Short-Chain Fatty Acids Impact Neonatal Regulatory T Cells

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T cells are specialised lymphocytes that play a pivotal role in the adaptive immune response and are marked by their surface expression of a T cell receptor (TCR). Conventional T cells are classically divided into one of two major subtypes based on the identity of their TCR co-receptor: CD4+ T cells or T helper (Th) cells play a key role in orchestrating adaptive immune responses via the production of effector cytokines; CD8+ T cells, also known as cytotoxic T lymphocytes (CTLs), are critical mediators in the elimination of virally infected or tumour cells, which they achieve through the release of cytotoxic granules that induce apoptotic or lytic death. Over the first weeks of life, the neonatal gastrointestinal tract is rapidly colonised by a diverse range of microbial species that come to form the ‘gut microbiota’. Microbial colonisation of the neonatal gut is a well-established regulator of several physiological processes that contribute to immunological protection in postnatal life, including the development of the intestinal mucosa and adaptive immunity. However, the specific microbiota-derived signals that mediate these processes have not yet been fully characterised. Short-chain fatty acids (SCFAs), end-products of intestinal bacterial metabolism, as one of the key mediators of immune development in early life. Critical to neonatal health is the development of regulatory T (Treg) cells that promote and maintain immunological tolerance against self and innocuous antigens.

Keywords: SCFA ; regulatory T cells ; newborn

1. Introduction

At birth, the immunologically naïve neonate undergoes a drastic transition from the sterile environment of the maternal womb to the microbially rich extrauterine environment. Consequently, newborns are highly vulnerable to infections, which account for 40% of the 3 million neonatal deaths worldwide each year [1]. In addition, the first few weeks of life represent a period in which rapid and significant microbial colonisation of the neonatal gastrointestinal tract occurs [2]. As a result, the neonatal immune system is bombarded with a diverse array of novel antigens, which induce dynamic adaptive changes in immune function to accommodate the acquisition of symbiotic microbes while retaining the capacity to protect against infectious challenge. Microbial colonisation of the neonatal gut is a well-established regulator of several physiological processes that contribute to immunological protection in postnatal life, including the development of the intestinal mucosa and adaptive immunity [2][3]. However, the specific microbiota-derived signals that mediate these processes have not yet been fully characterised. Accumulating evidence suggests short-chain fatty acids (SCFAs), end-products of intestinal bacterial metabolism, as one of the key mediators of neonatal immune development.

Of particular importance during immune development in early life is the establishment of tolerance against self and innocuous antigens derived from exogenous sources such as nutrition, commensal bacteria and the mother. The suppression of immune activation against these antigens is largely mediated by regulatory T (Treg) cells, which represent a subset of CD4+ T cells that are characterised by their immune suppressive effects [4]. Various studies have shown that SCFAs can induce the differentiation and expansion of Tregs, but the exact mechanisms through which SCFAs regulate Treg development and the extent of their effects in the neonate remain poorly defined. Moreover, mounting evidence indicates that SCFAs may also yield pathological effects in neonates when present in abnormal amounts.

2. Neonatal Regulatory T Cells

As immune deviation to Th2 suppresses Th1/Th17 responses in early life, adaptive immune responses during the neonatal period are typically considered to be anti-inflammatory. Critical to the maintenance of this anti-inflammatory status are Treg cells, which are highly prevalent in the cord blood CD4+ T cell compartment (~12%) and neonatal lymphoid tissues (~8%) [5]. In utero, Tregs play an essential role in preventing fetal inflammation and rejection of semi-allogeneic maternal tissue and/or cells since they dampen pro-inflammatory T cell activity and promote tolerance to

maternal antigens [6]. Recent work by Wood et al. also demonstrated that the development of immune tolerance to breastmilk-derived non-inherited maternal antigens (NIMA) in early life was mediated by neonatal Tregs [7]. CD4+ CD25+ Treg cells also negatively regulate cytotoxic activity by alloreactive CD8+ CTLs in neonatal tolerant mice [8]. Moreover, Treg and Th17 cell differentiation are reciprocally regulated [9]. While Foxp3 and RORγt both require TGF-β for upregulation, in the absence of pro-inflammatory cytokines (e.g., IL-6), the Treg master TF dominates and Th17 differentiation is suppressed. This may represent a potential mechanism by which Th17 activity is downregulated in early life.

Accumulating evidence suggests that T cell immunity in early life exhibits a strong bias towards Treg development. For instance, fetal naïve CD4+ T cells proliferate more rapidly and exhibit a greater propensity for Treg differentiation following TCR stimulation compared to their adult counterparts [10]. In line with this, fetal lymphoid tissues are enriched in tolerogenic cytokines that favour Treg differentiation over pro-inflammatory T cell activity, including members of the TGF-β family, which are known to induce Foxp3 upregulation [11]. The TF Helios, which is not expressed in adult naïve T cells, was found to play an important role in contributing to this predisposition, as Helios-deficient fetal naïve CD4+ T cells were unable to differentiate into Treg cells [12]. A similar propensity is evident in neonates. Wang et al. demonstrated that CD4+ thymocytes and T cells in neonatal mice display a ‘default’ tendency to differentiate into Foxp3+ Treg cells irrespective of the TCR stimulus and without the need for exogenous addition of TGF-β [13]. In humans, naïve T cells in cord blood were shown to be more prone to differentiating into functional Foxp3+PD-1+ Treg cells in an antigen-presenting cell (APC)-dependent manner compared to those in adult peripheral blood [14]. These findings indicate that Treg cells play an important role in regulating immune reactivity early in life. However, despite their protective effects, Treg cells have also been implicated in increased susceptibility to infections and dampened vaccine efficacy in neonates. Hence, further research into neonatal Treg cells is crucial to gain a deeper understanding of their function and role in health and disease [15].

3. Short-Chain Fatty Acid Levels in the Neonate

Current understanding of the effects of SCFAs on Tregs during the neonatal period is limited, as most studies concerning the immunomodulatory effects of SCFAs have been largely conducted in adult animals.

Adults derive the substrates for SCFA production from the ingestion of dietary fibre, but for newborns, consumption of solid foods containing dietary fibre does not begin until the weaning period. It is therefore likely that neonates derive SCFAs or the substrates for SCFA production from the maternal breastmilk or formula feed. Maternal breastmilk is the optimal nutritional supply for the newborn infant [16].

Recent work by Stinson et al. demonstrated that human breastmilk contains detectable levels of the SCFAs acetate, butyrate and formate at 1 month postpartum [17], which is in line with previous studies of the human milk metabolome [18] [19]. All of these SCFAs were also detected in the breastmilk samples from a single woman using nuclear magnetic resonance (NMR) as early as 24 days postpartum [20], suggestive of the potential role of breastmilk SCFAs in the neonatal period. However, further studies with a larger cohort of women are required to characterise the SCFA profile of human breastmilk in the early lactation stage. Breastmilk SCFAs are likely produced by the maternal gut microbiota and distributed to the mammary gland via the circulation. They may also be produced by the broad range of bacteria that are resident in the human breastmilk, although evidence for this possibility is currently lacking [17].

Human breastmilk also contains a significant amount of complex non-digestible carbohydrates, which are collectively called human milk oligosaccharides (HMOs). HMOs serve as preferred substrates for certain gut microbiota in SCFA production, including certain species of *Bifidobacterium* [21]. *Bifidobacterium*, being the predominant bacterial genera in the HMO-enriched guts of breastfed neonates, have been implicated in directing immune system development in early life [22]. In line with this, infants colonised with *Bifidobacteria* are known to produce high levels of SCFAs [23], which is unsurprising as the primary products of *Bifidobacterium* fermentation are acetate and lactate [24].

4. The Clinical Role of Short-Chain Fatty Acids in the Neonate

The role of SCFAs has been well documented in immune-mediated disorders in adults, most extensively in asthma. For instance, evidence from mouse models indicates an association between increased maternal dietary microbiota-accessible carbohydrates, SCFA exposure during pregnancy, and reduced offspring asthma mediated by the induction of Tregs in the lung [25]. Human breastmilk samples from atopic mothers had significantly lower concentrations of acetate and butyrate than those of non-atopic mothers. This reduced exposure to human milk SCFAs in early life may program atopy or overweight risk in breastfed infants [17][18].

In contrast, the only neonatal complication where the contribution of SCFAs was investigated is necrotising enterocolitis (NEC), an inflammatory condition of the bowels characterised by decreased epithelial barrier function, translocation of gut bacteria causing sepsis, and perforation of the intestine. He et al. examined the effects of human-to-mouse fecal microbiota transplants (FMT) on intestinal histological injury in mice receiving the microbiome isolated from fecal samples of patients with NEC and control infants matched by gestational age, birth weight, date of birth, mode of delivery and feeding patterns. FMT in germ-free mice with samples from NEC patients achieved higher histological injury scores when compared to mice that received FMT with control samples. The prevalence of Treg cells was reduced in both NEC patients and mice modelling NEC following FMT. NEC patients had increased *Proteobacteria* and decreased SCFA-producing *Firmicutes* and *Bacteroidetes* compared to fecal control samples, and the level of butyrate in the NEC group was lower than the control group. Alterations in microbiota and butyrate levels were maintained in mice following FMT [26].

Roy et al. developed a piglet model which replicates neonatal NEC with the aim of characterising the importance of bacterial fermentation of formula and SCFAs in its pathogenesis. SCFA levels were increased in *Escherichia coli*-fermented formula-treated porcine bowels, which demonstrated inflammation, coagulative necrosis and pneumatosis resembling human NEC. The authors concluded that while *E. coli* treatment alone can initiate intestinal inflammation, injury and apoptosis, bacterial fermentation of formula by *E. coli* generates SCFAs, which contribute to the pathogenesis of NEC. However, these results are difficult to compare to the findings of the previous study or interpret as the effect of acetate, propionate and butyrate was studied together following fermentation rather than on a separate basis. The authors do report, however, that butyrate levels were <9.91 µg/mL, propionate levels were 36.68 ± 4.83 µg/mL and acetate levels were 1783.82 ± 43.61 µg/mL following fermentation, suggesting that harmful effects might be associated with high acetate (and to a lower extent propionate), while protective effects might be linked to high butyrate levels in NEC [27]. A study by Nafday et al. suggests that higher concentrations of SCFAs applied by colonic instillation in a rat model cause colonic mucosal injury, particularly in the early postnatal period. Acetate, butyrate and propionate or a combination of these SCFAs were instilled at high concentrations of 300 nM, and histologic injury scores in the colon were recorded 24 h later. The severity of mucosal injury decreased as the rats matured, with significant injury on days 3 and 9 but minimal injury by postnatal day 23. However, in comparison to the above studies, the applied concentrations of acetate, butyrate and propionate were beyond those reported by Roy et al. by 10×, >2500× and 600× times, respectively, by far exceeding physiological levels, thus making clinical interpretation challenging [28]. Nevertheless, the above three reports do suggest that SCFAs can reach concentrations toxic to mucosal cells, and various SCFAs have different effects on gut health at various concentrations.

References

1. Liu, L.; Johnson, H.L.; Cousens, S.; Perin, J.; Scott, S.; Lawn, J.E.; Rudan, I.; Campbell, H.; Cibulskis, R.; Li, M.; et al. Global, Regional, and National Causes of Child Mortality: An Updated Systematic Analysis for 2010 with Time Trends since 2000. Lancet 2012, 379, 2151–2161.
2. Moore, T.A.; Hanson, C.K.; Anderson-Berry, A. Colonization of the Gastrointestinal Tract in Neonates A Review. Child. Obes. Nutr. 2011, 3, 291–295.
3. Kalbermatter, C.; Fernandez Trigo, N.; Christensen, S.; Ganal-Vonarburg, S.C. Maternal Microbiota, Early Life Colonization and Breast Milk Drive Immune Development in the Newborn. Front. Immunol. 2021, 12, 1768.
4. Debock, I.; Flamand, V. Unbalanced Neonatal CD4+ T-Cell Immunity. Front. Immunol. 2014, 5, 393.
5. Michaëlsson, J.; Mold, J.E.; McCune, J.M.; Nixon, D.F. Regulation of T Cell Responses in the Developing Human Fetus. J. Immunol. 2006, 176, 5741–5748.
6. Burt, T.D. Fetal Regulatory T Cells and Peripheral Immune Tolerance In utero: Implications for Development and Disease. Am. J. Reprod. Immunol. 2013, 69, 346–358.
7. Wood, H.; Acharjee, A.; Pearce, H.; Quraishi, M.N.; Powell, R.; Rossiter, A.; Beggs, A.; Ewer, A.; Moss, P.; Toldi, G. Breastfeeding Promotes Early Neonatal Regulatory T-Cell Expansion and Immune Tolerance of Non-Inherited Maternal Antigens. Allergy 2021, 76, 2447–2460.
8. Gao, Q.; Rouse, T.M.; Kazmerzak, K.; Field, E.H. CD4+CD25+ Cells Regulate CD8 Cell Anergy in Neonatal Tolerant Mice. Transplantation 1999, 68, 1891–1897.
9. Bettelli, E.; Carrier, Y.; Gao, W.; Korn, T.; Strom, T.B.; Oukka, M.; Weiner, H.L.; Kuchroo, V.K. Reciprocal Developmental Pathways for the Generation of Pathogenic Effector TH17 and Regulatory T Cells. Nature 2006, 441, 235–238.

10. Bronevetsky, Y.; Burt, T.D.; McCune, J.M. Lin28b Regulates Fetal Regulatory T Cell Differentiation through Modulation of TGF-β Signaling. J. Immunol. 2016, 197, 4344–4350.
11. Mold, J.E.; Michaëlsson, J.; Burt, T.D.; Muench, M.O.; Beckerman, K.P.; Busch, M.P.; Lee, T.H.; Nixon, D.F.; McCune, J.M. Maternal Alloantigens Promote the Development of Tolerogenic Fetal Regulatory T Cells In utero. Science 2008, 322, 1562–1565.
12. Ng, M.S.F.; Roth, T.L.; Mendoza, V.F.; Marson, A.; Burt, T.D. Helios Enhances the Preferential Differentiation of Human Fetal CD4 + Naïve T Cells into Regulatory T Cells. Sci. Immunol. 2019, 4, eaav5947.
13. Wang, G.; Miyahara, Y.; Guo, Z.; Khattar, M.; Stepkowski, S.M.; Chen, W. “Default” Generation of Neonatal Regulatory T Cells. J. Immunol. 2010, 185, 71–78.
14. de Roock, S.; Hoeks, S.B.E.A.; Meurs, L.; Steur, A.; Hoekstra, M.O.; Prakken, B.J.; Boes, M.; de Kleer, I.M. Critical Role for Programmed Death 1 Signaling and Protein Kinase B in Augmented Regulatory T-Cell Induction in Cord Blood. J. Allergy Clin. Immunol. 2011, 128, 1369–1371.
15. Semmes, E.C.; Chen, J.L.; Goswami, R.; Burt, T.D.; Permar, S.R.; Fouda, G.G. Understanding Early-Life Adaptive Immunity to Guide Interventions for Pediatric Health. Front. Immunol. 2021, 11, 595297.
16. Victora, C.G.; Bahl, R.; Barros, A.J.D.; França, G.V.A.; Horton, S.; Krasevec, J.; Murch, S.; Sankar, M.J.; Walker, N.; Rollins, N.C.; et al. Breastfeeding in the 21st Century: Epidemiology, Mechanisms, and Lifelong Effect. Lancet 2016, 387, 475–490.
17. Stinson, L.F.; Gay, M.C.L.; Koleva, P.T.; Eggesbø, M.; Johnson, C.C.; Wegienka, G.; du Toit, E.; Shimojo, N.; Munblit, D.; Campbell, D.E.; et al. Human Milk From Atopic Mothers Has Lower Levels of Short Chain Fatty Acids. Front. Immunol. 2020, 11, 1427.
18. Prentice, P.M.; Schoemaker, M.H.; Vervoort, J.; Hettinga, K.; Lambers, T.T.; van Tol, E.A.F.; Acerini, C.L.; Olga, L.; Petry, C.J.; Hughes, I.A.; et al. Human Milk Short-Chain Fatty Acid Composition Is Associated with Adiposity Outcomes in Infants. J. Nutr. 2019, 149, 716–722.
19. Smilowitz, J.T.; O’Sullivan, A.; Barile, D.; German, J.B.; Lönnerdal, B.; Slupsky, C.M. The Human Milk Metabolome Reveals Diverse Oligosaccharide Profiles. J. Nutr. 2013, 143, 1709–1718.
20. Wu, J.; Domellöf, M.; Zivkovic, A.M.; Larsson, G.; Öhman, A.; Nording, M.L. NMR-Based Metabolite Profiling of Human Milk: A Pilot Study of Methods for Investigating Compositional Changes during Lactation. Biochem. Biophys. Res. Commun. 2016, 469, 626–632.
21. Bode, L. The Functional Biology of Human Milk Oligosaccharides. Early Hum. Dev. 2015, 91, 619–622.
22. Laursen, M.F.; Sakanaka, M.; von Burg, N.; Mörbe, U.; Andersen, D.; Moll, J.M.; Pekmez, C.T.; Rivollier, A.; Michaelsen, K.F.; Mølgaard, C.; et al. Bifidobacterium Species Associated with Breastfeeding Produce Aromatic Lactic Acids in the Infant Gut. Nat. Microbiol. 2021, 6, 1367–1382.
23. Henrick, B.M.; Rodriguez, L.; Lakshmikanth, T.; Pou, C.; Henckel, E.; Arzoomand, A.; Olin, A.; Wang, J.; Mikes, J.; Tan, Z.; et al. Bifidobacteria-mediated immune system imprinting early in life. Cell 2021, 184, 3884–3898.e11.
24. Pokusaeva, K.; Fitzgerald, G.F.; van Sinderen, D. Carbohydrate Metabolism in Bifidobacteria. Genes Nutr. 2011, 6, 285–306.
25. Gray, L.E.K.; O’Hely, M.; Ranganathan, S.; Sly, P.D.; Vuillermin, P. The Maternal Diet, Gut Bacteria, and Bacterial Metabolites during Pregnancy Influence Offspring Asthma. Front. Immunol. 2017, 8, 365.
26. He, Y.; Du, W.; Xiao, S.; Zeng, B.; She, X.; Liu, D.; Du, H.; Li, L.; Li, F.; Ai, Q.; et al. Colonization of Fecal Microbiota from Patients with Neonatal Necrotizing Enterocolitis Exacerbates Intestinal Injury in Germfree Mice Subjected to Necrotizing Enterocolitis-Induction Protocol via Alterations in Butyrate and Regulatory T Cells. J. Transl. Med. 2021, 19, 1–12.
27. Roy, S.K.; Meng, Q.; Sadowitz, B.D.; Kollisch-Singule, M.; Yepuri, N.; Satalin, J.; Gatto, L.A.; Nieman, G.F.; Cooney, R.N.; Clark, D. Enteral Administration of Bacteria Fermented Formula in Newborn Piglets: A High Fidelity Model for Necrotizing Enterocolitis (NEC). PLoS ONE 2018, 13, e0201172.
28. Nafday, S.M.; Chen, W.; Peng, L.; Babyatsky, M.W.; Holzman, I.R.; Lin, J. Short-Chain Fatty Acids Induce Colonic Mucosal Injury in Rats with Various Postnatal Ages. Pediatr. Res. 2005, 57, 201–204.