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CD8+ Effector T Cells at the Fetal–Maternal Interface, Balancing Fetal Tolerance and Antiviral Immunity

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Abstract

During pregnancy CD8+ effector T cells need optimal immune regulation to prevent a detrimental response to allogeneic fetal cells while providing immune protection to infections. A significant proportion of (prospective) mothers carry naïve or memory CD8+ T cells with a TCR that can directly bind to paternal MHC molecules. In addition, a high percentage of pregnant women develop specific T cell responses to fetal minor histocompatibility antigens (mHags). Under normal conditions, fetal–maternal MHC and mHag mismatches lead to elevated lymphocyte activation but do not induce pregnancy failure. Furthermore, viral infections alter the maternal CD8+ T cell response by changing the CD8+ T cell repertoire and increasing the influx of CD8+ T cells to decidual tissue. The normally high T cell activation threshold at the fetal–maternal interface may prevent efficient clearance of viral infections. Conversely, the increased inflammatory response due to viral infections may break fetal–maternal tolerance and lead to pregnancy complications. The aim of this review is to discuss the recent studies of CD8+ T cells in pregnancy, identify potential mechanisms for antigen-specific immune recognition of fetal extravillous trophoblast (EVT) cells by CD8+ T cells, and discuss the impact of viral infections and virus-specific CD8+ T cells during pregnancy.

Keywords

CD8+ T cells; effector memory; fetal–maternal tolerance; HLA-C; mHag; virus

Introduction

To establish a healthy pregnancy, the maternal immune system must tolerate fetal alloantigens yet remain competent to respond to infections. CD8+ T cells are key cells to provide protective immunity against viral infections and are the most important cells that can directly recognize allogeneic MHC class I molecules. CD8+ T cells can also contribute to alloimmune recognition of mHags through the indirect antigen presentation pathway.

During human pregnancy, invading fetal extravillous trophoblast (EVT) cells are the most important cells to which a maternal alloimmune response can be directed. Hence, EVT cells play a central role in the establishment of fetus-specific immune tolerance and the prevention of a detrimental immune response to fetal alloantigens. Extravillous trophoblast cells lack expression of HLA-A and HLA-B molecules, which are the main cause of CD8+

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T cell mediated transplant rejection. Extravillous trophoblast cells do express the non-polymorphic HLA-E and HLA-G molecules which can prevent NK cell mediated cytotoxicity and may induce T cell tolerance.^{1–3} However, EVT cells also express highly polymorphic HLA-C molecules for which over 1600 alleles and 1200 proteins have been identified so far (IMGT/HLA sequence database: <http://www.ebi.ac.uk/imgt/hla/stats.html>).⁴ HLA-C expression prevents NK cell-mediated cytotoxicity through ligation with Killer Immunoglobulin-like Receptors (KIRs). However, HLA-C can also elicit a direct cytotoxic response by CD8+ T cells during allogeneic organ and hematopoietic stem cell (HSC) transplantation.^{5,6}

How maternal immune tolerance to paternally encoded HLA-C is established is a topic of extensive research and likely involves CD4+CD25+FOXP3+ Treg cells. In humans, fetal–maternal HLA-C mismatched[#] pregnancies contain a higher percentage of activated T cells and higher levels of functional CD4+CD25^{bright}FOXP3+ Treg cells at the fetal–maternal interface compared to HLA-C-matched pregnancies.^{7,8} In mice, depletion of CD4+CD25+ T cells leads to increased fetal resorption in allogeneic but not in syngeneic matings.⁹ In addition to CD4+CD25^{bright}FOXP3+ Treg cells, recent studies highlight that highly differentiated CD8+ effector memory T cells are present at the fetal–maternal interface and that fetus-specific CD8+ T cell response can be initiated during uncomplicated pregnancies.^{10–12} Moreover, the presence of highly differentiated CD8+ effector memory T cells in decidual tissue implies that antigens are present at the fetal–maternal interface that can attract antigen-specific responses.¹⁰ Whether or not maternal CD8+ T cells are able to directly recognize and elicit an allogeneic response to HLA-C, or other polymorphic proteins expressed by fetal EVT cells, has not been addressed in a systematic way. Therefore, the aim of this review is to discuss the possible ways and the potential risks of fetus-specific allorecognition mediated by CD8+ T cells during pregnancy. Although the target specificity of decidual CD8+ T cells is unknown, this may include MHC antigens (HLA-C in humans), mHags or non-self pathogen-derived antigens.

CD8+ T cells in peripheral blood and decidual tissue

CD8+ T cells are a heterogeneous subset of cells, and many cell surface markers are used to categorize CD8+ T cell subtypes during different stages of an immune response.^{13–15} In addition, T cells are highly migratory and their ability to fight infections depends on their tissue localization and their capacity to traffic through different lymphoid and peripheral tissues.¹⁶ To make this review more accessible for non-expert readers we include here a brief introduction to the process of CD8+ T cell activation, differentiation, their migratory properties as well as the cell surface markers that identify the main CD8+ T cell subtypes. Furthermore, we include an overview of the phenotype of decidual CD8+ T cells and discuss the differences between peripheral blood and decidual CD8+ T cells.

CD8+ T cell activation, differentiation, and migration

A typical CD8+ T cell response is characterized by three phases: (I) the initial T cell activation and expansion phase, (II) the contraction or death phase, and (III) the generation of T cell memory.^{13–15} During initial activation, naïve CD8+ T cells recognize their cognate antigen presented by MHC class I on antigen-presenting cells (APC). After costimulation of CD28 by APC-expressed CD80 and CD86, CD8+ T cells expand up to 10⁵-fold and acquire effector T cell functions. Effector T cell functions includes the ability to secrete pro-inflammatory cytokines (e.g., IFN and TNF) and the synthesis of cytolytic molecules

[#]HLA mismatch: The situation where the fetus (or organ donor) expresses an HLA allotype that the mother (or recipient) does not express. In a previous study, we found that approximately 70% of the mother/child combinations analyzed were mismatched for HLA-C whereas ~30% were HLA-C matched.⁷

(e.g., perforin and granzymes) to enhance the cytotoxic potential. During T cell activation, CD8⁺ T cells strengthen their TCR-signaling pathways so that they no longer require costimulatory signals and gain the ability to directly kill target cells that express their specific MHC class I/peptide complex. CD4⁺ T helper (Th) cells are not essential for the initial CD8⁺ T cell activation. However, the presence of different types of CD4⁺ Th cells, for example, Th1, Th2, Th17, and Treg cells can influence (enhance or suppress) the CD8⁺ T cell response by secreting cytokines, such as IL-2 (Th1), IFN (Th1), IL-4 (Th2), IL-17 (Th17), or IL-10 (Th2, Treg). Some Treg cell subsets can also provide cell contact dependent inhibition of CD8⁺ T cell activation and proliferation.

After the expansion phase, the large majority (90–95%) of the CD8⁺ effector T cells die by apoptosis in the contraction phase. The remaining CD8⁺ T cells differentiate into several types of memory T cells which can rapidly respond to reinfections with the same pathogen.^{17,18} A population of long-lived circulating memory cells, called central memory cells, is induced. Central memory cells express the lymph node homing receptors chemokine C-C motif receptor type 7 (CCR7) and L-selectin (CD62L). Thereby, they can enter lymph nodes and systematically scan the whole body for reinfection. Simultaneously, a population of tissue resident effector memory cells is established. Effector memory CD8⁺ T cells reside in peripheral tissues where they control latent (viral) infections (e.g., human cytomegalovirus (HCMV) and herpes simplex virus (HSV)) and can rapidly respond when reactivation occurs.

Extensive studies have identified many useful cell surface markers to identify and categorize CD8⁺ T cells in the different stages of the immune response. CD45RA and CD45RO can be used to identify naïve versus memory cells, respectively. The presence of the lymph node homing receptors CCR7 and CD62L can distinguish circulating cells (e.g., naïve and central memory cells) from tissue resident cell types (e.g., effector and effector memory cells).¹⁹ In addition, the loss of costimulatory molecules CD28 and CD27 identifies antigen-experienced T cells, whereas T cells-expressing CD28 and CD27 are still sensitive to costimulation and require a higher activation threshold (e.g., naïve cells). CD28 and CD27 also identify distinct subtypes of CD8⁺ effector memory T cells.²⁰ A summary of the CD8⁺ T cell subtypes, their functions, and phenotypes are summarized in Table I.

T cell activation also programs T cells to express distinct sets of integrins, selectins, and chemokine receptors. Expression of a particular combination of these receptors enables T cells to migrate to (inflamed) lymphoid or peripheral tissues that express the complementary integrin ligands, selectin ligands, and chemokines.¹⁶ The target tissue then further shapes the subsequent T cell differentiation process, and this may reprogram a proportion of activated T cells to alter their migratory properties, recirculate and enter different tissues to scan for infections. Limited data are available as to which chemokine receptor, integrin, and selectin combinations are required for the trafficking of T cells into specific tissues. In some tissues, redundancy may allow multiple T cell subsets to be recruited, whereas in other tissues, the homing receptor expression requirements may be more stringent.¹⁶

For example, in lymph nodes, the fibroblastic reticular cells (FRCs) express the chemokine CC-ligand-19 (CCL19) and CCL21 to guide CCR7⁺ T cells to interact with dendritic cells.^{21,22} Dendritic cells themselves express chemokines such as CCL3 and CCL4 which can attract CCR5⁺ T cells.^{23,24} To migrate to the skin T cells express E-selectin ligands (e.g., CD44 and CD43) which can bind E-selectin expressed on skin endothelial cells.^{25,26} In this process, CD4⁺ T cells depend on CCR4 expression whereas CD8⁺ T cells depend on CCR10 expression.^{27,28} In murine placental tissue, the recruitment of antigen-specific T regulatory cells has been shown to depend on CCR5 expression.²⁹ The lack of CXCL9 and CCL5 expression by decidual stromal cells may prevent recruitment of other T cell types to

decidual tissue in mice.³⁰ Regulating T cell trafficking and balancing local versus systemic immunity guides activated T cells to sites of infection and may prevent influx of potentially harmful autoreactive (or fetus-reactive) T cells to other tissues.¹⁶

Decidual CD8⁺ T Cells are highly differentiated effector memory T Cells

In early pregnancy, T cells constitute 5–20% of total decidual lymphocytes, and this increases with gestational age up to 40–80% at full-term pregnancy.³¹ Decidual T cells are a heterogeneous subset of cells with major differences compared to peripheral blood T cells. Differences include increased percentages of T cell receptor (TCR)- α T cells, CD4-CD8-TCR- α T cells, and CD4⁺CD25^{bright}FOXP3⁺ Treg cells as well as increased percentages of activated CD4⁺CD25^{dim} and CD8⁺CD28- T cells.^{32,33} Thus far, no clear function or antigen specificity has been identified for many of the decidual T cell types.

In contrast to peripheral blood, where CD4⁺ T cells form the predominant T cell subset, CD8⁺ T cells are the most abundant T cell subset in decidual tissue at term pregnancy. Approximately half of the peripheral blood CD8⁺ T cells are unprimed naïve cells whereas all other CD8⁺ subtypes (effector, central memory, and effector memory T cells) are less abundant.^{14,20} The large majority of CD8⁺ T cells at the fetal–maternal interface are activated effector memory T cells and unprimed naïve cells are virtually absent.¹⁰ A main feature of peripheral blood CD8⁺ effector and effector memory T cells is the high expression of cytolytic molecules like perforin and granzyme B.²⁰ However, decidual CD8⁺ effector and effector memory cells express significantly lower levels of perforin and granzyme B proteins. Interestingly, mRNA for perforin and granzyme B was found to be significantly higher in decidual CD8⁺ effector and effector memory T cells than in their peripheral blood counterparts. This indicates that alternative differentiation of decidual CD8⁺ T cells takes place, in part, at the post-transcriptional level and may implicate a role for microRNAs, which have recently been shown to control effector memory T cell differentiation.^{34,35} Decidual CD8⁺ T cells do express FASL and low levels of granulysin, which may provide alternative means to mediate cytotoxicity.^{10,36} Together these data suggest that decidual CD8⁺ T cells are antigen experienced and are highly differentiated cells; however, they display unique properties compared to peripheral blood CD8⁺ T cells.¹⁰ A summary of the differences between CD8⁺ T cell subtypes in peripheral blood and decidual tissue is included in Table I.

Antigen specificity of CD8⁺ T cells during pregnancy

The presence of highly differentiated CD8⁺ effector memory (EM) T cells in decidual tissue implies that antigens are present at the fetal–maternal interface that can induce an antigen-specific CD8⁺ T cell response. Thus far, the target specificity of decidual CD8⁺ T cells is unclear but may include MHC (HLA-C in humans), mHags, or non-self pathogen-derived antigens. All three possibilities, and the potential of each to contribute to antigen-specific recognition of fetal EVT cells, are illustrated in Fig. 1 and are discussed in the following sections.

Direct MHC recognition during pregnancy

Invading fetal EVT cells do not express HLA-A and HLA-B molecules that are the main candidates to elicit a CD8⁺ T cell response during allogeneic organ and HSC transplantation. However, EVT cells do express HLA-C, HLA-E, and HLA-G molecules. Of these molecules, only HLA-C is polymorphic and is thus the primary candidate to attract an antigen-specific response by CD8⁺ T cells.

From HSC transplant studies, it is known that donor/patient pairs with a single HLA-C mismatch can yield a cytotoxic T lymphocyte (CTL) response.^{5,6} CTL cytotoxicity assays

mainly detect direct T cell cytotoxicity to mismatched MHC antigens. The percentage of donor/patient pairs with a detectable cytolytic response was lower in the HLA-C mismatched group compared to donor/patient pairs with a single HLA-A and HLA-B mismatch. Nevertheless, in this study, 35% of the donors displayed direct T cell cytotoxicity to the patients with a single HLA-C mismatch.⁶ Similarly, maternal CD8⁺ T cells may be able to directly recognize non-self paternal HLA-C expressed on fetal EVT cells (Fig. 1a).

Direct allorecognition of MHC class I molecules largely depends on three features, (I) the differences in amino acid motifs (between donor/patient) in the 1 and 2 domains which are relevant for MHC-TCR binding,^{6,37} (II) the selection of peptides that can be presented by the foreign MHC molecules^{38,39} and (III) the TCR repertoire of the responder T cell pool. The TCR repertoire of each person is tightly selected during thymic maturation. In addition, the proportion and activation state of antigen-specific T cells is strongly influenced by lifelong exposure to pathogens and vaccinations. A substantial proportion of pre-existing and virus-specific CD4⁺ and CD8⁺ memory T cells (for e.g., Epstein-Barr virus (EBV), HCMV, varicella zoster virus (VZV) and influenza virus) were shown to cross-react against non-self allogeneic HLA molecules. In this case, the allogeneic HLA reactivity and virus specificity were mediated via the same TCR.^{40–42} Cross-reactivity was shown against a wide variety of HLA-A, HLA-B, and MHC class II molecules and it cannot be ruled out that virus-specific and HLA-C cross-reactive T cells exist. These cells may be able to directly recognize non-self paternal HLA-C expressed on fetal EVT cells (Fig. 1b).

The first study using a mouse model to assess direct allorecognition of paternal MHC molecules used the Des-TCR transgenic model. Des-TCR transgenic females have T cells with direct specificity for the MHC allotype H2-K^b which is expressed on mouse trophoblast cells.^{43,44} When H2-K^b negative and Des-TCR expressing females were mated with H2-K^b expressing males, a large number of maternal splenic T cells were activated. In addition, the H2-K^b negative females carrying H2-K^b expressing fetuses accepted H2-K^b tumor grafts during gestation.⁴⁴ A more recent study used CD8⁺ T cells from BM3-TCR transgenic mice that also have direct alloreactivity for H2-K^b molecules.⁴⁵ However, adoptive transfer of these H2-K^b specific CD8⁺ T cells to H2-K^b negative females carrying a H2-K^b expressing fetus did not induce CD8⁺ T cell proliferation or upregulation of the T cell activation markers CD69 or CD44 in the spleen, lymph nodes, uterine draining lymph nodes or at the implantation site.⁴⁵ More importantly, in both studies the presence of maternal CD8⁺ T cells with a direct specificity for fetal MHC molecules did not induce pregnancy failure. From both the human and mouse studies, it appears likely that at least a proportion of (prospective) mothers carry naïve or memory CD8⁺ T cells with a TCR that can directly bind and respond to paternal MHC molecules (HLA-C in human) expressed by fetal EVT cells. MHC mismatches between mother and fetus may lead to increased levels of lymphocyte activation but under normal conditions direct recognition of paternal MHC has not been shown to lead to pregnancy failure.

In mice, adoptive transfer of CD4⁺CD25⁺ Treg cell depleted lymphocytes to pregnant mice resulted in an increased resorption rate in allogeneic pregnancies compared to similarly handled syngeneic pregnancies.⁹ Thus, CD4⁺CD25⁺ Treg cells play a crucial role in preventing rejection of allogeneic MHC-mismatched fetuses. In addition, it demonstrates that maternal allospecific lymphocytes are present and can mediate fetal rejection in the absence of CD4⁺CD25⁺ Treg cells. In humans, HLA-C mismatched pregnancies have been shown to induce functional CD4⁺CD25^{bright}FOXP3⁺ Treg cells in decidual tissue, whereas HLA-C-matched pregnancies did not.⁷ These findings indicate that paternal MHC/HLA-C molecules are specifically recognized by maternal leukocytes. They can elicit an MHC/HLA-C specific response that is controlled by the induction CD4⁺CD25⁺ Treg cells. Whether MHC/HLA-C specific CD8⁺ T cells play a clinical role in human pregnancy

complications, such as miscarriages, and how CD4⁺CD25⁺ Treg cells suppress fetus-specific T cells is currently the focus of many studies.

Minor Histocompatibility Antigen (mHag) recognition during pregnancy

mHags are peptides derived from polymorphic (non-MHC) proteins that can be recognized as non-self by allogeneic T cells in cases of organ transplantation, HSC transplantation, or pregnancy.⁴⁶ An antigen-specific T cell response to mHag peptides can occur in two manners. First, donor and recipient (or fetus and mother) share the same MHC class I allele in which the mHag peptide can be presented. In this case, the MHC on donor (or fetal) cells can directly present the mHag peptides to responder (or maternal) CD8⁺ T cells that have a specificity for this MHC class I/mHag peptide complex (Fig. 1c). Second, a mHag-specific CD4⁺ and/or CD8⁺ T cell response can be initiated through the indirect antigen recognition pathway. Thereby, mHags are taken up by maternal APCs which, after antigen processing and presentation activate mHag-specific CD4⁺ and/or CD8⁺ T cells. In this case, donor and recipient (or fetus and mother) do not need to share the MHC class I molecules in which the mHag peptides can be presented (Fig. 1d–e). The route of indirect antigen recognition is strongly influenced by the type of APC that presents the antigen to T cells, as well as the nature of the particle or cell that carries the antigen. As such, alternatively activated macrophages at the fetal–maternal interface as well as inhibitory molecules (e.g., HLA-G) expressed by EVT cells and trophoblast particles influence T cell activation (Fig. 1f).^{47,48}

Several proteins encoded on the male Y chromosome have been shown to generate Y chromosome mHag peptides (HY) which can initiate an HY-specific CD8⁺ T cell response when female HSCs are transferred to male recipients and in mothers carrying a male fetus.^{49,50} Besides HY, many other mHags have been identified, but the majority have a tissue specific distribution; therefore, not all may be expressed on fetal trophoblast cells.⁵¹ Recently, the autosomal mHags (HMHA1, KIAA0020, and BCL2A1) and the HYs (KDM5D, DDX3Y, and RPS4Y1) were shown to be expressed on placental EVT cells as well as syncytiotrophoblast cells.⁵² Several of these mHags, including the HMHA1 antigen that is highly expressed on syncytiotrophoblast cells, can elicit an antigen-specific CD8⁺ T cell response in HSC transplantation.^{52,53} However, syncytiotrophoblast cells do not express any MHC molecules and therefore lack the capacity to directly present mHag peptides to T cells. Thus, syncytiotrophoblast-derived mHags can be presented by APC to elicit mHag-specific T cell priming. But, primed mHag-specific T cells will fail to recognize or kill syncytiotrophoblast cells due to the absence of all MHC molecules on these cells.

A recent study demonstrated that HY-specific T cells can be found in peripheral blood of 50% of women who are pregnant with a male fetus and can be detected as early as the first trimester of pregnancy.⁵⁴ This study demonstrated that the majority of HY-specific T cells are of the CCR7-CD45RA⁺ effector and the CCR7-CD45RA-CD28⁺CD27⁺ effector memory-1 CD8⁺ T cell types and can secrete IFN- γ as well as mediate direct cytotoxicity against male target cells.⁵⁴ Another study demonstrated that both HY-specific CTLs and HY-specific T cells with an immune suppressive phenotype can be induced as a consequence of pregnancy.⁵³ In this case, the HY-specific Treg cells have a low HY tetramer binding affinity and Treg mediated suppression functioned in a CTLA-4 dependent manner.⁵³ Besides the appearance of HY-specific CD8⁺ T cells in maternal peripheral blood, our data show that HY-specific CD45RA-CD8⁺ T cells can be found in decidual tissue (Fig. 2). It is not clear whether decidual HY-specific T cells are predominantly regulatory or cytotoxic. In all studies, the HY-specific T cells were detected using HLA-A or HLA-B tetramers loaded with HY peptides and consequently only HLA-A and HLA-B restricted T cells can be recognized. HLA-A and HLA-B proteins are not expressed by EVT cells, and therefore, these HY-specific and HLA-A/HLA-B restricted T cells are not able to

attack fetal EVT cells. The question whether HLA-C can present mHag peptides and if mHag-specific HLA-C restricted T cells exist requires further investigation.

Recent studies using the OVA-specific TCR transgenic mouse model,^{##} which mimics an mHag-specific alloresponse, demonstrated that paternally encoded OVA antigens can activate maternal OVA-reactive CD8⁺ OT-I and CD4⁺ OT-II cells.^{30,45,55} Seminal fluid containing OVA-induced proliferation of maternal OVA-specific CD8⁺ OT-I and CD4⁺ OT-II cells in the uterine draining lymph nodes as soon as 12 hrs post-mating.⁵⁵ CD8⁺ OT-I and CD4⁺ OT-II T cell proliferation in spleen and distant lymph nodes was not detectable after mating but from day 6.5 of gestation, a significant expansion of both CD8⁺ OT-I and CD4⁺ OT-II cells was observed in spleen and distant lymph nodes.⁵⁵ OVA presentation to maternal OT-I cells occurred through indirect antigen presentation that required maternal H2-K^b expressing APC and involved TAP dependent antigen processing. However, direct recognition of MHC class I/mHag peptide complexes expressed on fetal trophoblast cells has not been shown.^{45,55} Although CD8⁺ OT-I T cells are H2-K^b restricted and H2-K has been shown to be expressed on mouse trophoblast cells, no adverse effects on pregnancy outcome were observed.^{43,55} Another study using the same OVA/OT-I mouse model demonstrated that activated mHag-specific CD8⁺ OT-I cells could not accumulate in decidual tissue due to epigenetic silencing of key T cell attracting chemokines (CXCL9 and CCL5) in decidual stromal cells.³⁰ Peripheral challenge with OVA and adjuvant induced a large accumulation of CD3⁺ T cells in the myometrium as well as in undecidualized endometrium between implantation sites but not in decidual tissue. Simultaneous overexpression of both CXCL9 and CCL5 by lentivirus vectors increased the T cell density in deciduomas of pseudopregnant mice.³⁰

In conclusion, it is clear that paternally encoded mHags are recognized by the maternal immune system and can induce activation and proliferation of mHag-specific T cells. However, these fetus-specific T cells do not induce adverse effects on pregnancy outcome. The restricted chemokine expression in decidual tissue may reduce the influx of these fetus-specific T cells to the fetal-maternal interface, whereas the presence of alternatively activated macrophages and the expression of inhibitory molecules and anti-inflammatory cytokines may prime maternal T cells such that both CTLs and T cells with a regulatory function are induced.

Viral infections during pregnancy and the decidual CD8⁺ T cell response to viral antigens

Viral infections, such as HSV, HCMV, human immunodeficiency virus (HIV), and influenza viruses, can cause severe maternal and fetal disease and morbidity when they occur during pregnancy.^{56–60} Fetal infections have been reported to cause recurrent spontaneous abortions (RSAs) at a rate lower than 4%. However, difficulties in demonstrating the pathogenic role of a wide variety of viral and bacterial pathogens may result in under diagnosis.⁶¹ HCMV is the most common congenital infection and represent 0.5–2% of all live births. Human cytomegalovirus infects the placenta before it infects the fetus and causes placental thickening and placental insufficiency.⁶² A recent study demonstrated that the HCMV specific T cell response did not significantly differ between pregnant women and non-pregnant individuals at any time point during primary infection.⁶³ However, a significantly delayed lymphoproliferative response was observed in mothers that transmitted the HCMV infection to their fetus compared to non-transmitting mothers.⁶³

^{##}The OVA-specific TCR transgenic mouse model is a model where mice, in this case the males, contain the transgene act-mOVA encoding for the chicken egg ovalbumin (OVA). When act-mOVA transgenic males are mated with wild-type females, OVA is highly expressed by placental trophoblast cells. Exposing the maternal immune system to paternally derived OVA mimics an indirect mHag-specific alloresponse during pregnancy. Furthermore, CD8⁺ OT-I and CD4⁺ OT-II T cells that express OVA-specific transgenic TCRs can be given to females and this way the maternal immune response to fetal antigens can be monitored.^{45,84}

Furthermore, HCMV transmitting mothers contained lower percentages of CD4+CD45RA+IFN + and CD8+CD45RA+IFN + T cells compared to non-transmitting women.^{64,65} Another study demonstrated that in HCMV IgG sero-positive pregnant woman the proportion of HCMV specific T cells strongly increased during the third trimester of pregnancy, whereas no change was observed in pregnant and HCMV IgG sero-negative women.⁶⁶ No signs of HCMV reactivation in maternal peripheral blood cells were detected in these patients but unfortunately, no data were available on virus replication in placental tissue.⁶⁶ These data demonstrate that primary HCMV infection during pregnancy significantly alters the dynamics of maternal T cell responses. However, no systematic evaluation of the impact of placental viral infections on the decidual T cell repertoire and antiviral response at the fetal–maternal interface has been presented so far in humans.

A murine study examining lymphocytic choriomeningitis virus (LCMV) infection during gestation, demonstrated that LCMV virus was cleared in all tissues examined (liver, lung, spleen, thymus, and serum), but persisted in the placenta until delivery.⁶⁷ A significant infiltration of CD8+ T cells to the fetal–maternal interface was observed, and memory CD8+ T cell development in mice that were pregnant during primary infection was normal. Pregnancy outcome in LCMV infected mice was poor with a high rate of maternal mortality, fetal resorption, and mortality of the newborn pups.⁶⁷ Another study demonstrated that when deciduomas of pseudopregnant mice were injected with a control lentiviral vector-expressing GFP only, the CD3+ T cell density in decidual and endometrial tissue significantly increased.³⁰ Uninfected mice were protected from a large influx of T cells to decidua because of the absence of T cell attracting chemokines CXCL9 and CCL5.³⁰

Another way by which viral infections influence CD8+ T cells responses is through downregulation of HLA molecules from the cell surface of infected target cells. HSV and HCMV have been shown able to downregulate HLA-A, HLA-B, HLA-C, and HLA-E molecules in infected cells as well as HLA-G molecules from the cell surface of virus-infected choriocarcinoma (JEG-3) cells.^{68–70} These viruses block viral peptide loading on MHC class I molecules and are able to escape CTL mediated immune control. The loss of MHC class I molecules from the cell surface can render infected cells susceptible to NK cell mediated killing.⁷¹ HIV can specifically target HLA-A and HLA-B molecules for degradation but maintain HLA-C expression on the cell surface.^{68,72,73} Although expression of HLA-C on the cell surface prevents NK cell mediated cytotoxicity, HIV specific HLA-C restricted CD8+ CTLs can be generated and are able to mediate strong cytotoxic activity and produce high levels of IFN .⁷⁴ Similarly, HLA-E restricted CD8+ CTLs specific for viral and bacterial peptides presented in HLA-E can be generated and mediate direct cytotoxicity against infected target cells.^{75–77} These data demonstrate that viral infections of placental tissue may alter the HLA expression profile of infected trophoblast cells and render them susceptible to NK cell killing or lysis by HLA-C or HLA-E restricted CD8+ T cells (Fig. 1g-h). In this regard, decidual NK cells may play a crucial role in the antiviral immune response, but they may utilize distinct cytotoxic mechanisms compared to peripheral blood NK cells.^{78,79} Furthermore, pre-existing virus-specific and HLA-C or HLA-E restricted memory T cells that were generated during a previous infection may rapidly respond when reinfection or reactivation of a latent viral infection occurs during pregnancy.

In conclusion, these studies demonstrate that viral infections during murine and human gestation can lead to severe pregnancy complications. The presence of many anti-inflammatory molecules, cells, and cytokines at the fetal–maternal interface may prevent efficient clearance of viral infections in placental and fetal tissue. However, viral infections during pregnancy can alter the dynamics of the decidual CD8+ T cell response by changing the CD8+ T cell repertoire and increasing the influx of CD8+ T cells to decidual tissue. Moreover, viral infections can alter MHC expression profiles of infected trophoblast cells

and increase the production of pro-inflammatory cytokines and chemokines. This increased inflammatory response due to viral infections may break fetal–maternal tolerance and lead to pregnancy complications. However, whether or not virus specific CD8+ T cells in decidual or endometrial tissue can directly kill healthy or virus-infected trophoblast cells and result in pregnancy failure is a question that remains to be answered.

Summary and conclusions

From both murine and human studies, it appears likely that a significant proportion of (prospective) mothers carry naïve or memory CD8+ T cells with a TCR that can directly bind and respond to paternal MHC molecules (HLA-C in human) that are expressed by fetal trophoblast cells. In addition, a high percentage of pregnant women develop fetus-specific T cell responses to mHags through the indirect allorecognition pathway. Under normal conditions, fetal–maternal MHC and mHag mismatches lead to increased levels of lymphocyte activation but do not seem to induce pregnancy failure. The wide variety of immune regulatory mechanisms at the fetal–maternal interface may, in part, prevent a detrimental maternal CD8+ T cell response to fetal alloantigens (Fig. 3a–e).

Immune regulation at the fetal–maternal interface may be mediated by several mechanisms. Influx of T cells to decidual tissue may be reduced due to epigenetic silencing of key T cell attracting chemokines, CXCL9, and CCL5, in decidual stromal cells³⁰ (Fig. 3a). Inhibitory molecules including HLA-G, IDO, B7-H3 molecules, expressed by EVT cells, or other cells at the fetal–maternal interface, may directly inhibit or reduce CTL mediated cytotoxicity^{2,80–82} (Fig. 3b). High expression of anti-inflammatory cytokines like TGF- in decidual tissue may increase the T cell activation threshold⁸³ (Fig. 3c). The presence of a high percentage of CD4+CD25^{bright}FOXP3+ Treg cells at the fetal–maternal interface may inhibit or reduce allogeneic lymphocyte reactions^{7,8} (Fig. 3d). The presence of anti-inflammatory APCs in decidua and/or uterine draining lymph nodes may activate T cells to become regulatory cells (Fig. 3e).

Viral infections can skew the CD8+ T cell repertoire and alter the dynamics of the CD8+ T cell responses during pregnancy (Fig. 3f–j). Increased levels of pro-inflammatory cytokines and chemokines, induced by viral infections, increase the influx of T cells to decidual tissue^{30,67} (Fig. 3f). CD4+CD25^{bright}FOXP3+ Treg cells may not be able to efficiently inhibit allogeneic lymphocyte reactions under pro-inflammatory conditions. Conversely, CD4 + CD25^{bright} FOXP3+ Treg cells may prevent an optimal virus clearance in placenta tissue (Fig. 3g). The increased levels of pro-inflammatory cytokines may reduce the T cell activation threshold and strengthen pro-inflammatory responses (Fig. 3h). Similarly, the presence of inhibitory molecules and alternatively activated APCs may prevent optimal viral antigen presentation and subsequent virus clearance from placenta tissue (Fig. 3i–j).

Under normal conditions, pre-existing or newly induced fetus-specific cytotoxic CD8+ T cells do not seem able to kill healthy trophoblast cells or cause pregnancy failure. Whether a viral infection coinciding with the presence of fetus-specific CD8+ CTLs leads to trophoblast cytotoxicity and pregnancy complications remains to be determined. CD8+ effector T cells are key cells to provide protective immunity and key mediators of the alloimmune responses. Striking the correct balance between immune tolerance and viral immunity by CD8+ cytotoxic T cells in the decidua is therefore crucial for healthy progression of pregnancy. Further investigation into the mechanisms that prevent a detrimental fetus-specific CD8+ T cell response and the impact of viral infections on the CD8+ T cells response at the fetal–maternal interface will be crucial for a better understanding of pregnancy complications, such as miscarriages and pre-eclampsia.

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Fig. 1.

Multiple ways of antigen-specific immune-recognition of fetal extravillous trophoblast (EVT) cells by maternal CD8+ T cells.

- HLA-C is the only polymorphic MHC molecule expressed by fetal EVT cells. At least a portion of (prospective) mothers carry naïve or memory CD8+ T cells with a TCR that can directly bind and respond to paternal HLA-C molecules expressed by EVT cells.⁵⁻⁷
- Cross-reactive T cells are pre-existing virus-specific CD4+ and CD8+ T cells that are able to cross-react against foreign MHC molecules. Alloreactivity and virus specificity by these T cells are mediated via the same TCR.⁴⁰⁻⁴² HLA-C cross-reactive T cells have not been identified so far, but based on the high prevalence and broad diversity of cross-reactive cells it is likely that HLA-C cross-reactive T cells exist and are able to directly bind and respond to paternal HLA-C molecules expressed by EVT cells.
- Fetal minor histocompatibility antigens (e.g. HY antigens) may be presented in maternal HLA-C molecules expressed on fetal EVT cells. Mouse models have shown that mHag recognition required maternal antigen presenting cells (APCs). Direct recognition of MHC class-I/mHag complexes on fetal or placental trophoblast cells did not occur.^{45,55} In humans, the question whether HLA-C can present mHag peptides and if mHag specific HLA-C restricted T cells exist remains open.
- Indirect antigen recognition of major and minor histocompatibility antigens expressed by fetal EVT cells. Maternal APCs in decidual tissue and draining lymph nodes can pick up trophoblast cell debris, process allogeneic antigens, and present peptides through MHC class-II to maternal CD4+ T cells.^{45,55}
- Similar to (d) major and minor histocompatibility antigens can activate maternal CD8+ T cells through indirect antigen recognition. Thereby maternal APCs cross present peptides in MHC class-I to maternal CD8+ T cells.^{45,55}

- f) HLA-G can influence the alloimmune response by binding to HLA-G receptors (ILT-2 and KIR2DL4) expressed by APCs and NK cells.^{47,85} This may polarize APCs to secrete more anti-inflammatory cytokines and subsequently induce alternative CD4+ and CD8+ T cells differentiation that leads to more immune suppressive T cell phenotypes.
- g) Placental and trophoblast tissue can harbor several types of viral, bacterial, and parasitic infections. HLA-C molecules expressed on fetal EVT cells have been shown able to present viral peptides to virus-specific CD8+ T cells.⁷⁴
- h) Similar to (g), HLA-E molecules expressed on fetal EVT cells have been shown able to present viral and bacterial peptides to Ag-specific CD8+ T cells.⁷⁵⁻⁷⁷

Fig. 2.

Presence of HY-specific T cells at the fetal-maternal interface. Maternal peripheral blood lymphocytes and decidual lymphocytes from decidua basalis and decidua parietalis were stained for CD45, CD14, CD19, CD56, CD4, CD8, and a HY-specific tetramer and analyzed on LSR-II flow cytometer. CD45⁺ CD14⁻ CD19⁻ CD56⁻ CD4⁻ CD8⁺ cells were selected and HY tetramer is depicted within the CD8⁺ T cell population.

Fig. 3.

Placental viral infection changes T cell dynamics at the fetal–maternal interface.

Under normal placental condition (left):

- a) Low T cell influx to decidual tissue due to the limited expression of chemokines such as CXCL9 and CCL5 by decidual stromal cells.³⁰
- b) Inhibitory molecules (e.g., HLA-G, IDO, B7-H3) expressed by extravillous trophoblast (EVT) cell or other cells at the fetal–maternal interface may inhibit or reduce CTL mediated cytotoxicity.^{3,80,81}
- c) The presence of anti-inflammatory cytokines like TGF- increase the T cell activation threshold.⁸³
- d) High percentages of CD4+CD25^{bright}FOXP3+ Treg cells at the fetal–maternal interface can inhibit allogeneic lymphocyte reactions.^{7,8}
- e) The presence of anti-inflammatory APCs in decidua and uterine draining lymph nodes may activate T cells to become immune regulatory cells.⁴⁸

During placental infection like a viral infection (right):

- f) Increased levels of pro-inflammatory cytokines and chemokines increase the influx of T cells to decidual tissue.^{30,67}
- g) CD4+CD25^{bright}FOXP3+ Treg cells may not be able to efficiently inhibit allogeneic lymphocyte reactions under pro-inflammatory conditions. Furthermore, CD4+CD25^{bright}FOXP3+ Treg cells may prevent an optimal virus clearance in placenta tissue.
- h) Increased levels of pro-inflammatory cytokines can reduce the T cell activation threshold and induce pro-inflammatory responses.
- i) Inhibitory molecules (e.g., HLA-G, IDO, B7-H3) expressed by EVT cells or other cells at the fetal–maternal interface may not be able to efficiently inhibit allogeneic lymphocyte

reactions under pro-inflammatory conditions. Conversely, the presence of these inhibitory molecules may prevent an optimal virus clearance from placenta tissue.

j) Under inflammatory conditions, APCs in decidua and/or uterine draining lymph nodes may strengthen activation of cytotoxic T cells.

Table I

CD8+ T cell subsets in peripheral blood and decidual tissue

CD8 subset	Function	CDD45RA	CD45RO	CCR7	CD62L	CD28	CD27	Periphery ^a	Decidua ^a	Differences between peripheral and decidual CD8+ T cells
N	Unprimed cell, IL-2 secretion	+	-	+	+	+	+	47%	3%	Significantly reduced percentage of naive cells in decidua
E	Cytotoxicity, IFN- γ , TNF secretion performin+, granzyme B+	+	-	-	-	-	-	16%	13%	Reduced granzyme B and performin in decidual effector T cells
CM	Circulating memory cells, Lymph node homing potential	-	+	+	+	+	+	4%	2%	
EM	Tissue resident memory cells	-	+	-	-	-	-	23%	65%	Percentage of EM T cells of all subtypes are significantly increased in decidua
EM-1	Related to CM cells, granzyme K+	-	+	-	-	+	+	14%	19%	
EM-2	Partial effector functions, Performin ^{low} , granzyme B ^{low}	-	+	-	-	-	+	2%	25%	Reduced performin expression in decidual EM-2 cells
EM-3	Effector-like functions, Performin+, granzyme B+	-	+	-	-	-	-	2%	15%	Reduced granzyme B and performin in decidual EM-3 cells
EM-4	Related to CM cells, granzyme K+	-	+	-	-	+	-	4%	7%	

N, naive; E, effector; CM, central memory; EM, effector memory.

^aMedian percentage as found by Tilburgs *et al.*, J. Immunol. 2010 in term pregnancy decidual CD8+ T cells.