

Review

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Pregnancy and gamma/delta T cells: Taking on the hard questions

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Introduction

Considering the allograft rejection as one of the basic features of the immune system, the mammalian pregnancy is still a puzzling situation where the semiallogeneic embryo, a mating product of non-histocompatible individuals is not rejected. How are the demands of pregnancy solved in the context of the maternal immunity? How is the competent maternal immune system modulated during pregnancy? These are hard questions to answer and an intriguing challenge for immunologists to explain. Historically, the mammalian fetus has been regarded as a successful allograft, a tumor or a parasite [1,2]. Although the mechanisms that promote the survival of the conceptus are at large still unknown, it has become increasingly clear that the maternal immune tolerance towards the fetus is the result of the interactions of a jigsaw puzzle of actors – cells, serum proteins, hormones, cytokines, enzymes and neurotransmitters.

The fetus is never in direct contact with uterine/maternal tissues. Instead, the contact is mediated through the placenta, a transient organ expressing preferentially paternal genes. Placental trophoblast cells come in close contact with the maternal tissues forming the so-called feto-maternal interface. There is no doubt that the maternal immune system is able to recognize and react to fetally derived antigens. However, the fetus is recognized in such a way that the major histocompatibility complex (MHC) – specific, acquired arm of the maternal immunity is suppressed [3,4]. Instead, the maternal innate, first-line defense immune mechanisms are used and promoted during gestation [5,6]. The $\gamma\delta$ T cells are an important component of the innate immune system recognizing allo- and/or self-antigens upon cell infection, stress or transformation. Both an effector and a regulatory role for $\gamma\delta$ T cells

in vivo are well documented. Their overall function is to maintain homeostasis in the tissues where they reside [7,8]. The constitutive presence of $\gamma\delta$ T lymphocytes at the feto-maternal interface [9-11] implies a possible role in the adaptation of the maternal immune system to the requirements of pregnancy.

The leukocyte population at the feto-maternal interface – decidual associated lymphoid tissue (DALT)

The decidual associated lymphoid tissue in human early pregnancy, DALT, is shown in Fig. 1 after staining with the leukocyte common antigen CD (cluster of differentiation) 45. As can be seen the immune cells are abundant in decidua. Approximately 10–15 % of all decidual cells belong to the lymphoid cell lineages [11]. DALT comprises mainly of CD56⁺/CD16⁻ natural killer (NK)-like cells, T cells bearing T-cell receptor (TCR) $\alpha\beta$ or TCR $\gamma\delta$, dendritic cells and macrophages [11]. B cells are scarce or absent. The human DALT consist of lymphoid cell clusters (LCCs) of closely packed activated cells, subepithelially positioned cells in close contact with the basolateral portion of the glandular epithelium and individual cells randomly dispersed between the stromal cells [11]. In contrast to the gut mucosa, there are no truly intraepithelial lymphocytes in decidua; i.e. immune cells located above the basal membrane and between the glandular epithelial cells. However, numerous decidual lymphocytes, both CD56⁺ and T cells are localized in close vicinity of the basal membrane of the glandular epithelium.

The largest of the leukocyte populations in decidua are the bone marrow derived CD56^{bright}/CD16⁻ NK-like large granular lymphocytes (LGLs). These cells populate the

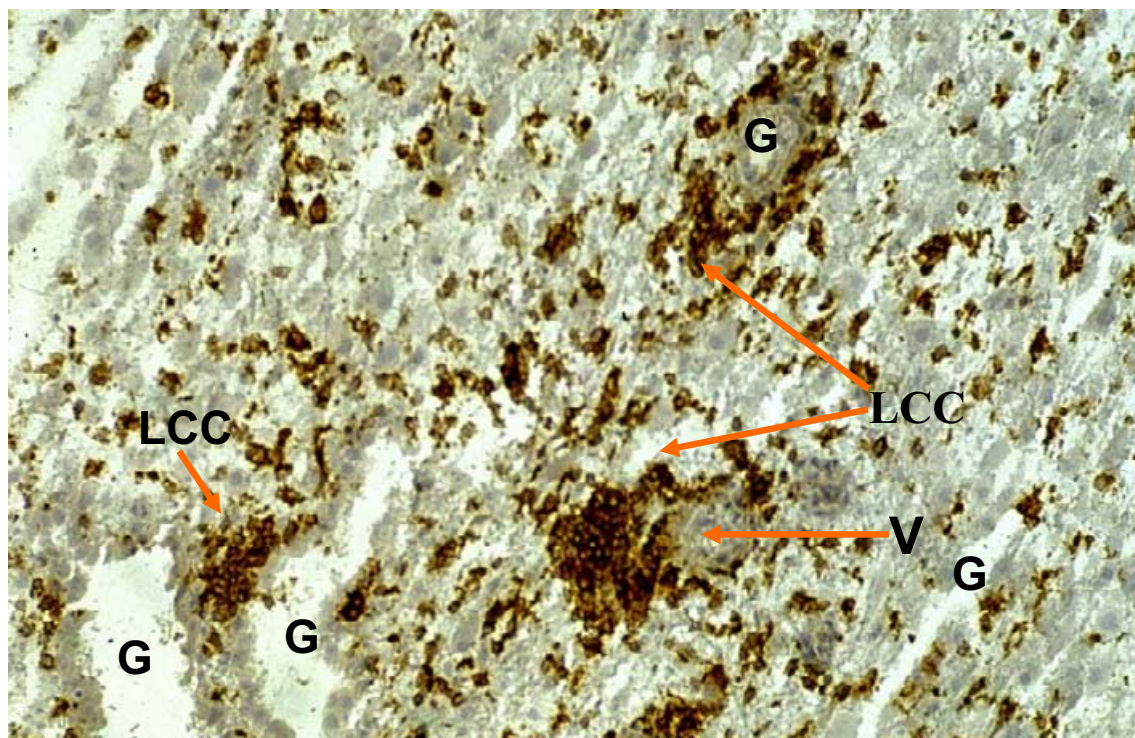


Figure 1

Immunohistochemical staining of immune cells in human early pregnancy decidua with monoclonal antibody against the leukocyte common antigen CD45 showing the histologic organization of DALT. LCC = lymphoid cell cluster, G = endometrial / decidual gland, V = vessel. Magnification $\times 32$.

uterine mucosa prior to implantation suggesting that the fetus does not play a direct role in their homing to the endometrium. Instead, circumstantial evidence implicates ovarian steroids and uterine decidualization as the main factors for the homing process [12]. Their phenotype is CD16⁻ CD56^{bright} CD57⁻ CD2⁺ CD3⁻ CD7⁺ CD4⁻ CD8⁻, *c-kit*⁺, CD94⁺, resembling that of the circulating CD56^{bright}/CD16⁻ NK cells [in [13]]. The murine counterpart of the CD56^{bright} cells does not express the CD56 molecule and its phenotype is Thy 1.1⁺, asialo-GM1⁺, interleukin (IL)-15R⁺ [14]. The CD56^{bright}/CD16⁻ decidual NK cells produce a variety of cytokines, including granulocyte-macrophage-colony stimulating factor (GM-CSF), transforming growth factor (TGF) β 1, interferon (IFN) γ , tumor necrosis factor (TNF) α , IL-2 and leukemia inhibitory factor (LIF) [15].

Although extensively studied the role of the CD56^{bright}/CD16⁻ cells in human pregnancy is not yet established. In mice, a role for the decidual NK-like cells in the modification of the uterine blood vessels in the process of placenta formation has recently been suggested [16]. In humans, an intriguing observation is that the CD56^{bright}/CD16⁻ cells, numerous at early pregnancy, drastically drop at the second and third trimester and are practically absent at term. Moreover, they express *c-kit* and the recombinase activating genes (RAG) 1 and RAG2, suggesting TCR rearrangement processes or/and a progenitor nature of these cells [13,17,18].

General characteristics of the $\gamma\delta$ T cells

Two lineages of T lymphocytes can be defined by their expression of TCR-TCR $\alpha\beta$ - and TCR $\gamma\delta$ cells. The TCR $\alpha\beta$ cells comprise the majority of T lymphocytes in the blood

Table 1: Comparison between TCR $\gamma\delta$ and TCR $\alpha\beta$ lymphocytes

Characteristics	Comparison between the $\gamma\delta$ T and $\alpha\beta$ T lymphocyte lineage		
	<i>Resident</i>	$\gamma\delta$ T lymphocytes <i>Circulating</i>	$\alpha\beta$ T lymphocytes
Immunity affiliation	innate	innate/adaptive(?)	adaptive
Ontogeny	develop earlier	develop earlier	develop later
Development	thymus/ extra-thymus	thymus	thymus/extra-thymus
Ag-receptor configuration	CD3+V δ 1	CD3+V δ 2	CD3+TCR $\alpha\beta$
Phenotype	different from $\alpha\beta$ T cells CD4-/CD8 ⁻ (most) CD8 ⁺ or CD8 $\alpha\alpha$ (some)	similar to $\alpha\beta$ T cells CD4-/CD8 ⁻ (most)	CD4 ⁺ or CD8 ⁺
MHC restriction	no	no	yes
Antigen recognition	self-antigens	non-peptide antigens from bacteria and plants	peptide+MHC
Frequency in blood	very few	1–10%	65–75%
Tissue distribution	mucosae, epithelia, lymphoid tissues	blood lymphoid tissues (?)	blood, lymphoid tissues, mucosae
Effector functions	cytotoxic potency cytokine release	cytotoxic potency cytokine release	cytotoxic potency cytokine release (Th1/Th2)
Biological functions	immunoregulation immunosurveillance pathogen eradication wound repair (mice)	pathogen eradication	pathogen eradication immune protection

and lymphoid tissues. About 90 to 95% of circulating T cells use the TCR $\alpha\beta$ while 5–10% use the alternate heterodimeric TCR composed of γ and δ chains. A portion of the $\gamma\delta$ T cells are generated in the thymus, but a major fraction appear to be generated in an extrathymic compartment [19]. Since their discovery during the 1980s, the $\gamma\delta$ T cells have been a focus for extensive research but still remain an enigma. In adult animals and humans $\gamma\delta$ T cells can be roughly divided into two groups – (i) circulating lymphocytes comprising 1–10% of the peripheral blood mononuclear cells and (ii) resident cells of the mucosal surfaces of the digestive-, respiratory-, urogenital tracts and the murine skin. In some reports the $\gamma\delta$ T cells are as abundant as 50% of the T cells in the murine skin epithelia or gut mucosa [7,20]. The $\alpha\beta$ - and $\gamma\delta$ T cells, seem to be different in their immune biology and belong to separate branches of the immune system – the TCR $\gamma\delta$ cells act like innate immune cells while the TCR $\alpha\beta$ cells play a central role in the adaptive immune system [21]. The major properties of the TCR $\alpha\beta$ and $\gamma\delta$ cells are shown in Table 1.

V δ usage – a landmark for circulating and resident $\gamma\delta$ T cells

One major difference between the circulating and resident $\gamma\delta$ T cells is that they are using different variable (V) δ chains – the resident $\gamma\delta$ T cells are V δ 1⁺ while the circulating counterpart is V δ 2⁺[20]. Recent studies of the phenotype of these two subsets in humans reveal that the V δ 2⁺ cells are similar in surface markers to the $\alpha\beta$ T cells while V δ 1 T cells have a phenotype more like mucosal lymphocytes and IELs [reviewed in [20]]. Resident $\gamma\delta$ T cells in epithelia are quite different in T-cell receptor repertoire and distribution from circulating $\gamma\delta$ T cells or $\alpha\beta$ T cells. These cells take up residence in the epithelial surfaces of the lung, intestine, uterus, vagina, tongue and murine skin [20]. Although $\gamma\delta$ T cells develop in a thymic dependent

manner, resident $\gamma\delta$ T cells can be thymus-independent and are detectable in athymic mice. Fetal liver and bone marrow progenitors can reconstitute the resident intraepithelial lymphocytes (IELs) in thymectomized recipients suggesting an alternative developmental pathway for the resident $\gamma\delta$ T cells [22]. Thus, these two $\gamma\delta$ T cell subsets may develop from distinct lineages.

Antigen recognition by $\gamma\delta$ T cells

What do $\gamma\delta$ T cells see? Unlike the $\alpha\beta$ T cells, the TCR $\gamma\delta$ cells are not MHC restricted. They seem to recognize antigens in a fundamentally different way than that of $\alpha\beta$ T cells, more similar to antibodies [7,21]. Furthermore, there are differences in the recognition pattern of the circulating and resident $\gamma\delta$ T cells. Human resident V δ 1⁺ T cells seem to be inherently self-reactive. Some of these cells recognize CD1c, a member of non-polymorphic cell-surface glycoproteins structurally and evolutionarily related to MHC class I molecules. It is not clear if the CD1c-reactive V δ 1⁺ cells respond to CD1c molecules alone or with a self-lipid molecule [23]. V δ 1⁺ cells have also been shown to recognize the newly defined MHC class I chain-related sequences A and B (MICA and MICB). These antigens are restricted to certain cell types of epithelial origin and are modulated by stress, inflammation, infection and cancer [7,20,24,25].

In contrast to resident $\gamma\delta$ T cells, the human circulating $\gamma\delta$ T cells have been shown to recognize non-peptide antigens derived from microbes and plants. The well-defined non-peptide antigens recognized by circulating $\gamma\delta$ T cells are prenyl pyrophosphates, bisolphonates, and alkylamines [20]. Thus, the recognition manner of $\gamma\delta$ T cells is dependent of the V δ usage.

Functional characteristics of the $\gamma\delta$ T cells

What do the $\gamma\delta$ T cells do? The function of the $\gamma\delta$ T cells should again be discussed in the context of their location in the blood or in the tissues. Various mucosae are the natural habitat of resident, $V\delta 1^+\gamma\delta$ T cells. It is however not known if these cells take up residence as naive or as antigen-experienced memory-type of cells. Several reports [20,26] have shown that resident $\gamma\delta$ T cells express cytotoxic molecules-perforin, granzymes and Fas ligand. Chemokines such as lymphotactin, MIP-1 α and MIP-1 β , the chemokine receptors CCR5 and CXCR3 and adhesion molecules are also expressed by $\gamma\delta$ T cells [20]. Taken together, these data indicates the "activated yet resting" state of the $\gamma\delta$ T cells. The ability of the resident $\gamma\delta$ T cells to rest but at the same time display molecules engaged in effector functions is consistent with the presumption that these cells function as first-line defense rather than as a component of the adaptive immunity.

The circulating $\gamma\delta$ T cells, on the other hand, can react rapidly with non-peptide antigens upon encountering infections and thereby activate the innate immune cells and subsequently facilitate adaptive immune responses of $\alpha\beta$ T cells. Several reports have shown that circulating $V\delta 2^+\gamma\delta$ T cells play a role in the elimination of infections with certain microbial pathogens such as intracellular bacteria like *Mycobacterium tuberculosis*, *Francisella tularensis*, *Legionella micdadei*, parasites like *Plasmodium falciparum* and *Schistosoma Mansonii* and the HIV virus [7,27,28]. Most studies have shown that the $\gamma\delta$ T cells play a role in bridging innate and adaptive immune responses. However, a fundamental question is whether circulating $\gamma\delta$ T cells have immunologic memory and can contribute to adaptive immune responses. In a non-human primate model of macaques infected with *Mycobacterium bovis* (BCG) strain was shown that circulating $V\delta 2^+$ cells which have undergone polyclonal expansion during a primary BCG vaccination can mount a memory/recall response following a secondary BCG infection [reviewed in [20]]. These studies provide evidence that $V\delta 2^+$ cells like $\alpha\beta$ T cells are able to contribute to adaptive immune responses.

Accumulating evidence has been indicative of yet another, not less important and sophisticated role for primarily the resident $\gamma\delta$ T cells, than infection protection: tumor-surveillance- and immunoregulatory functions [7,20,25,29]. Resident $\gamma\delta$ T cells may have a unique role in immune surveillance against malignancy. This immune function may have advantage over the $\alpha\beta$ T cells since resident $\gamma\delta$ T cells can directly recognize molecules expressed on cancer cells without antigen processing and presentation. The $\gamma\delta$ T cells have the ability to migrate as infiltrating lymphocytes in solid tumors [24,30] and have been shown to react on inducible MICA/B molecules, thus recognizing and eliminating damaged/malignant/stressed (epithelial) cells and

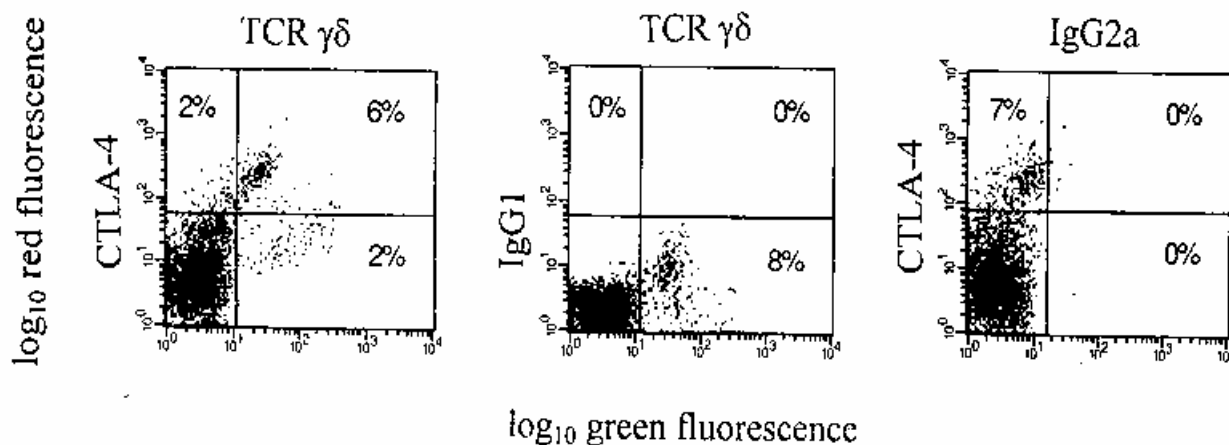
participating in the maintenance of homeostasis. Moreover, they can interact and modulate the activity of other immune cells directly or by cytokine production and thus function as regulatory cells. Although the exact mechanisms of these functions remain unclear, their ability to influence other immune cells provides them with the opportunity to modulate the course and outcome of a variety of immune and non-immune responses and to act in different ways depending on the particular microenvironment in which they are present.

 $\gamma\delta$ T cells in pregnancy

The immunological challenge of viviparity is to exert immunosuppression of specific responses towards the fetus without compromising the ability to fight infection. From this point of view, the $\gamma\delta$ T cells, which combine unique functions of infection protection and immunoregulation (Table 1), are of particular interest during pregnancy. Classical polymorphic MHC molecules are absent in the trophoblast cells and class II molecules cannot be induced even after stimulation with IFN γ [31], thus a direct allostimulation of the maternal $\alpha\beta$ T cells is avoided. In line with this finding it has been proposed that lack of polymorphic MHC molecule expression on the trophoblast is a way to "hide" pregnancy from the immune system. There is, however, abundant hard evidence refuting this hypothesis. The successful pregnancy is indeed recognized by the immune system in a way promoting immunotolerance. TCR $\alpha\beta$ -mediated recognition of fetal antigens, restricted to classical MHC molecules might provoke cytotoxic reaction toward the fetus and is unlikely to be promoted. The $\gamma\delta$ T cells, however, recognize a distinct group of ligands and antigens in a MHC-unrestricted manner and might play a key role in the immunological recognition of pregnancy.

Circulating $\gamma\delta$ T cells in human pregnancy

Human $\gamma\delta$ T cells in peripheral blood of women with normal pregnancy and recurrent abortions have been studied by Szekerez-Bartho et al. In healthy pregnant women, there was an accumulation of $V\delta 1^+$ circulating cells, in contrast to women with recurrent abortions where the $V\delta 2^+$ circulating cells dominated. The ratio of activated $\gamma\delta$ TCR $^+$ cells was significantly increased in normal pregnancies compared to that of recurrent abortions [32,33]. A bias towards circulating $V\delta 1^+\gamma\delta$ T cells seemed to be required for a successful normal pregnancy [32,33]. However, the precise role of circulating $\gamma\delta$ T cells in pregnancy is not yet completely established. Although convenient to study the $\gamma\delta$ T subsets during pregnancy in the peripheral blood, it cannot be excluded that the circulating $V\delta 1^+$ cells might simply be a spill over from the fetomaternal interface, where they are resident constitutive inhabitants.

**Figure 2**

Flow cytometric analysis of the regulatory cells-associated marker CTLA4 expression on freshly isolated, non-permeabilized human decidual $\gamma\delta$ T cells from early normal pregnancy.

$\gamma\delta$ T cells at the fetomaternal interface

Phenotype and morphology

The $\gamma\delta$ T cells are present in endometrium of all mammals throughout pregnancy [9]. It has been shown that these cells specifically colonize the non-pregnant murine and sheep endometrium and show a dramatic increase during pregnancy suggesting a special role at the fetomaternal interface [34]. The number of $\gamma\delta$ T cells in the uterus is higher in allogeneic than syngeneic pregnancy, and the expression of the TCR $\gamma\delta$ in the pregnant uterus is shown to be hormonally controlled [33]. The decidual $\gamma\delta$ T cells are large granular lymphocytes rich in intracytoplasmic granules and express neither CD4 nor CD8 (double negative) [10,11,13]. They are CD2⁺, express the activation marker CD69, the memory/activation marker CD45RO, the NK receptors CD94 [10,13] and NKG2D (manuscript in preparation), and CTLA 4 (fig. 2), a marker associated with regulatory T cells. The human $\gamma\delta$ T cells comprise a heterogeneous population: double positive TCR $\gamma\delta$ ⁺/CD56⁺ cells and TCR $\gamma\delta$ single positive cells [13]. The counterpart of these cells in the murine system seems to be the TCR $\gamma\delta$ ⁺/asialoGM⁺ cells and the single positive murine $\gamma\delta$ T cells as described by Arck et al [2]. The surface density of the TCR/CD3 is low in freshly isolated decidual $\gamma\delta$ T cells (10, 35) but can be up-regulated *in vitro* [10].

The vast majority of the human decidual $\gamma\delta$ T cells are V δ 1⁺ [13,36]. Itohara et al. [37] and Heyborne et al. [42] have shown that the V δ 1 chain is also preferentially used by $\gamma\delta$ T

cells in the uterus of normal and pregnant mice. Thus, the $\gamma\delta$ T cells in pregnant uterine mucosa, like other mucosa-associated $\gamma\delta$ T cells, are resident V δ 1⁺ cells.

Similar to resident $\gamma\delta$ T cells at other mucosal sites [20], the decidual $\gamma\delta$ T cells are activated but resting. We have shown that they possess a cytotoxic potency and express five major cytolytic molecules: perforin (Pf), granzyme A, granzyme B, granulysin and Fas ligand (FasL), and store them in microvesicles in intracytoplasmic cytolytic granules [26]. Like other cytotoxic lymphocytes [39] the decidual $\gamma\delta$ T cells do not express FasL on their surface but store preformed FasL in the granules, and can rapidly mobilize it to the cell surface upon stimulation. Thus, the two major cytotoxic mechanisms – Pf- and FasL-mediated – are performed by one common secretory pathway based on cytolytic granule exocytosis [26]. Cytotoxic mechanisms play a crucial role in the clearance of viral and bacterial infections, tumor surveillance, transplant rejection, homeostatic regulation of immune responses and peripheral tolerance [40]. Logically these mechanisms should have an important function at the fetomaternal interface by protecting the maternal-fetal unit against pathogens, controlling invasion of placental trophoblast, and creating a local transient immunotolerance toward the semiallogeneic conceptus through deletion of fetus-reactive lymphocyte clones. Indeed, recent studies of Pf- and FasL-deficient mice have shown that although functional deletion of Pf or FasL alone does not appear to affect fertility,

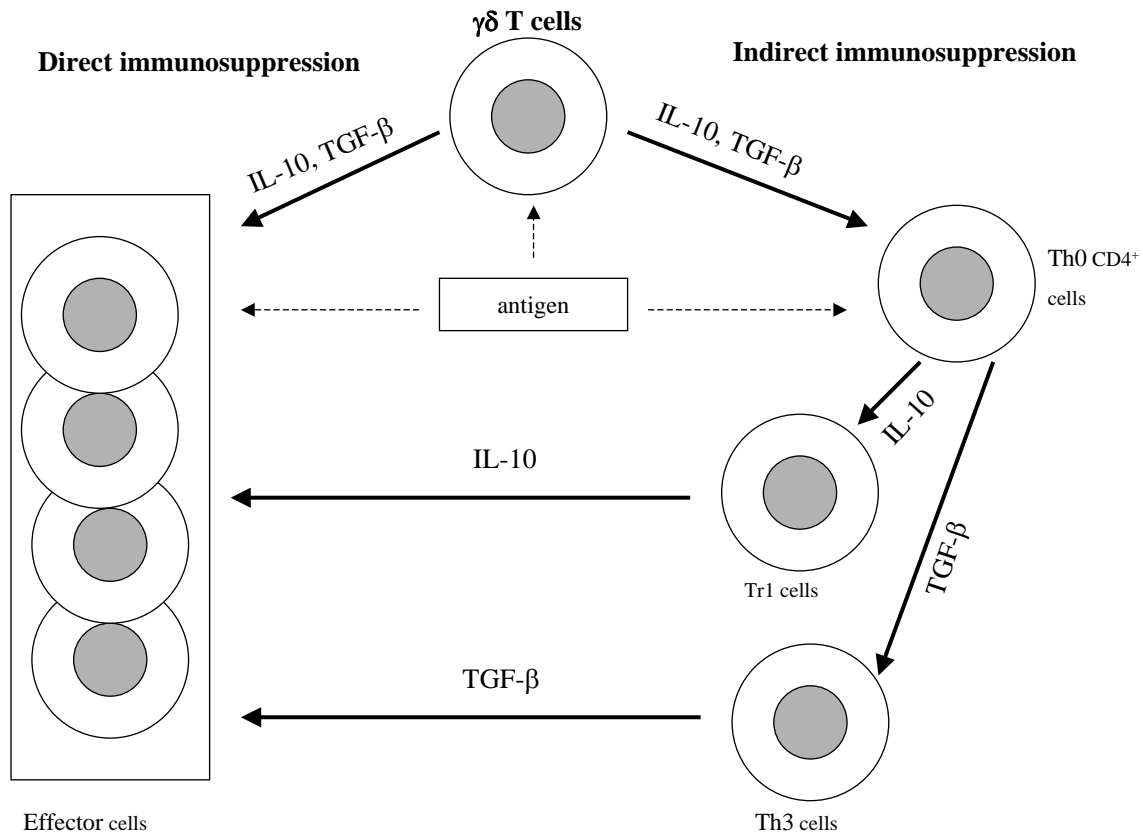


Figure 3
Schematic presentation of the regulatory role of decidual $\gamma\delta$ T cells in human early pregnancy (with permission from ref. 50).

the combined absence of these two effector molecules induces infertility [40].

Decidual $\gamma\delta$ T cells proliferate and differentiate in situ – decida as an extrathymic maturation site

Interestingly, we were able to stain $\gamma\delta$ T cells in mitosis [13] proving that the $\gamma\delta$ T cells divide in human decidua. As a rule, the plasma membrane of the mitotic cells was strongly stained with the reaction product indicating a high level of $\gamma\delta$ T cell receptor expression [13]. Our finding of $\gamma\delta$ T cells dividing *in situ* is in line with previous suggestion that $\gamma\delta$ T cells might expand in epithelial sites exposed to external environmental antigens, and, in some cases, recognize self-antigens, specific to a particular local environment [7,20,27]. By analogy, decidual $V\delta 1^+$ T cells may recognize trophoblast-related antigens and be involved in controlling trophoblast invasion during placenta formation [41].

In previous reports, Hayakawa et al. [17] in the human system and Kimura et al. [18] in the murine system have shown expression of mRNA for RAG-1 and RAG-2 proteins, which are required for TCR rearrangement, in human $CD56^{bright}/CD16^-$ cells and in murine decidual mononuclear cells respectively. We have confirmed and extended these results showing that transcripts of RAG can be easily detected in purified $CD56^+$, $CD2^+$, *c-kit*⁺ or $IL-7R^+$ decidual cells implying an ongoing process of TCR gene rearrangement [13]. There is no doubt that ongoing rearrangement of $TCR\gamma\delta$ takes place in decidua, probably for two purposes: 1) local extrathymic differentiation of $\gamma\delta$ T cells by TCR receptor rearrangements and 2) secondary $TCR\gamma\delta$ rearrangement, permitting editing of antigen receptors on mature cells, thus adjusting the decidual $\gamma\delta$ T-cell repertoire to the ongoing pregnancy. Re-induced RAG expression, involved in receptor editing is a phenomenon observed in immature T cells in thymus and seems to be

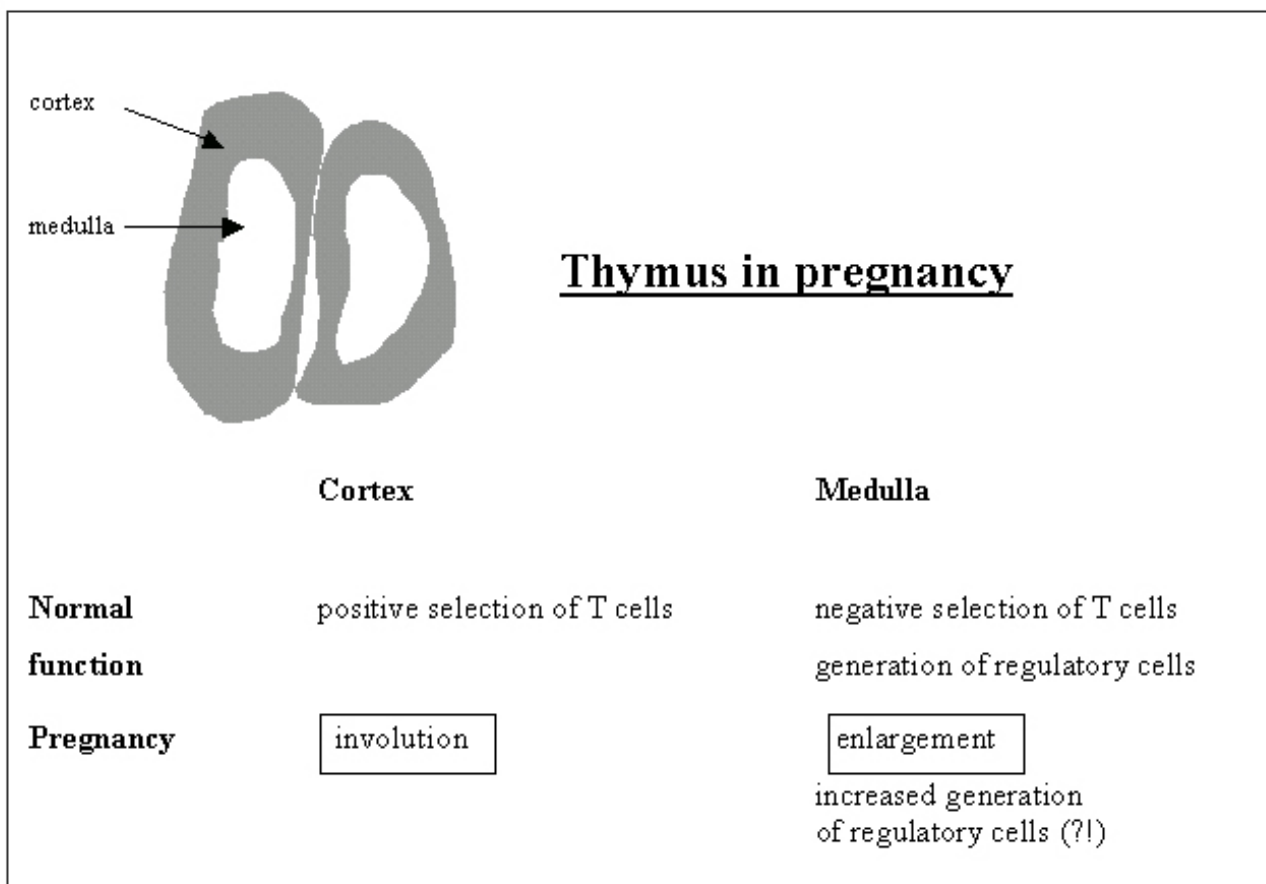


Figure 4
Correlation between the cortex and medulla of thymus and its main functions in normal situation and in pregnancy

required for the generation of normal T-cell repertoire [42]. Although not proven yet it is reasonable to assume that both local TCR $\gamma\delta$ receptor rearrangement and editing are equally used in decidua.

Is there a need for T-cell differentiation in decidua? What purpose and biological significance there might be for extrathymic T-cell differentiation during pregnancy?

We can argue for at least two different reasons for extrathymic maturation in pregnancy. The first reason is priming the maternal immune system to the fetus. The meeting between the mother and the fetus is dual: 1) between the maternal blood and syncytiotrophoblast cells of the chorion villi of the placenta and 2) between the extravillous trophoblast and the maternal epithelial, stromal, endothelial and immune cells in decidua when placenta is formed. It is reasonable to assume that the first encounter

and antigen presentation of fetal antigens to the immune system takes place in decidua. Decidua/endometrium might enrich CD56⁺ progenitor cells of bone marrow origin which will further differentiate/rearrange locally (or naive thymus-derived T cells will edit their TCR) upon the encounter of fetal antigens. The extrathymic maturation in decidua might be one of the mechanisms adjusting the immune system and the T-cell repertoire towards acceptance of the ongoing pregnancy. Heyborne et al have shown that murine decidual $\gamma\delta$ T cells recognize trophoblast-derived antigens. Immune cells, locally primed in decidua might then repopulate the peripheral blood of the pregnant woman as suggested by published reports [reviewed in [32]].

The second reason for extrathymic maturation in decidua might be the temporary thymic involution taking place during pregnancy. A great loss in thymic weight during

Table 2: Summary of some characteristics of the T regulatory cells

T regulatory (TREG) cells	
1. Definition	Cells with regulatory function that produce IL-10 and TGF- β and play a critical role in the control the immune response and the generation and maintenance of tolerance.
2. Properties	<ul style="list-style-type: none"> • <u>heterogeneous group</u> of lymphocytes • exist in <u>very low numbers</u> • <u>respond poorly</u> to stimulation through TCR • <u>unique and diverse mechanisms</u> of action • <u>none</u> common specific phenotypic marker
3. Some subtypes by phenotype	<ul style="list-style-type: none"> • CD4⁺CD25⁺ • CD4⁺CD45RB⁺ • CD8⁺ • TCR$\gamma\delta$⁺ • NT/NKT cells – e.g. Vα24-JαQ • CTLA4⁺
4. Subtypes by cytokine profile	<ul style="list-style-type: none"> • <u>Th3 cells</u>: differentiate from naive CD4⁺ or CD8⁺ cells under the influence of TGF-β, produce TGF-β > IL-10, varying IL-4 • <u>Tr1 cells</u>: differentiate from naive CD4⁺ or CD8⁺ cells under the influence of IL-10, produce IL-10 > TGF-β, no IL-4

pregnancy occurs due to increased cell death of small lymphocytes from the cortex. It appears that primarily CD4⁺/CD8⁺ cortical thymocytes are lost whereas most other subsets are retained; B-lymphopoiesis is depressed [43]. The cortical involution is at its greatest by the end of pregnancy and is maintained until lactation ceases. The implications of the observed thymic changes can be anticipated if they are correlated to the known thymic function [[44], Fig. 4]. It is reasonable to assume that such radical rearrangements are likely to have effects on the maternal immune system and to influence the mother's ability to protect the fetus from harmful maternal responses to paternally inherited fetal antigens. The involution of cortex might mean deleting clones or unresponsiveness to paternally derived antigens [45]. It is tempting to interpret the enlargement of the medulla as a potential increase of regulatory T cells needed to modulate the immune responses. Is there a role for decidua in the context of the thymic involution during pregnancy? The decidua as an extrathymic maturation site can be complementary to the thymic changes in at least two ways: 1) The need for positive selection ablated by cortex involution might be compensated for by extrathymic differentiation of T cells which will be primed on pregnancy-derived antigens in the decidual microenvironment and will allow to eliminate/silence fetus-reactive T cell clones. 2) Naive T cells generated in the medulla (e.g. regulatory cells) might be re-edited in the decidua thus adjusting their T-cell receptor repertoire to the ongoing pregnancy. The $\gamma\delta$ T cells, differentiated locally in decidua [13,17,18], will thus be specifically primed on the ongoing pregnancy.

The cytokine profile of the decidual $\gamma\delta$ T cells suggests regulatory functions

Cytokines at the fetomaternal interface play a pivotal role for the establishment and maintenance of normal pregnancy. Several well-performed studies in humans and mice have shown beyond doubt that there is a T-helper (Th) 2 bias in the cytokine response [reviewed in [46]]. But the role of cytokines in pregnancy cannot solely be explained by the Th2/Th1 paradigm. Although very attractive, there is a serious risk of oversimplifying this concept. First, a critical feature of the Th1/Th2 model is that the two cell types counter regulate one another via cytokine production. But the polarization of the Th1 versus Th 2 effector cells is rarely complete and simultaneous Th1 and Th2 responses are possible. Second, this concept is derived from results of *in vitro* experiments and experimental models with immunologically inactive, inbred laboratory mice. In reality, when faced with established responses, the Th1 effectors have little ability to down-regulate Th2 responses [47]. Similarly, Th2 effector cells, carefully separated from the Th2-like regulatory cells, have been shown to aggravate, rather than inhibit Th1-mediated inflammatory responses [47]. There is a compelling body of evidence that the T-cell function at the fetomaternal interface in successful pregnancy is modulated by a cytokine environment of IL-10 and TGF- β , cytokines that are not always viewed as Th2-type only [48]. Abandoning the Th1/Th2 bias one can ask the question if other, non-Th1/Th2 cells and responses operate at the fetomaternal interface. Careful studies of decidual $\gamma\delta$ T cells in the murine system have shown that, TCR $\gamma\delta$ ⁺/asialoGM1⁺ cells and TCR $\gamma\delta$ single positive cells [2] play a decisive role in pregnancy outcome depending on their cytokine response. At early preimplantation stage, murine $\gamma\delta$ T cells

produce TNF- α , IFN- γ and probably IL-2 and promote abortions by activation of decidual NK cells and macrophages. At a later stage, during the time of implantation and placenta formation, the $\gamma\delta$ T cells in murine decidua produce TGF- β and IL-10 and exert anti-abortogenic effect [2,49]. Using quantitative RT-PCR we have analyzed the cytokine profile of the two main subpopulations of $\gamma\delta$ T cells in human decidua: TCR $\gamma\delta^+$ /CD56 $^+$ and TCR $\gamma\delta$ single positive cells [10,13]. Our results [50] show that the TCR $\gamma\delta^+$ /CD56 $^+$ cells almost exclusively express mRNA for TGF- β 1 and IL-10 cells suggesting orientation towards an immunosuppressive profile [48]. Then as they further develop into primed TCR $\gamma\delta$ single positive cells their IL-10 and TGF- β 1 expression is strongly enhanced. Additionally, the TCR $\gamma\delta$ single positive cells transcribe two more cytokines-IL-6, suggesting an orientation toward the pregnancy-promoting Th 2 response and IL-1 β , a cytokine considered in general to have a function promoting maturation and clonal expansion of other lymphocyte subpopulations. In pregnancy in particular, IL-1 β is considered to be an important factor for the implantation of the blastocyst in the uterine cavity acting through up-regulation of adhesion molecule expression [51]. Our results [50] based on quantitative cytokine mRNA measurement in these two subpopulations of human decidual $\gamma\delta$ T cells indicates that these cells, by virtue of the strong dominance /exclusivity of IL-10/TGF- β mRNA expression can be ascribed to the newly "reborn" suppressor/regulatory T (Treg) cells. Furthermore, these cells express the regulatory T cell marker CTLA4 (Fig. 2). A brief summary of some of the characteristics of the Treg cells is given in Table 2.

Recent data provide convincing evidence that a specialized population of Treg cells both do exist and play a critical role in the generation and maintenance of immunological tolerance in humans [reviewed in [48,52]]. The Treg cells exert their suppressive activities on effector cells in remarkably low numbers [53]. Several subsets of Treg cells have been described in a variety of experimental models. In the context of this discussion it is interesting to note that two human CD4 $^+$ Treg-cell subsets exert their regulatory effect via secretion of the immunosuppressive cytokines IL-10 and TGF- β . One subset includes the so-called T helper 3 (Th3) cells, distinguished from other T helper cells by their ability to produce high levels of TGF- β and varying levels of IL-10 and IL-4 [52]. The other subset, termed T regulatory type 1 (Tr1) cells produces a significant level of IL-10, various levels of TGF- β and no IL-4 [54]. The decidual $\gamma\delta$ T cells produce high amount of IL-10 followed by TGF- β and no IL-4 [50] and thus belong to the Tr1 regulatory type of cells. The selective generation of different Treg cell subsets is determined by the cytokine microenvironment in which naive CD4 $^+$ T cells encounter antigen. This means

that if priming of naive Th0 cells occurs in the presence of IL-10 or TGF- β they differentiate to Tr1 or Th3 cells respectively and become polarized to synthesis of IL-10 or TGF- β [reviewed in [55]]. The cell type(s) responsible for the creation of such unique cytokine microenvironments *in vivo* is a subject of discussions. $\gamma\delta$ T cells are excellent candidates for this role, because these cells can respond to broadly distributed self-antigens in stressed, damaged and transformed tissues and do not require classical antigen processing and MHC-restricted presentation [27]. $\gamma\delta$ T cells have been implicated in the down regulation of immune responses in various inflammatory disorders and may acquire immunoregulatory properties at mucosal sites [reviewed in [56]]. A population of $\gamma\delta$ T cells producing the Tr1/Th3 - type cytokines IL-10 and TGF- β has been isolated from tumor-infiltrating lymphocytes. These $\gamma\delta$ T cells could play a role in the inhibition of immune responses to tumors [56]. It was shown that aerosol or nasal inoculation of intact insulin resulted in expansion of $\gamma\delta$ T cells with an immunosuppressive anti-diabetogenic effect, mediated by IL-10 [56]. Remarkably, only a small fraction of $\gamma\delta$ T cells was enough to prevent adaptive transfer of diabetes [56]. Furthermore, $\gamma\delta$ T cells producing IL-10/TGF- β were reported to be a critical cell population for the induction of allograft- and testicular tolerance [57,58].

Summing up the accounted data above an attractive hypothesis is that $\gamma\delta$ T cells act as cytokine-producing cells to create a decidual environment that actively tolerates the fetus [50]. We suggest that pregnancy-related antigen(s) can activate decidual $\gamma\delta$ T cells causing them to release the immunosuppressive Tr1- and Th3-type cytokines IL-10 and TGF- β . Figure 3 illustrates two possible mechanisms by which these cells could induce local uterine tolerance towards the fetus. In the direct pathway the effector cells (cytotoxic T lymphocytes, NK cells, macrophages, dendritic and B cells) at the fetomaternal interface could be directly inhibited by IL-10 and TGF- β [50]. In this pathway $\gamma\delta$ T cells function as Treg cells [56]. In the indirect pathway $\gamma\delta$ T cells could mediate the tolerogenic effect through generation of primed Th0, mainly TCR $\alpha\beta^+$ CD4 $^+$ (and probably CD8 $^+$) cells. Under the influence of IL-10 and TGF- β , these cells differentiate into IL-10 producing Tr1-type of cells and TGF- β producing Th3 type of cells which in their turn act suppressively on the effector cells. In this pathway the $\gamma\delta$ T cells are needed for generation of efferent suppressor cells, but are not suppressors themselves [56]. These two pathways might function in parallel and exert immunosuppression in concert with each other. It cannot be excluded that other types of decidual cells such as dendritic cells could also participate in the immunoregulation [59]. The model presented [[50], Fig. 3] is simplified but comprises one important mechanism for immunomodulation at the fetomaternal interface.

Conclusions

An evolutionarily important process such as the mammalian pregnancy is a paradox and a challenge for the immune system and must rely on several mechanisms acting in concert to modulate the maternal immunity. However, enough convincing evidence shows that the immune system *per se* is not necessary for reproduction. Mammals have to reproduce despite their immune system.

The dual mission of the immune system during pregnancy is to down-regulate the specific, adaptive immune responses without compromising the ability to fight infections and protect against tumor transformation. In this process the innate immunity is activated and used to compensate for the impairment of the adaptive immunity, and to fulfill the requirements of a competent maternal immune defense during pregnancy. From immunological point of view pregnancy is an innate immunity event and one of its components, the $\gamma\delta$ T cells with their unique properties among the lymphoid cells of the immune system, are particularly suited to effectuate in parallel specific effector mechanisms combined with immunoregulatory functions. The $\gamma\delta$ T cells in pregnancy are resident V δ 1⁺ lymphocytes that are permanent inhabitants of the decidual mucosa. They comprise about half of the decidual T-cell population, differentiate locally in uterus and thus prime on the ongoing pregnancy. Activated but silent, they have the potency to protect the fetomaternal unit against stressed, infected and/or transformed cells. Moreover, they express high amounts of mRNA for the immunoregulatory cytokines IL-10 and TGF- β in a pattern characteristic of Tr1 regulatory lymphocytes. This property delineates their other major role in reproduction – to function as direct or indirect immunosuppressors thus modulating the maternal immune system towards tolerance of the fetus.

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