The immune compartment at the maternal-fetal interface throughout human pregnancy

Zwan, A. van der

Chapter 05

Cytotoxic potential of decidual NK cells and CD8$^+$ T cells awakened by infections

Ângela C. Crespo, Anita van der Zwan,
João Ramalho-Santos, Jack L. Strominger, Tamara Tilburgs

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Abstract

To establish a healthy pregnancy the maternal immune system must tolerate fetal allo-antigens, yet remain competent to respond to infections. The ability of decidual NK cells (dNK) to promote migration of fetal extravillous trophoblasts (EVT) and placental growth as well as the capacity of EVT to promote immune tolerance are topics of high interest and extensive research. However, the problem of how dNK and decidual CD8+ T cells (CD8+ dT) provide immunity to infections of the placenta and the mechanisms that regulate their cytolytic function has thus far largely been ignored. Fetal EVT are the most invasive cells of the placenta and directly interact with maternal decidual immune cells at this maternal-fetal interface. Besides the expression of non-polymorphic HLA-E and HLA-G molecules that are associated with immunetolerance, EVT also express highly polymorphic HLA-C molecules that can serve as targets for maternal dNK and CD8+ dT responses. HLA-C expression by EVT has a dual role as the main molecule to which immune tolerance needs to be established and as the only molecule that can present pathogen-derived peptides and provide protective immunity when EVT are infected. The focus of this review is to address the regulation of cytotoxicity of dNK and CD8+ dT, which is essential for maternal-fetal immune tolerance as well as recent evidence that both cell types can provide immunity to infections at the maternal-fetal interface. A particular emphasis is given to the role of HLA-C expressed by EVT and its capacity to elicit dNK and CD8+ dT responses.

1. Decidual NK cells

The discovery of high numbers of large granular lymphocytes (LGL) in human decidua, later identified as decidual Natural Killer cells (dNK), led to the hypothesis that fetal placental cells actively inhibit maternal dNK and avoid immunologic rejection (1,2). The characterization of dNK as poor cytotoxic lymphocytes and major cytokine and growth factor producers distinguished dNK function from that of cytotoxic peripheral blood NK cells (pNK) (3, 4). The main role for dNK was established as cells that facilitate implantation, trophoblast invasion and vascular remodeling, processes that are of key importance for placental development and pregnancy success (4). The role of dNK in clearance of virus infections, a main function of pNK, has been ignored until recently, Siewiera et al. (5), demonstrated the ability of dNK to clear Human Cytomegalovirus (HCMV)-infected cells. Our lab has built upon this observation and highlighted the dual role of dNK, capable of mounting cytolytic responses during viral infections as well as both providing immune tolerance to the fetus and facilitating placental growth (6).

1.1 dNK paradox – high levels of cytotoxic granules but low cytotoxicity

dNK form a distinct NK cell population that has many differences in gene expression, cytokine secretion and expression of cell surface receptors compared to pNK. However, dNK contain equally high levels of the cytolytic molecules perforin and granzyme B as pNK (7, 3). In addition, dNKexpress increased levels of the cytolytic molecule granulysin compared to pNK (3). In contrast to pNK, in freshly isolated dNK, granulysin and perforin rarely co-localized (8) and dNK but not pNK constitutively secrete granulysin in high levels without prior stimulation (9). Granulysin is produced as an inactive 15 kDa pro-peptide that is processed in cytotoxic granules to a 9 kDa membranolyticpeptide. Although the function of granulysin expression in dNK is not completely understood, the 15 kDa, was shown to act as an alarmin involved in leukocyte recruitment whereas the 9 kDa isoform was shown to bind and disrupt cholesterol-poor membranes, i.e. bacterial, fungal and parasite membranes and enhance clearance of these infections (10, 11, 12, 13). Despite the abundance of cytolytic granules, dNK are not able to kill Major Histocompatibility Antigen (MHC) Class I negative target cells (e.g. cell lines K652 or 721.221) efficiently as do pNK. The low cytotoxicity of dNK is due to an intrinsic block in the polarization of cytolytic granules to the immune synapse that can be overcome by incubating dNK with IL-15 (14, 6). Thus dNK require additional activation by cytokines or activating NK receptor-ligand interactions to display their full cytotoxicity.

1.2 dNK – EVT interactions result in immune tolerance

Human Leukocyte Antigen (HLA)-G+ extravillous trophoblasts (EVT) are the most invasive cells of fetal origin that migrate deeply into maternal tissues and establish direct contact with maternal dNK (15). In vitro co-culture of primary EVT and dNK obtained from the same pregnancy sample demonstrated an abundance of contacts formed

between EVT and dNK. In the contacts between dNK and EVT, perforin did not localize to the immune synapse and both dNK and pNK were unable to kill EVT, even when activated by pro-inflammatory cytokines (6).However under pro-inflammatory conditions (i.e. IL-2 hyperstimulation) dNK were able to induce apoptosis in the trophoblast cell line HTR-8/SV40neo. dNK-derived granulysin actively accumulated in the nuclei of EVTs, causing the death of EVTs due to apoptosis (16). Interaction of dNK with primary EVT led to the acquisition of HLA-G by dNK through trogocytosis that was followed by a cycle of internalization, degradation, and reacquisition of HLA-G (6). Cytokine activation of dNK facilitated degradation of trogocytosed HLA-G and coincided with increased cytotoxicity by dNK. Thus the HLA-G cycle may provide a direct inhibition of cytotoxicity at an individual EVT–NK synapse as well as a prolonged but temporary inhibition of the dNK cytolytic machinery during HLA-G internalization and signaling events. Signaling may involve the HLA-G receptor Killer cell immunoglobulin-like Receptor-2DL4 (KIR2DL4) as well as the activating HLA-C receptor KIR2DS1 that were both increased on HLA-G+ dNK. The inhibition of dNK cytotoxicity as well as other mechanisms of immune regulation such as the direct induction of FOXP3+ regulatory T cells (Treg) by EVT are important facets of maternal–fetal tolerance (17). However, it raises the problem of how the pregnant female is able to respond to events that require participation of cytotoxic dNK and/or decidual effector T cells, for example during a placental HCMV infection, a common viral infection at this site.

1.3 The role of activating NK receptors in pregnancy complications and viral infections

Killer cell Ig-like Receptors (KIR) are the major MHC Class I receptors expressed by NK cells and can be inhibitory or activating depended on the presence of Immunoreceptor tyrosine-based inhibitory motifs (ITIM) or Immunoreceptor tyrosine-based activating motifs (ITAM). Both inhibitory and activating KIR have specificity for discrete groups of MHC Class I alleles (18). Several viruses, including HCMV, impair the expression of MHC molecules to avoid immune recognition by T cells. The lack of MHC ligands for inhibitory KIR expressed by NK cells allows for activation of NK cytotoxicity through missing self-recognition. On the other hand viruses can induce the expression of activating ligands (e.g. MICA and MICB) on the surface of infected cells that directly bind activating NK receptors and promote NK cytotoxicity. Whether or not an NK cell kills a target cell is the result of the balance of inhibitory and activating signals between NK and target cells (19). While the presence of activating ligands on the infected cell with activating receptors on the NK cells will promote cytotoxicity, the presence of MHC molecules that interact with inhibitory receptors will prevent cytotoxicity (Fig. 1) (19). Of high importance for pregnancy is the activating KIR2DS1 receptor that binds HLA-C2 group allotypes of HLA-C. Both KIR2DS1 as well as inhibitory KIR for HLA-C, KIR2DL1 and KIR2DL2/3 that respectively bind HLA-C2 and HLA-C1 group allotypes, are significantly higher expressed by dNK compared to pNK. These observations suggest that there is a

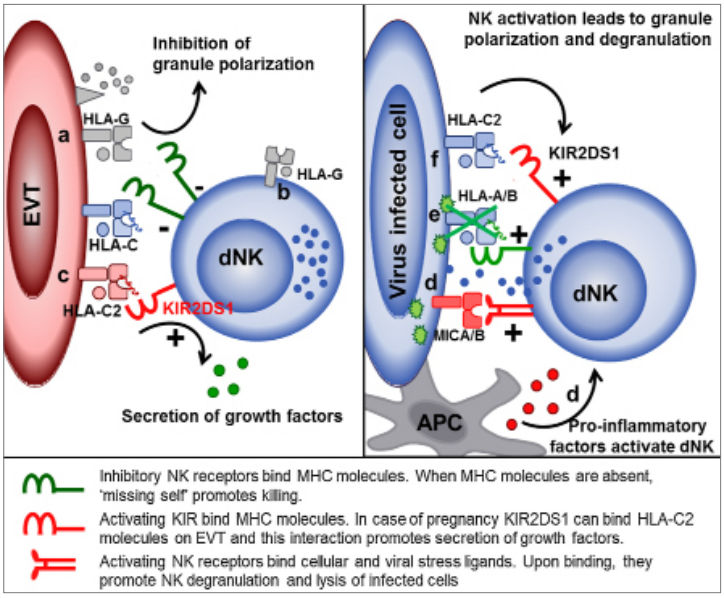

Figure 1. Three functions of dNK include support of trophoblast invasion and placental growth, maintenance of maternal-fetal tolerance and to provide immunity to infections.
(a) HLA-G provides direct inhibition of cytotoxicity at an individual EVT–NK synapse and (b) prolonged but temporary inhibition of the dNK cytolytic machinery after HLA-G trogocytosis; (c) Interaction of KIR2DS1 and HLA-C2 may activate dNK to secrete cytokines and growth factors that benefit trophoblast migration and placental growth; (d) Virus-induced stress ligands and pro-inflammatory factors activate dNK cytotoxicity; (e) virus mediated down regulation of MHC activates NK cytotoxicity through inhibitory KIR and 'missing self' and (f) activating KIR may provide additional NK activation and increase clearance of virus-infected cells.

skewing of KIR expression by dNK towards re cognition of HLA-C, and especially the potential of dNK to develop an activating response to HLA-C2 (20, 21). The presence of KIR2DS1 in the maternal genome was associated with a lower risk for pregnancy complications such as miscarriage, fetal growth restriction and preeclampsia (15, 22). This was most obvious when the fetus expressed HLA-C2, the ligand for KIR2DS1. Recently, the presence of KIR2DS5 has also been associated with lower risk to develop pregnancy complications in African women that may imply a wider role for activating KIR during pregnancy (23). Although the underlying molecular mechanisms that explain the genetic associations between KIR2DS1, HLA-C2 and pregnancy complications remain largely unknown, the current hypothesis suggests that dominance of the inhibitory HLA-C receptors (KIR2DL1, KIR2DL2 and KIR2DL3) reduces the activation of dNK by HLA-C expressed by EVT. Activation of KIR2DS1 on dNK was shown to enhance granulocyte-monocyte colony stimulation factor (GM-CSF) secretion, a growth factor important for trophoblast migration and placental growth (21). The increase in GM-CSF production was observed when dNK were stimulated with anti-KIR2DS1 antibodies and classical NK

target cells that expressed HLA-C2. However, we recently demonstrated that primary EVT do not elicit cytokine responses by dNK even when KIR2DS1 and HLA-C2 are present (17). Furthermore, a murine model to address inhibition and activation of dNK by MHC expressed on trophoblasts demonstrated that the parental origin of the inhibitory MHC was irrelevant for pregnancy outcome (24). Therefore, these genetic associations demand further investigation into the molecular and cellular mechanisms underlying the reduced pregnancy risk linked to activating KIR, and in particular KIR2DS1.

A hint may lie in the observation that individuals who carry the KIR-B haplotype with more activating KIR also have a significantly improved outcome after viral infections such as HCMV, HIV and HPV (25, 26, 27). Of particular interest is that activating KIR were shown to play a role in NK mediated clearance of HCMV infection following hematopoietic stem cell (HSC) or solid organ transplantation (28, 27, 29). Primary HCMV infection or reactivation of latent HCMV infection can cause major problems during pregnancy. HCMV causes placental thickening and insufficiency, leading to fetal growth restriction (30, 31, 32). Placental HCMV infection interferes with trophoblast invasion and placental development and can give rise to placental pathology and congenital syndromes. HCMV sero-positive woman also have a 1.5 fold increased risk to develop preeclampsia (33). CD8+ T cells represent only 2–7% of CD45+ lymphocytes in decidual tissue in 1st trimester pregnancy and thus dNK are the prime candidates to respond to viral infections at this site (34, 35). Siewiera et al., provided the first evidence that dNK are able to clear HCMV-infected decidual stromal cells (5).We recently demonstrated that dNK can clear HCMV infection of maternal decidual stromal cells but that HCMV-infected EVT can-not be cleared (6). The failure of dNK to kill EVT, even when infected with HCMV, may reduce the risk of immune rejection of EVT and placental tissue (Crespo et al. submitted for publication). However inefficient clearance of virus infected placental cells may result in virus-induced placental pathology and development of complications later in pregnancy. Therefore investigation of the benefits of activating KIR/MHC combinations on the ability of dNK to clear HCMV in placental tissue will be of high interest to identify factors that can reduce the risks of placental HCMV infection and associated immunopathology in the placenta. Furthermore, understanding the mechanisms that regulate the switch between immune tolerance and immunity in dNK will contribute to the development of novel strategies to limit virus-induced placental pathology and congenital infection.

2. Regulation of cytolytic activity of CD8+ dT

Besides dNK, decidual CD8+ T cells (CD8+ dT) are key cytolytic effector cells present at the maternal fetal interface. CD8+ dT form a minority of leukocytes present in first trimester decidua (~2 to 7% of CD45+ cells) but their proportion increases to ~30% in term pregnancy decidua (36). CD8+ dT have a predominant effector-memory (EM) phenotype (37, 34) but have reduced expression of perforin and granzyme B proteins in comparison with peripheral blood CD8+ T cells (CD8+ pT) (38, 34). Interestingly, CD8+dT

had increased levels of perforin and granzyme B mRNA content. This suggests post-translational modifications that block translation of perforin and granzyme B mRNA into functional proteins that may be mediated by miRNAs (34, 39). Interestingly, similar to dNK, CD8+ dT express increased levels of granulysin suggesting they are locally activated and may play a protective role against different extracellular or intracellular pathogens (34, 9, 16). Despite the low levels of cytolytic granules, upon stimulation with PMA, first trimester CD8+ dT were shown to be cytolytic and produce cytokines that affect invasion of trophoblasts (40). We recently demonstrated that CD8+ dT are fully functional and upon activation in ex-vivo cultures, upregulate expression of perforin and granzyme B proteins and degranulate in levels equal to CD8+ pT. Thus the cytolytic activity of CD8+ EM dT seems regulated by a blockade in the translation of mRNA into cytolytic proteins, possibly to prevent detrimental responses to foreign fetal and placental cells. However this blockade can be overcome by addition of pro-inflammatory cytokines and T cell receptor (TCR) stimulation that may allow clearance of infected cells (Fig. 2). This is in contrast to dNK that have an abundance of pre-stored intracellular granules containing cytolytic molecules and fail topolarize these granules to the immune synapse in the absence of pro-inflammatory signals. Further elucidation of the molecular mechanisms that regulate the expression of cytolytic molecules by CD8+ EM dT will be key to understanding how these cells provide immunity to infection yet maintain immune tolerance to fetal and placental cells.

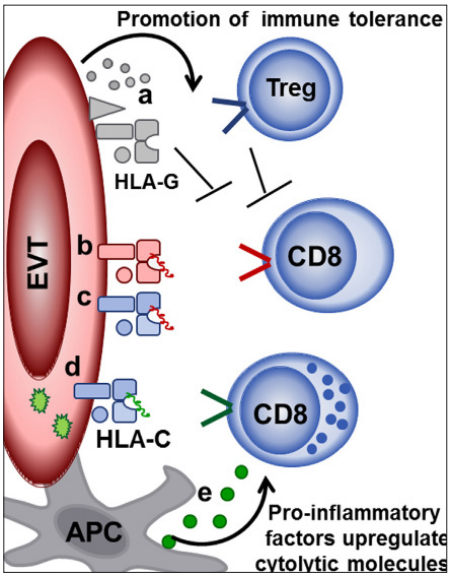

Figure 2. Two functions of CD8+ dT include maintenance of maternal-fetal immunetolerance and to provide for anti-viral immunity. (a) The presence of HLA-G, anti-inflammatory cytokines and high levels of Treg inhibit CD8+ dT cytotoxicity and promote immune tolerance; (b) The presence of allogeneic HLA-C and (c) fetal mHag provide targets for maternal effector T cell responses; (d) Virus-infected EVT can present viral peptides in HLA-C to HLA-C-restricted CD8+ CTL; (e) Virus-induced pro-inflammatory factors can enhance cytolytic activity of CD8+ dT.

2.1 Antigen specificity of CD8+ dT

The existence of highly differentiated CD8+ EM dT indicates the presence of antigens at the maternal-fetal interface that attract an antigen-specific CD8+ dT response. The antigen-specificity of CD8+ dT cells may include MHC molecules of paternal origin (HLA-C expressed by EVT), minor histocompatibility antigens (mHags) or pathogen-derived antigens (recently reviewed in (35)). Although a large proportion of women generate antibodies and cytotoxic T lymphocytes (CTL) specific for paternal MHC and paternal mHags that can be expressed by fetal and placental tissues, there is no evidence that these immune responses responses compromise pregnancy outcome in humans or mice (41, 42, 43, 44, 45, 46). There are many possibilities as to why these fetus-specific T cells do not cause rejection during pregnancy; (i) Incomplete activation of T cells during pregnancy may result in a lack of effector function (42, 34), (ii) High levels of Treg suppress CD8+T cell responses in decidua (47, 48), (iii) A diminished influx of effector cells due to silencing of key T cell attracting chemokines (49) (iv) High levels of anti-inflammatory cytokines such as TGF-β may increase the T cell activation threshold in decidua (50).

2.2 Anti-viral and bacterial immunity by CD8 T cells in pregnancy

Viral infections were shown to alter the dynamics of peripheral blood CD8+ T cell (CD8+ pT) responses during pregnancy (51). However, very few studies have addressed the role of decidual CD8+ EM T cells and their ability to clear placental infections. A murine study demonstrated that antigen-specific CD8+ Tcells in pregnant and non-pregnant mice during acute LCMV infection have similar function and proliferative capacity (52). However, despite the significant expansion and infiltration of antigen-specific CD8+ T cells into the uterus and placental tissues, viral infection persisted in the uterus and placenta of LCMV-infected mice. The virus was cleared in every other tissue (e.g. Serum, Spleen, Liver and Lung) assayed (52). The authors suggested that the lack of MHC class I expression on murine trophoblasts could prevent efficient clearance of the virus by MHC-restricted T cells (52). Another study demonstrated that fetal wastage in mice triggered by pre-natal Listeria monocytogenes infection was the result of placental recruitment of CXCL9-producing inflammatory neutrophils and macrophages that subsequently promote infiltration of maternal fetal-specific CD8+ T cells. Upregulation of CXCR3 expression bymaternal CD8+ T cells with fetal specificity was responsible for influx of these T cells to the decidual tissue (53). During infection with an OVA-expressing Listeria monocytogenes (LM-OVA), OVA-specific CD8+ T cells were shown to accumulate in higher proportions in the decidua compared to the spleen when mice were inoculated with LM-OVA prior to pregnancy (54). Despite the influx of T cells to decidua, bacteria were not efficiently cleared from placental tissue, confirming the study by Constantin et al. Furthermore, both studies suggest that despite chemokine gene silencing to prevent influx of T cells to decidual tissue in healthy murine

gestation (42), incomplete protection against T-cell recruitment to the maternal–fetal interface occurs when T cells are primed by infection (54).

Human EVT can be infected by intracellular pathogens such as HCMV and Listeria monocytogenes (55, 56). Thus, when EVT are infected, HLA-C is the only molecule that can present pathogen-derived peptides to antigen-specific CD8+ T cells. During HIV and HCMV infection HLA-C-restricted CTL responses were shown to comprise as much as 54% of the total response in peripheral blood. Moreover, HLA-C-restricted CTL were shown to be functionally and phenotypically identical to HLA-A- and HLA-B-restricted CTL (57, 58). In humans, HCMV sero-positivity profoundly influenced the peripheral blood T cell repertoire (51). HCMV sero-positive women demonstrated higher levels of CCR7-CD45RA+ effector cells as well as CCR7-CD45RA-CD28- effector memory cells in peripheral blood during late pregnancy compared to sero-negative pregnant women. Besides the studies of virus-specific CD8+ T cells in maternal blood, a recent study demonstrated increased percentages of HCMV and Epstein Bar virus (EBV)-specific CD8+ T cells in decidual tissue compared with peripheral blood after uncomplicated pregnancy. These virus-specific CD8+ memory T cells were able to produce IFN-γ and were restricted to recognize viral peptides in HLA-A or HLA-B molecules (59). Thus, these CD8+ dT may provide cellular immunity for infected maternal cells that express HLA-A andHLA-B. A crucial challenge is to investigate whether virus-specific and HLA-C-restricted CTL are present at the maternal-fetal interface and function to provide immunity when EVT are infected.

Besides directly eliciting maternal CD8+ T cell responses, viral infections have been shown to facilitate ascending bacterial infections and lead to fetal wastage (60, 61). Uncontrolled placental viral (and bacterial) infections also provide a pro-inflammatory milieu that can alter the stability and function of Treg and the activation status of dNK and CD8+ EM dT (62). Infections can result in enhanced alloreactivity, resistance to tolerance induction and destabilization of established tolerance. Similarly, infections in transplant recipients have been associated with failure to induce transplant tolerance and allograft rejection even after long periods of transplant tolerance (62, 63).

Conclusion

or CD8+ dT. Viruses, bacteria and parasites take advantage of the immune privileged status of the placenta and the presence of many immune suppressive mechanisms. Investigation of the mechanisms by which infections are controlled in the placenta as well as the effects of infection and inflammation on Treg function, effector T cell activity and EVT migration, will provide a deeper understanding of the development of pregnancy complications that are associated with infections. Developing strategies to enhance maternal immunity to common infections may prevent development of pregnancy complications and diminish the risk of transmission of infections to the fetus that may lead to severe congenital syndromes.

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