198(8): 1201.
- [20] Quenby S, Nik H, Innes B, Lash G, Turner M, Drury J, Bulmer J. Uterine natural killer cells and angiogenesis in recurrent reproductive failure. *Hum Reprod.* 2009; 24(1): 45.
- [21] Wang B, Biron C, She J, Higgins K, Sunshine MJ, Lacy E, Lonberg N, Terhorst C. A block in both early T lymphocyte and natural killer cell development in transgenic mice with high-copy numbers of the human CD3 ϵ gene. *Proc Natl Acad Sci USA.* 1994; 91(20): 9402.
- [22] Guimond M-J, Wang B, Croy BA. Immune competence involving the natural killer cell lineage promotes placental growth. *Placenta.* 1999; 20(5-6): 441.
- [23] Van den Heuvel MJ, Chantakru S, Xumei X, Evans EE, Tekpetey F, Mote PA, et al. Trafficking of circulating pro-NK cells to the decidualizing uterus: Regulatory mechanisms in the mouse and human. *Immunol Invest.* 2005; 34(3): 273.
- [24] Sentman CL, Meadows SK, Wira CR, Eriksson M. Recruitment of uterine NK cells: Induction of CXC Chemokine ligands 10 and 11 in human endometrium by estradiol and progesterone. *J Immunol.* 2004; 173(11): 6760.
- [25] Carlino C, Stabile H, Morrone S, Bulla R, Soriani A, Agostinis C, et al. Recruitment of circulating NK cells through decidual tissues: A possible mechanism controlling NK cell accumulation in the uterus during early pregnancy. *Blood.* 2008; 111(6): 3108.
- [26] Bogovic T, Crncic T, Laskarin G, Juretic K, Strbo N, Dupor J, et al. Perforin and Fas/FasL cytolytic pathways at the maternal-fetal interface. *American Journal of Reproductive Immunology.* 2005; 54(5): 241-248.
- [27] Ljunggren HG, Karre K. On search of the 'missing self': MHC molecules and NK cell recognition. *Immunology Today.* 1990; 11(7): 237-244.
- [28] Long EO. Regulation of immune responses through inhibitory receptors. *Annual Reviews of Immunology.* 1999; 17: 875-904.
- [29] Moretta L, Biassoni R, Bottino C, et al. Human NK-cell receptors. *Immunology Today.* 2000; 21(9): 420-422.
- [30] Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *J Clin Invest.* 1997; 99(9): 2152-2164.
- [31] Bischof P, Meisser A, Campana A. Paracrine and autocrine regulators of trophoblast invasion – a review *Placenta.* 2000; 21 (Suppl_A): S55-S60.
- [32] Spencer TE, Burghardt RC, Johnson GA, Bazer FW. Conceptus signals for establishment and maintenance of pregnancy. *Anim Reprod Sci.* 2004; 83: 537-550.
- [33] Vince GS, Johnson PM. Is there a Th2 bias in human pregnancy? *J Reprod Immunol.* 1996; 32(2): 101-104.
- [34] Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F, et al. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature.* 1992; 359(6390): 76-79.
- [35] Bilinski P, Roopenian D, Gossler A. Maternal IL-11/Ralpha function is required for normal decidua and fetoplacental development in mice. *Genes Dev.* 1998; 12(14): 2234-2243.
- [36] Ashkar AA, Di Santo JP, Croy BA. Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J Exp Med.* 2000; 192(2): 259-270.
- [37] Piccinni M-P, Scaletti C, Vultaggio A, Maggi E, Romagnani S. Defective production of LIF, M-CSF and Th2-type cytokines by T cells at fetomaternal interface is associated with pregnancy loss. *J Reprod Immunol.* 2001; 52(1-2): 35-43.
- [38] Raghupathy R. Th-1 type immunity is incompatible with successful pregnancy. *Immunology Today.* 1997; 18(10): 478-482.
- [39] Ng SC, Gilman-Sachs A, Thaker P, Beaman KD, Beer AE, Kwak-Kim J. Expression of intracellular Th1 and Th2

- cytokines in women with recurrent spontaneous abortion: Implantation failures after IVF/ET or normal pregnancy. *Am J Reprod Immunol.* 2002; 48(2): 77-86.
- [40] Li X, Lonard DM, O'Malley BW. A contemporary understanding of progesterone receptor function. *Mech Ageing Dev.* 2004; 125(10-11): 669-678.
- [41] Mulac-Jericevic B, Conneely OM. Reproductive tissue selective actions of progesterone receptors. *Reproduction.* 2004; 128(2): 139-146.
- [42] Polgar B, Kispal Gy, Lachmann M, Paar C, Nagy E, Csere P, et al. Molecular cloning and immunological characterization of a novel cDNA coding for PIBF. *J Immunol.* 2003; 171(11): 5956-5963.
- [43] Szekeres-Bartho J, Wegmann, TG. A progesterone-dependent immuno-modulatory protein alters the Th1/Th2 balance. *J Reprod Immunol.* 1996; 31(1-2): 81-95.
- [44] Yie SM, Xiao R, Librach CL. Progesterone regulates HLA-G gene expression through a novel progesterone response element. *Human Reproduction.* 2006; 21(10): 2538-2544.
- [45] Piccinni M-P, Beloni L, Livi C, Maggi E, Scarselli GF, Romagnani S. Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nat Med.* 1998; 4(9): 1020.
- [46] Piccinni M-P, Scaletti C, Vultaggio A, Maggi E, Romagnani S: Defective production of LIF, M-CSF and Th2-type cytokines by T cells at fetomaternal interface is associated with pregnancy loss. *J Reprod Immunol.* 2001; 52(1-2): 35.