Immunobiology of Reproduction: Role of Uniquely Abundant NK Cells in the Placenta

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Introduction

It was recognized first by Peter Medawar[1] that the human fetus is a natural allograft which is exceptionally successful, surviving much longer than any other allograft would in an immunologically competent recipient. Very soon it was recognized also that what is transplanted is not the fetus itself, but the trophoblast cells which are a real semiallogenic transplant. These strategically positioned cells are in direct and intimate contact with the maternal decidua. Trophoblastic cells regulate expression of MHC genes and their products in a unique fashion which is different with those in other tissues and may be responsible for special immunogenic properties of the placenta.

During the menstrual cycle and throughout the pregnancy, human endometrium is abundantly endowed with bone-marrow derived cells (leukocyte populations) including macrophages, T-cells and large granular lymphocytes.[2] Macrophages are present in both non-pregnant endometrium and basal decidua and their number does not change significantly during pregnancy.[2] Decidual macrophages are endowed with a potential for many immunological functions (antigen presentation, cytokine production, phagocytosis of tissue debris, etc.) but many of their in situ functions still are not clear.[3]

T-lymphocytes are scattered throughout human decidua, representing approximately 10 to 15% of decidual lymphocytes, and have an inverted CD4:CD8 ratio compared with peripheral blood T-cells.[2,4] Decreased expression of TCR/CD3 complex in the first trimester decidual T-cells, compared with peripheral blood T-cells has been described.[5-7]

Decidual large Granulated Lymphocytes

Large granulated lymphocytes are the predominant population of endometrial lymphocytes (eGL) at the end of the menstrual cycle during the late secretory phase and also in the first trimester of pregnancy (decidual large granulated lymphocytes [dLGL]).[8-10] dLGL are similar to granulated metrial gland (GMG) cells in the metrial gland present in uteri of pregnant rodents.[11] Decidual large granulated lymphocytes strongly express CD56 (CD56bright) which leads to the suggestion that these cells belong to the NK cell line, and resemble peripheral blood NK cells. However, detailed examinations clearly showed that dLGL are morphologically, phenotypically, and functionally distinct from peripheral blood NK cells.[12-15] Decidual LGL do not express CD3 although the ζ and ε chain of CD3 molecular complex could be found in cytoplasm of 90% of $CD3^-$ decidual NK clones.[16] The phenotype of dLGL matches that of a small subset of peripheral blood NK cells which are $CD56^+$ and $CD16^-$,[17] but they are agranular and have lower expression of CD56 marker.[13,14,17] $CD56^{bright}$ decidual lymphocytes are $CD3^-CD4^-$, and 20% of them are $CD8^+$. The most striking difference between peripheral blood and decidual $CD56^+$ lymphocytes is almost complete absence of CD16 molecule on decidual lymphocytes. In first trimester decidua, only 2% of $CD56^+$ cells have classical NK phenotype ($CD3^-CD56^+CD16^+$). Accordingly, 98% of decidual lymphocytes belong to the nonclassical NK cell subpopulation ($CD56^+CD16^-$). The prevalent phenotype of dLGL is $CD3^-CD2^+CD4^-CD8^-CD16^-CD56^{bright}$.[18] In non-pregnant endometrium, $CD56^+$ cells increase in their number from the proliferative to midsecretory phase of the menstrual cycle.[8] We have found $CD56^+$ cells in midsecretory endometrium at more than three times the number of mature T-lymphocytes ($CD3^+$ cells). Moreover, the number of $CD56^+$ cells showed a discernible increase in the first trimester basal decidua where they account for about 80% of the total leukocyte population and cause an increase in the CD56/CD3 cell ratio to more than five.[2] The number of these cells is reduced significantly by term (essentially absent), indicating that their primary function is during implantation and early pregnancy. The increase in eGLs from the early to the late secretory phase coincides with the rise in progesterone levels. If pregnancy does not occur, they die by apoptosis, which also suggests that these cells are under hormonal control.[19] It remains to be determined whether these effects of progesterone are a direct or indirect response.

Function of Decidual NK (dLGL) Cells

We have shown that decidual lymphocytes are functionally distinct from PBL. Spontaneous proliferation of decidual lymphocytes (DL) is significantly higher than the proliferation of PBL from the same patients.[20] This higher level of activity may be the result of either the presence of more growth promoting cytokines produced by LGLs within decidua,[21] or a greater proliferative capacity of decidual LGLs which share the same phenotype as a subpopulation of PBL showing high proliferative capacity.[17] However, induced proliferation of DL to polyclonal mitogens is many times lower than the proliferation of PBL.[20] This lower level of blastic transformation can be explained rather as a result of the lower number of responsive cells than the lower capacity to respond to polyclonal mitogens. Namely, we repeatedly have found that the relative numbers of CD3 positive DL in suspensions of DL preparations are regularly three to four times lower than in the corresponding PBL suspensions (unpublished). Accordingly, lower proliferation probably does not represent suppression on the level of the cell reactivity to polyclonal mitogens, in agreement with the conclusion of others.[22]

Investigating the role of decidua-trophoblast interactions, we have found that human decidual lymphocytes, both adherent (predominantly macrophages) and nonadherent (predominantly lymphocytes) cells from normal pregnancy and anembryonic pregnancy (viable trophoblast) have very strong immunosuppressive potential. However, adherent and

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nonadherent cells from missed abortion patients (dead trophoblast) had the opposite effect, i.e., the facilitation of immune reactivity.[20,23] It is well documented that dLGL are producers of a wide range of immunomodulatory cytokines. The expression of message as well as production of various cytokines including M-CSF, GM-CSF, G-CSF, IL-1ß, LIF, TNFα, IFNγ and TGFß1,[24-28] and TGFß2[29] was found.

Regulation of NK Cell Activity

The trophoblast is the only cell population of fetal origin that forms the ultimate interface with the maternal tissues, including maternal immunocompetent cells and antibodies. The trophoblast cell subpopulations contain different levels of HLA class I mRNA and express the protein antigen differently from one another.

On the basis of MHC antigen expression trophoblastic cells can be divided into three subpopulations: (i) There is no class I or class II MHC antigen expression on the syncytiotrophoblastic cells (neither mRNA nor protein), (ii) Villous cytotrophoblast cells express MHC class I mRNA, but not protein, and (iii) Invasive extravillous cytotrophoblast cells express mRNA and protein for non-classical HLA-G class I antigens, and additionally, the classical HLA-C molecule.[30-34]

Accordingly, both HLA class I classical (class Ia) and non-classical HLA class I (class Ib) antigens can be expressed on the trophoblastic cells. HLA-G is capable of binding peptides from viruses that are known to infect uterus during pregnancy and so limit virus spread to fetal tissues. Human placenta can be infected by a limited number of viruses[35] and low diversity of peptides presented by HLA-G may be sufficient to play a critical role in the presentation of viral peptides in placenta, together with HLA-C.[31] Very low levels of HLA-G polymorphism should avoid maternal alloimmune reaction, potentially harmful for the fetus.

Human NK cells express cell surface receptors capable of recognizing HLA class Ia molecules as ligands of lytic specificity.[36] Their role is crucial for immunosurveillance of cells that have downregulated or lack MHC class Ia expression at their cell surface, which is the case for most trophoblast cells at the maternal-fetal interface.[30] Numerous recent data confirmed that HLA-G is a ligand of some NK receptors: LIR-1/ILT-2, CD94/NKG2A[37] and p49.[38] These NK receptors belong to the group of killer inhibitory receptors (KIR), i.e., receptors inducing inhibitory transduction signals. As has been emphasized, NK cells are very abundant in the first trimester pregnancy decidua and ligation by HLA-G may modulate the secretion of cytokines from these cells. Accordingly, HLA-G expression could fulfill many local functions, acting against maternal alloimmune attack (by ligation to inhibitory receptors on NK and T-cells), against viral and other intracellular parasite infections, and promoting trophoblast growth by secreting cytokines.

It is believed that NK cell activity is regulated via signals that are transmitted through killer inhibitory receptors (KIR) and killer activatory receptors (KAR) on NK cells upon contact with their ligands, i.e., HLA class I molecules on trophoblast.[24,39] KIRs are also expressed on a small number of T-cells and can mediate inhibition of lysis of target cells expressing the specific HLA ligands.[40] This would be a potential mechanism for preventing lysis of trophoblast by decidual T-cells.[41]

As has been discussed, both NK cells and CTL are abundantly present in the decidua and in the close contact with trophoblastic cells. Recently we have shown that cytolytic cells at the maternal-fetal interface are fully equipped with appropriate cytolytic machinery. By immunohistochemistry and flow cytometric analyses, we have found perforin (P)-containing cells in both decidua basalis and decidua parietalis. The number of P^+ cells in decidua parietalis being even higher then in decidua basalis.[18] The percentage of P^+ cells in the suspension of decidual lymphocytes is twice that of PBL from the same women (55% vs. 27%). However NK activity of these $CD56^+$ cells is much lower than NK activity of PBL[18,42] in spite of the fact that they are full of perforin.[18] The question could be raised as to when and how these cells can be activated? This seems especially important in light of the data that in the same granules of decidual NK cells perforin, granzymes, and TIA-1 co-localize.[43]

The CD16 molecule is the IgG FcRIII that is exclusively responsible for lymphocyte-mediated antibody-dependent cellular cytotoxicity (ADCC) reactions[17,44] which may be important for some types of rejection of allogeneic grafts. Early pregnancy could be the period when the fetoplacental unit allograft needs to be protected against ADCC, which is a combination of both natural (innate) and adaptive immunity. It is important to emphasize that this protection (the downregulation of $CD16^+P^+$ cells) in the first trimester pregnancy is realized at both local (decidua)[18] and systemic (peripheral blood) levels. Namely, we have found profound changes in the $CD16^+$ subpopulation (a drastic decrease of: $CD16^+P^+$ cells; $CD16^+$ cells among P^+ cells; P^+ cells among $CD16^+$ cells).[45] In contrast, at the time of parturition, we have found a significant increase of $CD16^+P^+$ cells.[45] Investigation of the perforin expression in both decidual lymphocytes and peripheral blood lymphocytes in the first trimester of pathological pregnancies (anembryonic pregnancy and missed abortion) showed that significant decreases in the prevalence of perforin positive lymphoid cells, their subpopulations, and mean fluorescence intensity for perforin are associated with pregnancy failure.[46]

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