T Cells in Preterm Infants and the Influence of Milk Diet

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Preterm infants born before 32 weeks gestational age (GA) have high rates of late onset sepsis (LOS) and necrotizing enterocolitis (NEC) despite recent improvements in infection control and nutrition. Breast milk has a clear protective effect against both these outcomes likely due to multiple mechanisms which are not fully understood but may involve effects on both the infant’s immune system and the developing gut microbiota. Congregating at the interface between the mucosal barrier and the microbiota, innate and adaptive T lymphocytes (T cells) participate in this interaction but few studies have explored their development after preterm delivery. We conducted a literature review of T cell development that focuses on fetal development, postnatal maturation and the influence of milk diet. The majority of circulating T cells in the preterm infant display a naïve phenotype but are still able to initiate functional responses similar to those seen in term infants. T cells from preterm infants display a skew toward a T-helper 2 (Th2) phenotype and have an increased population of regulatory cells (Tregs). There are significant gaps in knowledge in this area, particularly in regards to innate-like T cells, but work is emerging: transcriptomics and mass cytometry are currently being used to map out T cell development, whilst microbiomic approaches may help improve understanding of events at mucosal surfaces. A rapid rise in organoid models will allow robust exploration of host-microbe interactions and may support the development of interventions that modulate T-cell responses for improved infant health.

Keywords: T-lymphocytes, infant, premature, mucosal immunity, necrotizing enterocolitis, extremely premature

INTRODUCTION

Infants born prematurely, especially before 32 weeks gestational age (GA) are susceptible to diseases associated with invasive bacterial infections, specifically late onset sepsis (LOS) and necrotizing enterocolitis (NEC). These occur in around 30 and 6% of very preterm infants, respectively, according to recent data (1), most occur in the first 4 weeks of life, and both diseases are associated with changes in the gut microbiota (2, 3). Feeding preterm infants with their mother’s breast milk has been shown to reduce the risk of NEC and LOS (4, 5). T lymphocytes (T cells) are an important component of the immune response to infection, however, this role is balanced against their emerging role in tissue regeneration and repair. The newborn, who is exposed to diverse microbes including many potential pathogens soon after birth, requires an appropriate T cell response to navigate the potentially conflicting requirements of growth, repair and infection control.

This review focuses on fetal T cell development, postnatal T cell maturation, and the potential for dietary modulation of gut mucosal T cells in the preterm environment. T cells of particular interest...
in the preterm infant comprise those with innate-like properties such as γδ T, invariant natural killer T (iNKT) and mucosa-associated invariant T (MAIT) cells, which have the capacity to deliver effector functions without prior clonal expansion. Among conventional TCRαβ-expressing T cells, regulatory T cells (Treg8) are known for their ability to mediate tolerance but also have emerging roles in tissue regeneration of potential relevance to NEC (Table 1).

A challenge of studying T cells in preterm infants is that it is difficult to obtain suitable samples purely for research purposes. Umbilical cord is often used to represent the immune system of preterm infants, however a recent systems level analysis suggests it is more representative of the fetal state than the ex-utero infant (6). Therefore, where possible, we focus on studies using postnatal blood, but supplement these data with studies on umbilical cord blood, fetuses, and animal studies as necessary.

**FETAL T-CELL DEVELOPMENT**

Bone marrow-derived T progenitor cells enter the thymus at around 8 weeks gestation (19). These early thymocytes do not display the full range of T cell receptors (TCR) until 16 weeks GA (20). Fetal T cells can be detected in peripheral blood from 8 weeks GA, with the capacity to proliferate and produce cytokines (21). T cell migration to secondary lymphoid tissue has been confirmed with identification of T cells in the mesenteric lymph nodes (MLN) from 12 weeks GA (22). Interestingly, fetal CD4 T (Th) cells in the intestinal lamina propria but not spleen or liver display a predominantly memory phenotype (22, 23). This suggests priming as a result of prior “antigen” exposure, the nature of which is not known (22). Fetal MLN-derived T cells have capacity to respond to stimulation by proliferating and secreting a broad range of cytokines. Both proliferation and cytokine production by fetal MLN-derived T cells are augmented upon the removal of Treg8 (20) suggesting Treg8 play an important role in limiting fetal tissue inflammation.

**SKEWING OF THE T-helper (Th) RESPONSE**

Naïve Th cells can differentiate toward alternative cell fates depending on the context in which they receive antigenic stimulation (8). Preterm infants have been suggested to have a skewed Th2 response based on their increased production of a classical Th2 cytokine, IL-5, and decreased production of a Th1 cytokine, IFN-γ, upon stimulation of peripheral blood compared to term cord blood (7). Postnatally, the ability of preterm Th cells to secrete IFN-γ remains low with single cell RNA-seq analysis of term and preterm infants at 12 weeks of age demonstrating up-regulation of genes that suppress IFN-γ, together with longitudinal sampling of preterm infants displaying

### Table 1: Summary of differences between peripheral blood T cell populations in preterm infants compared to term infants.

| T cell type | Description of role | Relative abundance (preterm compared to term infants, population as % of T cells) | Relative function (preterm compared to term infants) | Relevance of difference | References |
|-------------|---------------------|----------------------------------|--------------------------------|--------------------------|-----------|
| CD4+ T (Th) | Regulate immune response Help B cells produce immunoglobulin | ↑↑ | ↑ IL-5 ↓ IFN-γ | Limited Th1 response limits response to intracellular infections | (6, 7) |
| Treg | Suppress immune response | ↑↑ | ↑ IL-10 | Suppress immune response, leading to tolerogenic state (limiting inflammation) | (6, 8–10) |
| CD8+ T | Cytotoxic T cells | ↔ | Not known ↑ IFN-γ* ↑ TNF-α* ↓ IL-2* | Potentially increased ability to respond to viral infections | (11, 12) |
| γδ T | Innate-like, predominantly mucosa-based range of cytotoxic functions | ↓↓ | ↑ IFN-γ ↑ IL-10 | Potentially compensates for decreased Th1 response. | (13–15) |
| iNKT | Innate-like, some roles similar to natural killer cells | Not known/↑↑ | Not known | Unclear if beneficial or detrimental | (16) |
| MAIT | Innate-like, cytotoxic and inflammatory functions | ↓ | Not known | Unclear if beneficial or detrimental | (17, 18) |

*Umbilical cord blood. ↑Increased; ↑↑Greatly increased; ↓Decreased; ↓↓Greatly decreased; ↔Similar.

Unsurprisingly, the majority of T cells derived from cord blood in newborns are naïve (24). The proportion of naïve T cells has been shown to remain high (median 85%) at 6–8 weeks age [postnatal age (PNA)] in preterm infants, however this is significantly less than term infants at this PNA (25). This may reflect increased antigen exposure or reduced thymic output in preterm infants.

The size, composition, and function of the immune cell compartment varies from one tissue to another (26, 27). Interestingly, fetal CD4 T (Th) cells in the intestinal lamina propria but not spleen or liver display a predominantly memory phenotype (22, 23). This suggests priming as a result of prior “antigen” exposure, the nature of which is not known (22). Fetal MLN-derived T cells have capacity to respond to stimulation by proliferating and secreting a broad range of cytokines. Both proliferation and cytokine production by fetal MLN-derived T cells are augmented upon the removal of Treg8 (20) suggesting Treg8 play an important role in limiting fetal tissue inflammation.
low expression (6, 13). An effective T<sub>reg</sub>1 response is key to preventing intracellular infections, including bacteria, and this may explain why preterm infants have increased susceptibility.

In contrast to the T<sub>reg</sub>2 skewing observed in peripheral blood, fetal intestinal T<sub>reg</sub> cells instead have a tendency to secrete TNF-α and IL-2 (T<sub>reg</sub>1 cytokine), when compared to term infant intestinal samples. TNF-α was shown in a human fetal organoid model to be important in intestinal epithelial growth by its effects on intestinal stem cells, albeit high levels of TNF-α suppress epithelial growth. Infants with NEC have an increased production of TNF-α from intestinal T<sub>reg</sub> cells, although the temporal relationship to disease is unknown (23).

The tissue T<sub>reg</sub> response is affected by human milk oligosaccharides (HMO's), which comprise 1–2% of human milk and are the third largest non-water component by weight (after lactose and lipids). HMO's are not digestible by the infant but are believed to modulate the gut microbiota. HMO's appear to promote the growth of Bifidobacteria and suppress potentially pathogenic organisms (28, 29). When adding HMO's to fetal in vitro organ cultures, gene transcripts associated with T<sub>reg</sub> differentiation were found only with HMO's from early, not mature human milk, believed to reflect variation in the concentration of the HMO's. These transcriptional changes were deduced to promote a T<sub>reg</sub>1 response whilst suppressing a T<sub>reg</sub>2, T<sub>reg</sub>17, and IL-8 expression (30). It is possible that these alterations play a role in the reduction of NEC and LOS seen in preterm infants fed breast milk.

**SUPPRESSION OF THE IMMUNE RESPONSE**

T<sub>reg</sub>8 are a population of T<sub>reg</sub> cells defined by their suppressive function toward effector T cell responses. T<sub>reg</sub> abundance is inversely correlated with GA and higher in infants compared to adults (9, 31). T<sub>reg</sub>8 have been identified in the thymus as early as 13 weeks GA and in the periphery (spleen) from 14 weeks GA (21). The proportion of T<sub>reg</sub>8 in the fetal MLN is significantly increased compared to adults and T<sub>reg</sub>8 are functional even at a fetal stage (20, 23).

Interleukin- (IL-) 10 is amongst the factors that mediate the suppressive function as well as induce expansion of T<sub>reg</sub>8 (8–10). IL-10 is produced following bacterial invasion or in stimulated tissues. IL-10 has been shown to contribute to bacterial clearance, yet minimize host damage from infection (10). Interestingly, germline mutations causing loss of function in the IL-10 receptor, as well as deficiency of the critical T<sub>reg</sub> transcription factor FOXP3, cause early-onset enterocolitis (32). The ability of lamina propria T<sub>reg</sub>8 to produce IL-10 is greatly reduced in preterm infants with NEC compared to term infants (23). An impaired T<sub>reg</sub> IL-10 response to bacterial invasion may have a role in development of NEC, or it may be that the microbial dysbiosis seen before the development of NEC leads to an impaired IL-10 response (2).

Similar to humans, mice harbor an increased density of T<sub>reg</sub>8 in the intestine compared with other organs. In mice, the introduction of gut bacteria induces an increase in the population of colonic but not small intestinal T<sub>reg</sub>8, apparently driven by Clostridium. These T<sub>reg</sub>8 are thought to be peripherally induced (rather than thymus-derived) as judged by their lack of expression of the transcription factor, Helios (33). Bacteroides fragilis has also been shown to induce T<sub>reg</sub>8 in mice through its production of polysaccharide A (PSA) in the presence of the toll-like receptor, TLR-2 (34, 35). In mice, induction of T<sub>reg</sub>8 upon treatment by PSA in the presence of Bacteroides fragilis is protective against not only intestinal inflammation but also encephalitis, suggesting there could be a systemic effect of this interaction (34–36). No preterm human work on this aspect currently exists.

**UNIMPAIRED CD8 EFFECCTOR POTENTIAL?**

CD8 T cells have been demonstrated in the fetal intestine as early as 16 weeks GA (20, 37). Whilst they display mainly a naïve phenotype in the first 12 weeks of postnatal life, little is known about their postnatal effector potential (11). Fetal (term and preterm cord blood) and adult CD8 T cells develop a similar memory phenotype and ability to produce perforin and cytokines in response to a common neonatal virus, cytomegalovirus (CMV) (38). Furthermore, using cord blood derived CD8 T cells from varying gestations (23–41 weeks GA), an increased ability to secrete IFN-γ, TNF-α, and IL-2 has been demonstrated at earlier gestations (12).

These limited data suggest prematurity does not prevent CD8 effector T cell function in newborns. Indeed, it is possible that they display an excessive response to viral infection, which may be harmful, particularly as many infants are postnatally exposed to CMV.

**INNATE-LIKE T CELLS**

Innate-like T cells can display effector function without prior antigen priming. This would suggest they could be important in early life (17, 39). Innate-like T cells include γδ T, iNKT, and MAIT cells.

**Gamma-Delta (γδ) T Cells**

Unlike αβ T cells, a large number of γδ T cells reside in non-lymphoid tissues including the gut, spleen and lungs. They have a small range of antigen receptors but are able to respond to a large repertoire of antigens including peptide not bound to classical major histocompatibility complex (MHC) molecules (39). They comprise 4–10%, 1–3%, and <1% of T cells in the peripheral blood of adults, term and preterm cord blood of infants, respectively (14).

γδ T cells develop a memory phenotype in the first month of life (15) and preterm γδ T cells have an increased ability to secrete IFN-γ and IL-10 upon stimulation with PMA and ionomycin compared to both term γδ T cells and preterm αβ T cells (15). The ability to secrete IFN-γ increases over time, which is in contrast to preterm T<sub>reg</sub> cells (13). However, when challenged with influenza virus, cord blood γδ T cells show a
decreased ability to proliferate and produce IFN-γ compared to term infants and adults (14). The differences in these results could be due to experimental design, however these data suggest at least the potential for preterm γδ T cells to contribute to the cellular immune response. Little is known about preterm mucosal γδ T cells or their responses to alternative ligands.

Invariant Natural Killer T (iNKT) Cells

Natural killer T (NKT) cells define a population of T cells that bear TCR’s restricted by a non-classical MHC molecule, CD1d, and express cell surface markers associated with NK cells. Invariant/ type 1 NKT (or iNKT) cells express the invariant TCR (Vα24-Jα18). iNKT cells vary in abundance depending on tissue, comprising 0.01–0.1% of lymphocytes in peripheral blood but 10% of lymphocytes in the omentum (40). There is an increased abundance of iNKT cells in preterm cord blood compared to term cord blood (16).

iNKT’s are able to secrete cytokines that mediate T3,1, T1,2, or T17 responses, and recognize specific glycolipid antigens presented by CD1d (41). The ability of preterm iNKT cells to produce these T3 cytokines is not known but has been demonstrated in the case of term cord blood iNKT’s. The secretion of IL-10 is particularly increased compared to adults suggesting a potential regulatory role of iNKT’s in term infants (41).

iNKT cells have been associated with colitis in mouse models. Germ-free mice have an increased proportion of iNKT cells in their colon and increased susceptibility to colitis, however colonization of the intestine with bacteria early in life leads to a reduction in iNKT density and provides protection from colitis (42). iNKT cells able to produce IL-13 have been implicated in the pathogenesis of colitis (43). Furthermore, iNKT-deficient mice differ in gut microbial composition compared to wild-type mice, as well as showing increased intestinal leukocyte infiltration (44). Whether iNKT cells are involved in the pathogenesis of NEC in preterm infants is not known.

Mucosa-Associated Invariant T (MAIT) Cells

MAIT cells are a population of T cells predominantly found in the lung and intestinal mucosa, that display a semi-invariant TCR (Vα7.2 – Jα3.3/20/12). MAIT cells have been demonstrated in the fetal thymus from 18 weeks GA. MAIT cells represent <1% of peripheral blood T cells in newborns compared to up to 10% in adults, suggesting a postnatal expansion (17). MAIT cells are of particular interest as they recognize MR-1, an MHC-1 like molecule that presents microbial derived metabolites of the essential vitamin, riboflavin (17). Riboflavin is synthesized by gut commensal bacteria, and has a variable concentration in human milk dependent on maternal intake (45, 46). Thus, the postnatal expansion of MAIT cells is likely driven both by bacteria and by diet.

In contrast to thymic MAIT cells, those in fetal intestine, spleen, and MLN express a marker of activation (PLZ1F), suggesting peripheral maturation. They develop this mature phenotype in the first 2 months of life (17, 47) and display inflammatory and cytotoxic abilities. Indeed, fetal intestinal, liver and lung but not thymic or spleen MAIT cells can produce IFN-γ following stimulation (18). Functional changes in MAIT cells have been associated with inflammation in adults with inflammatory bowel disease (48). However, no study has examined their relationship with NEC.

Dietary Interactions with Mucosal T Cells

When considering modulation of the preterm T cell populations, we have described how key dietary exposures such as HMOs and bacteria appear important. Breast milk contains a vast array of components that may directly or indirectly affect gut mucosal T cell populations, as demonstrated in both human and animal models of early life (Figure 1). The gut microbiota is likely to be a key mediator of such dietary effects, implying the possibility of therapeutic modulation either by dietary modifications or the use of prebiotics or probiotics.

Colostrum

Compared to more mature human milk,colostrum has a higher concentration of bioactive components including HMOs, lactoferrin, immune cells, and immunoglobulin. In a porcine model, pregnant sows were given NEC-inducing intra-amniotic lipopolysaccharide injections in-utero and offspring pups were then fed varying diets before euthanasia. Pups fed colostrum had decreased levels of distal ileal IL-8 and IL-1β, combined with increased abundance of blood T3 cells (as % of lymphocytes). The authors concluded that a colostrum diet led to maturation of the intestinal mucosa as well as the systemic immune system (50).

Translocation of Milk Derived Immune Cells

Human milk contains stem cells and leukocytes (51, 58). Studies using mice show that maternal immune cells can translocate from the intestine into the circulation or distal organs (53, 54, 59). Stem cells derived from milk have been shown to translocate into the brains of mice, and once there to traffic and differentiate (53, 54). In these mouse models, T cells are the predominant cell type that transfers across the intestinal epithelium despite their relative paucity in milk (58, 59). CD8 T cells, in particular, translocate into the intestinal Peyer’s patches, possibly based on their expression of a gut homing receptor (CCR9). Once there, they show an increased ability to produce cytokines upon stimulation compared with peripheral blood CD8 T cells (59).

It is unclear whether diet-derived T cells migrate beyond the MLN in mice as studies conflict (52, 59) but it is plausible that milk-derived maternal CD8 T cells translocate into the mucosa in humans and compensate for “deficiencies” of preterm T cells.

Lactoferrin

Lactoferrin is the major whey glycoprotein of human milk and subject to intense research for over 50 years due to its ability to inhibit bacteria by multiple mechanisms (55, 60). Lactoferrin has also been shown to bind to immune cells and influence their function (61). Most lymphocytes can express a lactoferrin receptor including stimulated γβ and γδ T cells.
Furthermore, T cells in the lamina propria of pigs bind lactoferrin (57). In a mouse model, T cells in the lamina propria proliferated upon administration of enteral bovine lactoferrin in the presence or absence of colon cancer. These T cells secreted IFN-γ and IL-18, suggesting that they were immunologically active (62).

**CONCLUSION**

Preterm infants have a T cell population that was designed for fetal life and therefore differs systematically from term infants (Table 1). In particular, they have a diminished Th1 response leaving them susceptible to infection by intracellular pathogens. Trgs protect the preterm infant from an excessive innate response which may reduce the risk of NEC but may also increase the risk of LOS. This variation in function of Trgs is modulated by IL-10.

There are multiple mechanisms by which the diet, gut microbiome, and T cell populations have been demonstrated to interact in human, mouse, and laboratory experiments demonstrated in Figure 1. However, there is a paucity of data comparing the effects of alternative feeding strategies on the preterm infant's immune system, including T cells, and there remains substantial uncertainty about when and how donor human milk and human milk derived fortifiers should be used. Clinical studies aimed at identifying changes in the immune system associated with these dietary interventions will improve understanding and enable more informed nutritional management.

The functions of innate-like cells such as iNKT and MAIT cells in preterm infants have yet to be unraveled but a number of compelling studies on animals imply an important role in preventing intestinal inflammation. Alterations in gut mucosal T cells may occur in preterm infants as a result of dietary or microbiome manipulations. A
better understanding of the interplay of diet, microbiome and host immunity will underpin efforts to develop interventions that modulate T-cell responses for improved infant health.

REFERENCES

1. Griffiths J, Jenkins P, Vargova M, Bowler U, Juszczak E, King, et al. Enteral lactoferrin supplementation for very preterm infants: a randomised placebo-controlled trial. The Lancet. (2019) 393:423–33. doi: 10.1016/S0140-6736(18)32221-9

2. Stewart CJ, Embleton ND, Marrs ECL, Smith DP, Smith DP, Nelson A, et al. Temporal bacterial and metabolic development of the preterm gut reveals specific signatures in health and disease. Microbiome. (2016) 4:67. doi: 10.1186/s40168-016-0216-8

3. Stewart CJ, Embleton ND, Marrs ECL, Smith DP, Fofanova T, Nelson, et al. Longitudinal development of the gut microbiome and metabolome in preterm neonates with late onset sepsis and healthy controls. Microbiome. (2017) 5:1. doi: 10.1186/s40168-017-0295-9

4. Patel AL, Johnson TJ, Engstrom JL, Fogg LF, Jegier BJ, Bigger R, et al. Impact of early human milk on sepsis and health-care costs in very low birth weight infants. J Perinatol. (2013) 33:514–19. doi: 10.1038/jp.2013.2

5. Corpeleijn WE, Kuivenhoven MP, Paap M, Vliet I, Scheerder I, Muizer Y, et al. Intake of own mother’s milk during the first days of life is associated with decreased morbidity and mortality in very low birth weight infants during the first 60 days of life. Neonatology. (2012) 102:276–81. doi: 10.1159/000341335

6. Olin A, Henckel E, Chen Y, Lakshmikanth T, Pou C, Mikes, et al. Stereotypic immune system development in newborn children. Cell. (2018) 174:1277–92.e14. doi: 10.1016/j.cell.2018.06.045

7. Dirix V, Vermeulen F, Mascart M. Maturation of CD4+ regulatory T lymphocytes and of cytokine secretions in infants born prematurely. J Clin Immunol. (2013) 33:1116–33. doi: 10.1007/s10875-013-9911-4

8. Raphael I, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their regulatory function in mucosal and lymphoid tissues. Mucosal Immunol. (2019) 12:455–65. doi: 10.1038/s41455-019-0155-1

9. Zahran AM, Saad K, Abdel-Rahme YE, Elsayh KI, El-Houfey AA, Abo ul-Khair MD, et al. Characterization of regulatory T cells in preterm and term infants. Archivum Immunologumae et Therapiae Experimentalis. (2019) 67:49–54. doi: 10.1007/s00005-018-0530-x

10. Penaloza HF, Noguera LP, Riedel CA, Bueno SM. Expanding the current understanding of the interplay of diet, microbiome and host immunity will underpin efforts to develop interventions that modulate T-cell responses for improved infant health. In: Proceedings of the 9th International Conference on Neonatal Inflammation and Immunity. DeZamora, L., Gonzalez, A., Editors. (2015) 161:65–74. doi: 10.1016/j.clim.2015.07.003

11. Sproat et al. T Cells in Preterm Infants

12. Scheible KM, Embleton ND, Marrs ECL, Smith DP, Smith DP, Nelson A, et al. Developmental reduction in CD31 during gestation is associated with decreased morbidity and mortality in very low birth weight infants during the first 60 days of life. Neonatology. (2012) 102:276–81. doi: 10.1159/000341335

13. Dirix V, Vermeulen F, Mascart M. Maturation of CD4+ regulatory T lymphocytes and of cytokine secretions in infants born prematurely. J Clin Immunol. (2013) 33:1116–33. doi: 10.1007/s10875-013-9911-4

14. Li J, Li H, Mao H, Yu M, Feng T, Yang F, et al. Vgamma9Vdelta2-T cells constitutively expressing the interleukin-2 receptor α chain early in life are primed to respond to lower antigenic stimulation. Immunology. (2010) 131:289–99. doi: 10.1111/j.1365-2267.2010.03304.x

15. Ben Youssef G, Tourret M, Salou M, Ghazarian L, Houdouin V, Mondot S, et al. Ontogeny of human mucosal-associated invariant T cells and related T cell subsets. J Exp Med. (2018) 215:459–79. doi: 10.1084/jem.20171739

16. Leansyah E, Loh L, Nixon DF, Sandberg JK. Acquisition of innate-like microbial reactivity in mucosal tissues during human fetal MAIT-cell development. Nat Commun. (2014) 5:5314. doi: 10.1038/ncomms4143

17. Haynes BF, Heiny CS. Early human T cell development: analysis of the human thymus at the time of initial entry of hematopoietic stem cells into the fetal thymic microenvironment. J Exp Med. (1995) 181:1445–58. doi: 10.1084/jem.181.4.1445

18. Michaelsson J, Mold JE, McCune JM, Nixon DF. Regulation of T cell responses in the developing human fetus. J Immunol. (2006) 176:5741–8. doi: 10.4049/jimmunol.176.10.5741

19. Darrasse-Jeze G, Marodon G, Salomon BL, Catala B, Klatzmann, D. Ontogeny of CD4+CD25+ regulatory/suppressor T cells in human fetuses. Blood. (2005) 105:8715–21. doi: 10.1182/blood-2004-10-4053

20. Li N, van Junen V, Abdelalal T, Guo N, Kasatksyay SA, Ladell K et al. Memory CD4(+) T cells are generated in the human fetal intestine. Nat Immunol. (2019) 20:301–12. doi: 10.1038/s41590-018-0294-9

21. Schreurs R, Baumdick ME, Sageafi AF, Kaufmann M, Mokry M, Klarenbeek PL, et al. Human fetal T/NF-α-T cell cytokine-producing CD4(+) effector memory T cells promote intestinal development and mediate inflammation early in life. Immunity. (2019) 50:662–76.e8. doi: 10.1016/j.immuni.2019.12.010

22. Quinello C, Silveira-Lessa AL, Ceccon, MEJR, Cianciarullo MA, Carneiro-Sampaio M, Palmeira P. Phenotypic differences in leucocyte populations among healthy preterm and full-term newborns. Scand J Immunol. (2014) 80:57–70. doi: 10.1111/sij.12183

23. Berrington JE, Barge D, Fenton AC, Cant AJ, Spickett GP. Lymphocyte subsets in term and significantly preterm UK infants in the first year of life analysed by single platform flow cytometry. Clin Exp Immunol. (2005) 140:289–92. doi: 10.1111/j.1365-2249.2005.02767.x

24. Wong MT, Lim FS, Teng KW, Mc Govern N, Narayanan S, et al. A high-dimensional atlas of human T cell diversity reveals tissue-specific trafficking and cytokine signatures. Immunity. (2016) 45:442–56. doi: 10.1016/j.immuni.2016.07.007

25. Thome JJ, Bickham KL, Ohmura Y, Kubota M, Matsuoka N, Gordon, et al. Early-life compartmentalization of human T cell differentiation and regulatory function in mucosal and lymphoid tissues. Nat Med. (2016) 22:72–7. doi: 10.1038/nm.4008

26. Gonia S, Tuepker M, Heisel T, Aturcan C, Bode L, Gale A, et al. Human milk oligosaccharides inhibit Candida albicans invasion of human premature intestinal epithelial cells. J Nutr. (2015) 145:1992–8. doi: 10.3945/jn.115.214940

27. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. (2010) 23:1147–55. doi: 10.1093/glycob/cws074

28. He Y, Liu S, Leone S, Newburg DS. Human colostrum oligosaccharides modulate major immunologic pathways of immature human intestine. Mucosal Immunol. (2014) 7:1326–39. doi: 10.1038/mi.2014.20

29. Pagel J, Hartz A, Figge J, Gille C, Eschweiler S, Petersen K, et al. Regulatory T cell frequencies are increased in preterm infants with clinical early-onset sepsis. Clin Exp Immunol. (2018) 165:219–27. doi: 10.1111/cei.12810

30. Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schaffer AA, Noyan F, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med. (2009) 361:2033–43. doi: 10.1056/NEJMoa0907206

31. Atarashi K, Tanoue T, Shim a T, Imaoka A, Kuwahara T, Momo se Y, et al. Induction of colonic regulatory T cells by indigenous clostridium species

AUTHOR CONTRIBUTIONS

TS wrote the initial manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.
- murine model of Treg and Th population being changed by microbiota. *Science.* (2011) 331:337–41. doi: 10.1126/science.1198469
34. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science.* (2011) 332:974. doi: 10.1126/science.1206095
35. Ramakrishna C, Kujawski M, Chu H, Li L, Mazmanian SK, Cantin M, et al. Bacteroides fragilis polysaccharide A induces IL-10 secreting B and T cells that prevent viral encephalitis. *Nat Commun.* (2019) 10:2153. doi: 10.1038/s41467-019-09884-6
36. Sittipo P, Lobionda S, Choi K, Sari IN, Kwon HY, Lee K, et al. Toll-like receptor 2-mediated suppression of colorectal cancer pathogenesis by polysaccharide A from *Bacteroides fragilis*. *Front Microbiol.* (2018) 9:1588. doi: 10.3389/fmicb.2018.01588
37. Zhang X, Moelzki B, Lemoine S, Deriaud E, Lim A, Zhivkai D, 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. doi: 10.1126/scitranslmed.3008748
38. Marchant A, Appay V, van der Sande M, Duphny N, Liesnard C, Kidd M, et al. Mature CD8+ T lymphocyte response to viral infection during fetal life. *J Clin Investig.* (2003) 111:1747–55. doi: 10.1172/JCI200317470
39. Vantourout, P, Hayday A. Six-of-the-best: unique contributions of γδ T cells in autoimmune diseases. *Immunol.* (2018) 9:1333. doi: 10.3389/fimmu.2018.01333
40. Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, et al. Bacteroides fragilis polysaccharide A induces IL-10 secreting B and T cells that prevent viral encephalitis. *Nat Commun.* (2019) 10:2153. doi: 10.1038/s41467-019-09884-6
41. 02.005
42. Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, et al. Bacteroides fragilis polysaccharide A induces IL-10 secreting B and T cells that prevent viral encephalitis. *Nat Commun.* (2019) 10:2153. doi: 10.1038/s41467-019-09884-6
43. Heller F, Fuss IJ, Niewenhuis EE, Blumberg RS, Strober W. Oxazolone induces differentiation of human invariant NKT cells. *J Exp Med.* (2003) 197:2485–93. doi: 10.1084/jem.20031071
44. Bezzina CP, Leen CC, Antia R, Wu H, Hunkapiller MT, Glimcher LH, et al. Invariant natural killer T cells shape the gut microbiota and regulate neutrophil recruitment and function during intestinal inflammation. *Front Immunol.* (2018) 9:3999. doi: 10.3389/fimmu.2018.03999
45. Hambel P, Beltran F, Chetwynd E, Stuebe AM, Twigger, A-J., et al. Breastmilk is a novel source of stem cells with multilineage differentiation potential. *Stem Cells.* (2012) 30:2164–74. doi: 10.1002/stem.1188
46. Sproat et al. T Cells in Preterm Infants
47. Walker LJ, Tharmalingam H, Klenerman P. The rise and fall of MAIT cells and function. *Trends Immunol.* (2015) 36:489–93. doi: 10.1016/S1471-4906(15)00390-5
48. Chiba A, Murayama G, Miyake S. Mucosal-associated invariant T cells in autoimmune diseases. *Front Immunol.* (2018) 9:1333. doi: 10.3389/fimmu.2018.01333
49. Le Bourhis L, Guerrin L, Dusseau X, Martin E, Soudais C, Lantz, et al. Mucosal-associated invariant T cells: unconventional development and function. *Trends Immunol.* (2011) 32:212–8. doi: 10.1016/j.it.2011.02.005
50. Ren S, Hui Y, Goerick-Prisch S, Pankratova S, Kot W, Pan X, et al. Gut and immune effects of bioactive milk factors in preterm pigs exposed to prenatal inflammation. *Am J Physiol Gastrointest Liver Physiol.* (2019) 317:G67–77. doi: 10.1152/ajpgi.00942.2019
51. Hassiotou F, Beltran F, Chetwynd E, Stuebe AM, Twigger A-J., et al. Breastmilk is a novel source of stem cells with multilineage differentiation potential. *Stem Cells.* (2012) 30:2164–74. doi: 10.1002/stem.1188
52. Ma LJ, Walter B, Deguzman A, Muller HK, Walker AM. Trans-epithelial immune cell transfer during sucking modulates delayed-type hypersensitivity in recipients as a function of gender. *PLoS ONE.* (2008) 3:e3562. doi: 10.1371/journal.pone.003562
53. Hassiotou F, Mobiley A, Geddes D, Hartmann P, Walkie T. Breastmilk imparts the mother's stem cells to the infant. *FASEB J.* (2015) 29:876.
54. Aydin MS, Yigit EN, Vatanasalar E, Erdogan E, Ozturk G. Transfer, and integration of breast milk stem cells to the brain of suckling pups. *Sci Rep.* (2018), 8:14289. doi: 10.1038/s41598-018-32715-5
55. Mincheva-Nilsson L, Hammarstrom S, Hammarstrom M. Activated human γδ T lymphocytes express functional lactoferrin receptors. *Scand J Immunol.* (1997) 46:609–18. doi: 10.1046/s0304-4165(68)90015-9
56. Nielsen SM, Hansen CH, Danielsen EM. Lactoferrin targets T cells in the small intestine. *J Gastroenterol.* (2010) 45:1121–8. doi: 10.1007/s00353-010-0271-z
57. Trend S, de Jong E, Lloyd ML, Kok CH, Richmond P, Doherty DA, et al. Leukocyte populations in human preterm and term breast milk identified by multicolour flow cytometry. *PLoS ONE.* (2015) 10:e0135580. doi: 10.1371/journal.pone.0135580
58. Cabinian A, Sinisimer D, Tang M, Zumba O, Mehta H, Toma A, et al. Transfer of maternal immune cells by breastfeeding: maternal cytotoxic T lymphocytes present in breast milk localize in the Peyer's patches of the nursed infant. *PLoS ONE.* (2011) 6:e20135. doi: 10.1371/journal.pone.0120135
59. Hassiotou F, Mobiley A, Geddes D, Hartmann P, Walkie T. Breastmilk imparts the mother's stem cells to the infant. *FASEB J.* (2015) 29:876.
60. Ward P, Conneely OM. Lactoferrin: role in iron homeostasis and host defense against microbial infection. *BioMetals.* (2004) 17:203–8. doi: 10.1023/B:BIOM.0000027693.60932.26
61. Legrand D. Overview of lactoferrin as a natural immune modulator. *J Pediatr.* (2016) 173(Suppl.).S10–5. doi: 10.1016/j.jpeds.2016.02.071
62. Wang, W.-P., Iigo M, Sato J, Sekine K, Adachi I, et al. Activation of intestinal mucosal immunity in tumour-bearing mice by lactoferrin. *Jap J Cancer Res.* (2000) 91:1022–7. doi: 10.1111/j.1349-7006.2000.tb0880.x

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