

# The sterile and tolerogenic fetal niche does not restrict the generation of CD4 T memory cells

Alexandre Morrot

Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21.941-590, Brazil

Correspondence to: Dr. Alexandre Morrot. Instituto de Microbiologia, Universidade Federal do Rio de Janeiro (UFRJ), CCS - Sala D1-035, Av. Carlos Chagas Filho, 373 - Cidade Universitária, CEP 21.941-902, Ilha do Fundão, Rio de Janeiro, RJ, Brazil. Email: morrot@micro.ufrj.br.

**Abstract:** T-cell activation requires a sequence of signals derived from co-stimulatory receptors and from immunogens that may or may not be of infectious origin. This scenario provides the threshold of inflammatory stimulus needed for the induction of antigen-specific T cell responses. One of the dogmas of immunology stipulates that the activation of T lymphocytes is prevented in immunosuppressed or tolerogenic environments. However, it was shown recently that a healthy uterine environment that is considered sterile, therefore not exposed to infection, is capable of generating T memory cells with the capacity to differentiate lineage-specific inflammatory effector T-cell responses. The implications of this finding are discussed in this editorial.

**Keywords:** Memory T cells; immunological tolerance; pregnancy; placenta

Submitted Jan 24, 2015. Accepted for publication Jan 29, 2015.

doi: 10.3978/j.issn.2305-5839.2015.02.02

View this article at: <http://dx.doi.org/10.3978/j.issn.2305-5839.2015.02.02>

Eutherian mammals are placental tetrapods that survived and diversified during one of the greatest global extinction events of all time, at or very close to the boundary between the Cretaceous and Paleogene periods, about 65 million years ago (1-3). As a consequence nearly all large vertebrates became extinct in what was clearly a geological, climatic and biological event with worldwide consequences (4). The evolution of the placenta is one of the most important vertebrate adaptations; it may have guaranteed the reproductive fitness in a geological period in which the environment was constantly changed by extinctions and the formation of new ecosystems. In contrast to other species, the placental mammals possessed a successful reproductive strategy in the fetal development, which may have enabled them to survive during long-distance migrations in search of new environments without a need to establish a local resident niche. The lack of this adaptation may have reduced the chances of other life-forms evading the catastrophic scene during the mass extinctions. The evolution of the placenta together with endothermy enabled the mammals to explore alternative productive environments during their evolutionary diversification, one of the most successful in the history of tetrapods (5).

The placenta is an embryonic attachment through which exchanges occur between mother and child. It is formed by the egg tissues, embryologically derived from the corium (6). Through the placenta the fetus exchanges oxygen and carbon dioxide, obtains nutrients directly by diffusion from the maternal blood, and excretes products of its metabolism such as nitrogen. The human placenta is composed of extravillous trophoblast cells that penetrate deeply into the uterine mucosa in the spongy layer of the decidua, the name given to the endometrium during pregnancy (6). Although the placental organ provides a physical barrier that shields the fetus from the maternal immune system, there is direct contact between extravillous trophoblast cells, maternal immune cells and decidual stroma cells. In this situation, the maternal organism carries a semi-allogeneic fetus, which is nevertheless tolerated (7,8).

The mechanisms responsible for this tolerance are poorly understood. There is evidence that the fetal-maternal relation is a symbiosis rather than representing temporary immune-suppressed state (9). The maternal immune system must harbor alloreactive lymphocytes that are not clonally deleted by central tolerance mechanisms since the thymic negative selection only depletes auto-reactive T cells.

Therefore, in order to avoid rejection of the fetal allograft the maternal immune cells at the maternal-fetus interface must be tolerant to foetal antigens while protecting the fetus from pathogen infections that potentially threaten the pregnancy (8-10).

Different mechanisms have been proposed to account for the lack of immune response at the maternal-placental interface. One important property of the interfacial region is the absence of the classical cell surface molecules encoded by the large gene family constituting the major histocompatibility complex (MHC) (11). These surface receptors bind to peptide fragments derived from pathogens or antigenic proteins and display them on the cell surface to permit recognition of T cells bearing complementary antigen receptors (12,13). These MHC molecules determine the compatibility between donor and recipient in organ transplantation, and are crucial for inducing alloreactive immune responses (14). In humans, the MHC is also referred to as the human leukocyte antigen (HLA). Unlike nucleated cells in other tissues, trophoblast cells express the nonclassical Class I HLA-G protein, which is found in soluble and membrane-bound form (15). The restricted tissue distribution of this nonclassical HLA protein is associated with fetal tolerance. Since the absence of expression of MHC Class 1 molecules would render the trophoblast sensitive to cell-mediated killing by natural killer cells, HLA-G molecules are able to interact with and inhibit the activation of the killer-cell immunoglobulin-like receptors (KIRs), a family of cell surface proteins found on NK cells, thus allowing the trophoblast cells to evade NK cell-mediated lysis (15).

Other mechanisms are thought to contribute to the immunosuppressive environment of the placenta during pregnancy. These mechanisms involve an altered Th1/Th2 profile resulting in the predominance of Th2 cytokines (16), which may restrict the activation of alloreactive Th1 cells that normally carry out tissue surveillance and are responsible for many miscarriages (10). Fetal protection from rejection by alloreactive T cells has also been attributed to a tolerogenic state of the dendritic cells (DC) in the placenta. The DCs found in the placenta have a reduced ability to present antigens as they produce lower levels of co-stimulatory molecules and IL-12—the key cytokine driving Th1 responses—while at the same time producing higher levels of the immunosuppressive cytokine, IL-10 (17,18). Tolerance of the fetus is also mediated by CD4<sup>+</sup> regulatory T (Treg) cells (10), which increase in frequency during pregnancy in the placentas of both mice

and humans (19). Recent studies have indicated that Treg cells specific for fetal antigens expand more than 100-fold during pregnancy (19). Together, these mechanisms restrict the local priming and blast expansion of fetal antigen-specific T cells, thus ensuring a normal pregnancy. This local restriction is also maintained by placental indoleamine 2,3-dioxygenase-mediated degradation of tryptophan, since this amino-acid is responsible for stimulating the proliferation of cells of the maternal immune system (8).

Although fetomaternal tolerance clearly plays a critical role in guaranteeing a healthy pregnancy, recent studies have shown that memory T cells are in fact generated in the fetal T cell compartment (20). In these studies, led by Richard Lo-Man, neonatal cells purified from the umbilical cord blood of placentas obtained from healthy donors were shown to possess an effector memory phenotype (TEM), expressing low levels of CD25 and high levels of CD127 (CD25<sup>lo</sup>CD127<sup>hi</sup>). As has been demonstrated in other systems, these activated and memory T lymphocytes express the short form of the protein tyrosine phosphatase CD45—the CD45RO variant (20). This isoform facilitates T cell activation and is present on conventional adult memory T cells. The fetal CD4<sup>+</sup> TEM were identified as CD45RO<sup>+</sup> cells; however they were distinct from regulatory T cells as they did not express Foxp3. They also displayed a polyclonal TCR $\alpha\beta$  repertoire with a broad distribution of lengths of the CDR3 variable regions of recombinant TCR genes. Importantly, the TEM cells analyzed in these studies mainly originated from the fetus, as demonstrated by X and Y chromosome FISH (20).

Furthermore, examination of the cytokine profile after T cell receptor activation revealed that the neonatal TEM cells produced tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and granulocyte-macrophage colony-stimulating factor (GM-CSF). Analysis of the T helper (TH) cytokine secretion profiles of these activated neonatal T cells identified interferon- $\gamma$  (IFN- $\gamma$ )-producing TH1 cells and interleukin-4 (IL-4)/IL-13-producing TH2-like cells (20). Moreover the molecular profiles of the neonatal TEM cells based on their patterns of transcription indicated that they consist of distinct clusters of T cell subsets expressing CCR6, CXCR3, or CCR4. Microarray analysis identified both CXCR3<sup>+</sup> TEM and CCR6<sup>+</sup> TEM cells with TH1 and TH17 profiles, respectively. Although the CCR6<sup>+</sup> TEM displayed the genetic profile characteristic of TH17 cells they failed to produce IL-17A. Nonetheless, the neonatal cells formed TH17 cells in the presence of IL-1 $\beta$  and IL-23. A fourth population was characterized as CCR<sup>-</sup> TEM cells

with a phenotype transitional between naïve and effector memory cells, possibly with distinct developmental fates in terms of TH commitment (20).

The findings of the Lo-Man group indicate that the neonatal cells in placental cord blood have effector memory phenotypes with inflammatory properties. One possibility is that they represent a pool of natural memory cells that have been generated in the thymus. However since they lack the expression of CD31 and PTK7, which is characteristic of recent thymic emigrants, this seems unlikely (20). Although the neonates in these studies were not reported to display any pathology, or to have any pathogen infections, recent studies have demonstrated the presence of nonpathogenic commensal bacteria in the placenta (21). They have shown that this specialized tissue harbors a unique microbiome composed of nonpathogenic commensal microbiota of the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fosobacteria phyla (21).

An interesting possibility, therefore, is that the placental microbiota are the source of an endogenous stimulus for the activation/differentiation of neonatal T cells. This would have an impact on strategies designed to modulate the neonatal TEM compartment for purposes of immunization. Since these cells can differentiate into TH1 cells, it is plausible to think they might provide immunity against intracellular pathogens that threaten fetal development. This might be achieved by modulating the immune responses by commensal bacteria in the placenta, which may be crucial for generation and maintenance of the neonatal inflammatory effector memory T cells. Such therapeutic strategies might be safer than using vaccination approaches, which might reverse the tolerance of the maternal-placental interface, thus creating a potential risk for the fetus.

### Acknowledgements

*Funding:* This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

*Disclosure:* The author declares no conflict of interest.

### References

- O'Leary MA, Bloch JJ, Flynn JJ, et al. The placental mammal ancestor and the post-K-Pg radiation of placentals. *Science* 2013;339:662-7.
- Wible JR, Rougier GW, Novacek MJ, et al. Cretaceous eutherians and Laurasian origin for placental mammals near the K/T boundary. *Nature* 2007;447:1003-6.
- Luo ZX, Yuan CX, Meng QJ, et al. A Jurassic eutherian mammal and divergence of marsupials and placentals. *Nature* 2011;476:442-5.
- Renne PR, Deino AL, Hilgen FJ, et al. Time scales of critical events around the Cretaceous-Paleogene boundary. *Science* 2013;339:684-7.
- Luo ZX. Transformation and diversification in early mammal evolution. *Nature* 2007;450:1011-9.
- Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. *Philos Trans R Soc Lond B Biol Sci* 2015;370:20140066.
- Arck PC, Hecher K. Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. *Nat Med* 2013;19:548-56.
- Koch CA, Platt JL. T cell recognition and immunity in the fetus and mother. *Cell Immunol* 2007;248:12-7.
- Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004;5:266-71.
- Alijotas-Reig J, Llorba E, Gris JM. Potentiating maternal immune tolerance in pregnancy: a new challenging role for regulatory T cells. *Placenta* 2014;35:241-8.
- Redman CW, McMichael AJ, Stirrat GM, et al. Class 1 major histocompatibility complex antigens on human extra-villous trophoblast. *Immunology* 1984;52:457-68.
- Rammensee HG. Peptides made to order. *Immunity* 2006;25:693-5.
- Charron D. Immunogenetics today: HLA, MHC and much more. *Curr Opin Immunol* 2005;17:493-7.
- Nagy ZA. Alloreactivity: an old puzzle revisited. *Scand J Immunol* 2012;75:463-70.
- Dahl M, Djuricic S, Hviid TV. The many faces of human leukocyte antigen-G: relevance to the fate of pregnancy. *J Immunol Res* 2014;2014:591489.
- Lin H, Mosmann TR, Guilbert L, et al. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. *J Immunol* 1993;151:4562-73.
- Segerer SE, Rieger L, Kapp M, et al. MIC-1 (a multifunctional modulator of dendritic cell phenotype and function) is produced by decidual stromal cells and trophoblasts. *Hum Reprod* 2012;27:200-9.
- Blois SM, Ilarregui JM, Tometten M, et al. A pivotal role for galectin-1 in fetomaternal tolerance. *Nat Med* 2007;13:1450-7.
- Rowe JH, Ertelt JM, Xin L, et al. Pregnancy imprints

- regulatory memory that sustains anergy to fetal antigen. *Nature* 2012;490:102-6.
20. Zhang X, Mozeleski B, Lemoine S, et al. CD4 T cells with effector memory phenotype and function develop

- in the sterile environment of the fetus. *Sci Transl Med* 2014;6:238ra72.
21. Aagaard K, Ma J, Antony KM, et al. The placenta harbors a unique microbiome. *Sci Transl Med* 2014;6:237ra65.

**Cite this article as:** Morrot A. The sterile and tolerogenic fetal niche does not restrict the generation of CD4 T memory cells. *Ann Transl Med* 2015;3(5):64. doi: 10.3978/j.issn.2305-5839.2015.02.02