the control of NMT1 expression is necessary for the development of strategies with which to therapeutically target NMT1 in CD4+ T cells in patients with RA. ❐

David K. Finlay
School of Biochemistry and Immunology and School of Pharmacy and Pharmaceutical Sciences, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland.
e-mail: finlayd@tcd.ie

Published online: 4 February 2019
https://doi.org/10.1038/s41590-019-0322-4

References

1. Udenwobele, D. I. et al. *Front. Immunol.* **8**, 751 (2017).
2. Wen, Z. et al. *Nat. Immunol.* https://doi.org/10.1038/s41590-018-0296-7 (2019).
3. Rampoldi, F. et al. *J. Immunol.* **195**, 4228–4243 (2015).
4. Weyand, C. M., Zeisbrich, M. & Goronzy, J. J. *Curr. Opin. Immunol.* **46**, 112–120 (2017).
5. Oakhill, J. S. et al. *Proc. Natl Acad. Sci. USA* **107**, 19237–19241 (2010).
6. Zhang, C. S. et al. *Cell Metab.* **20**, 526–540 (2014).
7. Lin, S. C. & Hardie, D. G. *Cell Metab.* **27**, 299–313 (2018).
8. Bruyn, G. A. et al. *Ann. Rheum. Dis.* **67**, 1090–1095 (2008).
9. Deng, L. et al. *Cell Death Dis.* **9**, 1143 (2018).

Competing interests

The author declares no competing interests.

MEMORY T CELLS

Pre-birth memory

The transition of the fetus from the womb to the external world represents an extraordinary challenge. The generation of memory T cells before birth may serve an important role in preparation for this fundamental transition.

Ai Ing Lim, Oliver J. Harrison and Yasmine Belkaid

Careful understanding of the developing human immune system is of fundamental importance. Indeed, growing evidence demonstrates that prenatal infections or early alterations in the ability of the offspring to respond to exogenous challenges such as the microbiota can have long-term and, in some cases, catastrophic consequences. In this issue of *Nature Immunology*, Li et al. demonstrate that the developing fetal immune system is already seeding the human intestine with memory-like T cells before birth[1].

Much of the understanding of the human immune system stems from studies conducted on peripheral blood samples that are usually derived from adult donors. Many groups have begun to detail the phenotype and development of innate and adaptive lymphocytes that populate the human intestine in early life[2–4]. Published findings have demonstrated that by as early as 5 years of age, the human intestine has accumulated a substantial population of effector- and memory-like T cells[3]. Furthermore, studies have highlighted the accumulation of innate lymphoid cell populations within the developing fetal intestine, which suggests that even before birth, the immune system is readying its barrier tissues for the outside world, including exposure to both nutrients and the microbiota[2]. In the current study, Li et al. extend the understanding of pre-birth development of the immune system by investigating the T cell populations present within the fetal human intestine. Using an array of advanced technological approaches, they characterize the CD4+ T cell populations present in human fetal tissues during the second trimester of pregnancy and demonstrate that the developing human fetal intestine already contains cells that express phenotypes compatible with all three major populations of CD4+ T cells, including CD45RA+ naive T cells, CD45RO+ effector- and memory-like T cells and CD25+CD127− regulatory T cells (Fig. 1). While CD45RA+ cells are considered phenotypically naive, expression of CD45RO marks both effector T cell subsets and memory T cell subsets. Bone fide memory T cells are defined as antigen-experienced cells able to proliferate and execute effector functions rapidly following re-exposure to antigen. In the context of the study by Li et al., in the absence of knowledge of antigen specificity and/or enhanced functionality, fetus-derived CD45RO+ T cells are referred to as 'memory-like' T cells[1]. Complementary usage of single-cell RNA sequencing and in vitro stimulation identifies considerable phenotypic and functional heterogeneity of human fetal intestinal CD4+ T cells and raises several interesting observations. First, fetal memory-like CD4+ T cells are readily capable of producing the cytokines IL-2, IFN-γ, IL-4 and granzyme B. The trace production of granzyme B by CD45RO+ CD4+ T cells also suggests the presence of cytolytic CD4+ T cells in fetal intestine, which has not been previously reported. Second, greater than 50% of fetal naive and memory-like CD4+ T cells can produce the cytokine TNF. In contrast, other studies have shown that adult intestinal CD45RA+ CD4+ T cells do not produce TNF after being stimulated with staphylococcal enterotoxin B[5]. The production of TNF by fetal naive CD45RA+ CD4+ T cells may suggest the early acquisition of effector functions in the fetal intestine. Third, while fetal intestinal CD4+ T cells can produce type 1 and type 2 cytokines, production of type 17 cytokines is noticeably absent, in line with single-cell RNA-sequencing data showing that only ~1% of CD4+ T cells express *RORC* (which encodes the transcription factor RORγt). This observation parallels a published report in which cord blood memory T cells were shown to be polyclonal, with potential to produce the cytokines TNF, GM-CSF, IFN-γ, IL-4 and IL-13 but not IL-17A[6]. Because IL-17A-producing CD4+ T cells constitute an abundant population in adult intestine[5], this suggests that IL-17A-producing CD4+ T cells, in contrast to other helper T cell subsets, are tightly dependent on post-natal factors and, in particular, on commensal colonization, for their development and accumulation within tissues.

The observations noted above raise the intriguing question of the nature of the antigen-presenting cells and antigens required for generation of the memory and memory-like T cell pool before birth. A published study found that fetal tissues, and in particular barrier tissues such as the skin, lungs and gut, were seeded with all dendritic cell (DC) subsets as early as at 12 weeks of gestation[7]. Further, by 16 weeks of gestation, both resident DCs and migratory DCs were already detectable in the mesenteric lymph nodes[7], in support of the idea that migration from the tissue in which antigen can be captured to the site of T cell priming may occur at a very early stage of development. This study also showed that fetal DCs not only were able to respond to microbe-derived ligands but also could promote

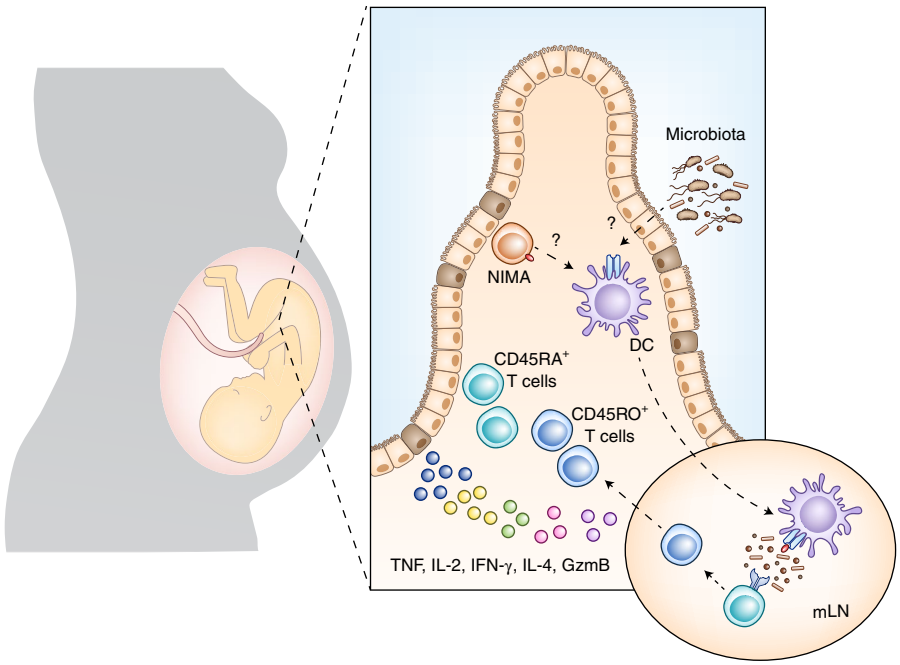

Fig. 1 | Naive and memory-like CD4⁺ T cells accumulate within the fetal intestine. Heterogenous populations of phenotypically naive (CD45RA⁺) and memory-like (CD45RO⁺) CD4⁺ T cells accumulate in the fetal intestine by the second trimester of pregnancy. The majority of naive and memory-like CD4⁺ T cells can produce TNF and IL-2. In addition, memory-like T cells can also produce IFN-γ, IL-4 and granzyme B (GzmB) but not IL-17A. Both resident DCs and migratory DCs are already present within the intestinal lamina propria and mesenteric lymph nodes (mLN) during gestation, but their role in the accumulation and function of fetal memory-like CD4⁺ T cells remains to be addressed. The antigen specificity of fetal memory-like T cells remains unknown, although commensal microbe–derived or genetically foreign non-inherited maternal antigens (NIMA) might serve a key role in the activation and accumulation of fetal intestinal T cells.

regulatory responses[7], a phenomenon that might contribute to the shaping of the 'tone' of the immune system after birth (that is, the balance of proinflammatory responses and regulatory responses), in particular at sites exposed to both nutrients and the microbiota. The intriguing observation that the majority of fetal intestinal T cells are capable of producing TNF also raises the possibility that these cells may contribute to protection against infection both before birth and after birth. How these cells are primed and the nature of the antigen responsible or even if exogenous antigens are required remain important questions to answer.

In their study, Li et al. utilize T cell antigen receptor–sequencing techniques to provide evidence of clonal T cell expansion among the fetal memory-like pool[1]; however, the nature of the antigens that drive this expansion, or whether this is indeed antigen driven, remains unclear. While the womb has until recently been considered a 'sterile' environment, this does not imply it is antigen free. Indeed, maternally derived cells have been identified within human fetal tissues from the second trimester of pregnancy onward, a phenomenon called 'microchimerism'. These maternally derived cells persist in the offspring throughout adulthood[7]; perhaps fetal intestinal memory T cells, as a consequence of microchimerism, recognize genetically foreign non-inherited maternal antigens. Alternatively, while this remains controversial, a published study detected the presence of microbial communities within the placenta, amniotic fluid and meconium[8], which suggests that the womb might not be an entirely sterile environment[9]. Perhaps exposure to trace microbes or microbe-derived antigens present in the womb trigger the accumulation of fetal memory T cells.

If indeed fetal intestinal CD4⁺ T cells display reactivity to microbial or commensal antigens, understanding the stringency of this specificity would be essential. Poly-reactive commensal-specific IgA antibodies, using a germline-encoded specificity and produced by intestinal B cells, promote the anatomical segregation of the intestinal epithelium and the commensal microbiota[10]. Perhaps fetal memory CD4⁺ T cells exhibit similar T cell antigen receptor cross-reactivity to multiple antigens, as has been described for human immunodeficiency virus– and influenza virus–specific memory CD4⁺ T cells recirculating in adult human blood[11]. It would be of interest to explore the possibility that clonally expanded, broadly reactive fetal intestinal CD4⁺ T cells might provide a first line of immunity to a large repertoire of microbes, including the microbiota.

The generation of 'virtual memory' T cells in the absence of foreign-antigen experience has been reported in mouse studies. It was also shown that human fetal splenic CD45RO⁺ T cells were able to proliferate in response to exogenous IL-2 but remained unresponsive to stimulation with the invariant signaling protein CD3[12], which provided the first suggestion that 'virtual memory' T cells could be found within the human fetus. The development of hybridoma-screening and yeast-display systems and isolation of ex vivo T cell clones will be key to better understanding of the specificity and potential cross-reactivity of memory-like or memory T cells in the fetal intestine.

Going forward, an equally intriguing aspect of future studies would be to address how anatomical tissue localization influences T cell effector function in the fetal intestine. While this is technically challenging for human studies, approaches to distinguishing the intravascular or parenchymal localization of distinct subsets of fetal intestinal T cells may aid the understanding of fetal T cell effector function. This might include whether tissue entry imposes or hinders the ability of naive or memory-like fetal T cells to produce TNF and other effector cytokines, or whether naive CD4⁺ T cells within the intestine could be derived from blood contaminants or are located within developing tertiary lymphoid structures. Answering such questions might aid in the identification of tissue factors that control the effector function of fetal T cells. Finally, perhaps fetal intestinal memory-like T cells are not all fetus derived. In line with the occurrence of pregnancy-induced microchimerism, perhaps some of the memory-like T cells that accumulate within the fetal intestine originate directly from the mother. HLA typing or genotyping from single-cell RNA-sequencing data may allow future studies to assign cells as being of maternal or fetal origin.

By leveraging recent technological advances, as demonstrated here by Li et al.[1]., researchers are increasingly able to perform sophisticated immunological studies to delineate the development of the human immune system. Deeper understanding of the accumulation and function of prenatal and neonatal human memory T cells, as well as definition of their antigen specificities

and potential cross-reactivities, will be key to understanding how the fetal immune system prepares for the transition from the womb to life-long challenges from the external world and how dysregulation in the repertoire or function of these cells may result in predisposition to disease states. ❐

Ai Ing Lim[1], Oliver J. Harrison[1] and Yasmine Belkaid[1,2,3*]

[1]*Metaorganism Immunity Section, Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA.* [2]*NIAID Microbiome Program, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA.* [3]*Center for Human Immunology, National Institutes of Health, Bethesda, MD, USA.*
**e-mail: ybelkaid@niaid.nih.gov*

Published online: 11 February 2019
https://doi.org/10.1038/s41590-019-0326-0

References

1. Li et al. *Nat. Immunol.* https://doi.org/10.1038/s41590-018-0294-9 (2019).
2. Li, N. et al. *J. Exp. Med.* **215**, 1383–1396 (2018).
3. Senda, T. et al. *Mucosal Immunol.* https://www.nature.com/articles/s41385-018-0110-8 (2018).
4. Thome, J. J. et al. *Nat. Med.* **22**, 72–77 (2016).
5. Hegazy, A. N. et al. *Gastroenterology* **153**, 1320–1337 (2017).
6. Zhang, X. et al. *Sci. Transl. Med.* **6**, 238ra272 (2014).
7. McGovern, N. et al. *Nature* **546**, 662–666 (2017).
8. Aagaard, K. et al. *Sci. Transl. Med.* **6**, 237ra265 (2014).
9. Kinder, J. M., Stelzer, I. A., Arck, P. C. & Way, S. S. *Nat. Rev. Immunol.* **17**, 483–494 (2017).
10. Bunker, J. J. et al. *Science* **358**, eaan6619 (2017).
11. Su, L. F., Kidd, B. A., Han, A., Kotzin, J. J. & Davis, M. M. *Immunity* **38**, 373–383 (2013).
12. Byrne, J. A., Stankovic, A. K. & Cooper, M. D. *J. Immunol.* **152**, 3098–3106 (1994).

Competing interests

The authors declare no competing interests.