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EDITORIAL COMMENTARY

The extra-lymphoid compartment of breast milk: Not a simple transfer of passive immunization

Recent evidence reported that breast milk in humans can be considered as an extra-lymphoid compartment enriched in CD8+ effector memory T cells (CD8$^+$TEMs), which are able to transfer immunity even as a cell dialyzed extract.[1] Ian A. Myles and Sandip K. Datta showed that in breast milk leukocytes can transfer passive immunity via a cell-free dialyzed component (less than or equal to) 10 kDa from CD8$^+$TEMs.[1] The ability of this dialyzed fraction (dialyzable leukocyte extract) to induce an antigen-specific immune response in infants is particularly intriguing, as milk is digested and absorbed by neonatal gut.[1] The authors sustained the thesis that besides to immune cells immunization can be transferred via a cell-derived dialyzed component, consistent with Lawrence' transfer factor. Briefly speaking, the novelty reported by the authors put in the spotlight the crucial importance of breastfeeding in immunity and contributed in raising important questions about the role of different lactation approaches in skewing the immune endowment in the early life.

The very close relationship between infants and their mothers should affect the complex mechanisms driving the development of neonatal immunity as a whole, probably selecting, at least initially, a kind of immunity much more similar to the mucosal immunity. According to our opinion, therefore, a kind of "extended gut control mechanism," between the mother and her child, may explain why CD8+ effector memory T cells (CD8$^+$TEMs) are particularly crucial for neonatal immune development, more than the expected task of a passive immunization from the immunocompetent mother we described so far (Fig. 1). Yet, before expanding further this hypothesis, we would like to summarize the evidence reported by the authors, highlighting the major impact of their observation on pediatric immunology.

Despite the role of memory CD8$^+$ T cells is still far to be fully elucidated, the presence of this T-cell subset in the colostrum is decidedly abudant.[2] Previous studies performed in our laboratories showed that colostrum is particularly enriched in CD45RA$^-$/CD27$^-$ CD8$^+$TEMs, but also in CD45RA$^+$/CD27$^-$ effector cells, assessing that these subpopulations should be really crucial for infants' immunity, even via their lysed immunogenic products.[1,3] Myles and Datta showed that dialyzable leukocyte extract (DLE) from colostrum or breast milk behaved as DLE from blood.[1] They demonstrated that pulsed dendritic cells (DCs) with *Candida albicans* antigen, upon DLE challenge increased the DC expression of IL-6, whereas the removal of T-cell receptor-beta from DLE reduced this effect.[1] According to the authors, milk lysates contain a segment of the TCR that should be able to stimulate antigen-matched DCs, and confer a passive immunity to the infant.[1]

The extra-lymphoid compartment of breast milk is endowed with hallmarks very similar to the immune microenvironment of gut, as we are going to detail further on. We are persuaded, therefore, that the evidence reported by Myles and Datta may shed light on the immuno-modulatory role exerted by colostrum in the full maturation of the infant's immune system. In this sense, Myles and Datta's paper further contributed to raise important questions about the actual role of CD8$^+$TEMs and their soluble products in the development of the immune microenvironment of the neonatal gut. Recent evidence supports this hypothesis. For example, infants have high contents of butyrate in the gut during their early life upon breastfeeding[4] and butyrate modulates the effector action of CD8$^+$ T memory cells in the gut.[5] Besides short chain fatty acids (SCFAs), fundamental evidence recently showed that milk contains a panoply of factors including cytokines, soluble receptors, oligosaccharides, microbiota, immune and stem cells, and other mediators that are able to enhance oral and mucosal tolerance.[6,7] The postpartum period is particularly crucial for the neonatal life and breast milk should play a pivotal role in skewing a proper oral tolerance and a functional immune response in infants. Milk components not only contribute to the "mother-to-infant" passive immunization but promote the shaping of mucosal tolerance and improve newborn's cell-mediated immunity. Some of these factors from milk may more easily penetrate the gut mucosal barrier early, while tight junctions are open. Actually, colostrum promotes the rapid progress of the intercellular tight junctions in the epithelial cells of intestine (IECs) and triggers the building of a gut immune barrier.[8]

The composition of colostrum and milk in the first 6 months of postpartum neonatal life resembles a typical gut microenvironment, with soluble factors, CD8$^+$ memory cells, and microbiota.[8] Some microbiota species in the breast milk, such as *Bacteroides fragilis*, are particularly important to induce the expansion of CD103$^-$ DCs in the lamina propria, alongside with vitamin A and TGF-β in the milk, as infant's IECs are immature, have a reduced gut microbiome a scanty milieu of cytokines.[8] In our opinion, immunity in neonatal gut is quite an extension of mother's mucosal immunity via the extra-lymphoid milk compartment, driving immune B-cell memory toward a mucosal T-cell memory.[9] In infants, breastfeeding causes, within 6 months a reduction in the CD27$^+$IgM$^+$, CD27$^+$IgA$^+$, and CD27$^-$IgG$^+$ B cells subsets, turning to CD8$^+$ T central memory cells (CD45RO$^+$, CCR7$^+$), which increase of about 19% in the early postpartum life, with a breastfeeding period lasting 3 months and 20% if lasting 6 months.[9]

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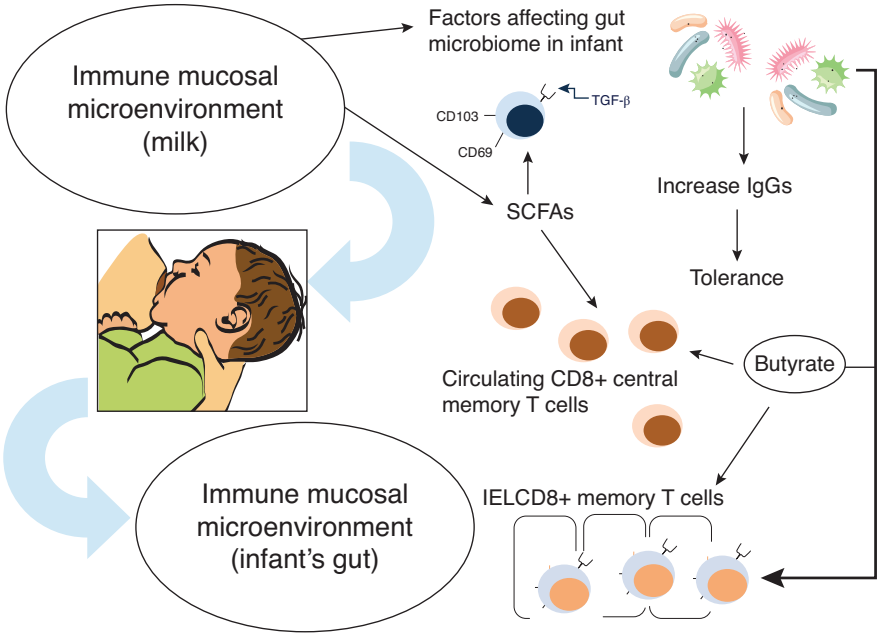

FIGURE 1 Diagram briefly describing the concept of "extended gut." Components in the mother's milk recall the immune microenvironment in the postpartum early life (left). These components are able to tune the composition of newborn's gut microbiome, influencing IgG production and the subsequent gut immune tolerance and tuning by SCFA, such as butyrate, the development of memory CD8+ T cells in the infant

The microbial composition of milk, which is affected also by mother's delivery, shapes the early composition of the microbiome in the neonatal period during breast-feeding and drives many further modifications leading to the infant's immune development. Breast-feeding neonates have mostly Lactobacillus and Bifidobacterium in their gut microbiome, species that easily degrades a kind of oligosaccharides, leading to SCFAs and an increase in the expression of IgGs, thus promoting immune tolerance in the gut. On the contrary, babies fed with milk formulas have mainly Enterbacteria, Bacteroides, Enterococcus, Clostiridia, and Streptococcus in the gut microbiota.[9]

It might be particularly intriguing to envisage which kind of microbes, foods, and bacterial strains are present in defined collected DLEs and to investigate about their role in infant immunity. Breast-feeding is not simply a passive immunization but the complete making of a gut immune system including mucosal tolerance.

In our "extended gut" hypothesis, the role of CD8+ memory cells is of the utmost importance and may shed light on the role exerted by TCR in DLE. The thymus leukemia antigen, a kind of nonclassical MHC I antigen, is able to induce on DCs cross-linking with CD8$\alpha\alpha$ upon activated CD8$\alpha\beta$ T cells, the selection of precursors of $CD8^+$ memory cells, a mechanism particularly important for generating antigen-sensitive CD8$\alpha\beta$ memory T cells.[10] The prolonged interaction via a TCR with antigen-presenting cells such as DCs promotes and enhances the expansion of $CD8^+$TEMs. While the DLE approach may induce an "immunogenic" increase in gut $CD8^+$TEMs, colostrum should involve mainly the ability of CD8$\alpha\alpha$ T cells to prevent apoptosis in CD8$\alpha\beta$ memory T cells, promoting their expression in the newborn intestine.[10] The rapid expansion of $CD8^+$TEMs by immunogenic factors is down-regulated by apoptotic signals, while CD8$\alpha\alpha$ T cells promotes the effector differentiation of CD8$\alpha\beta$ memory T cells in the gut, preventing them from rapid massive apoptosis.[10] Therefore, the puzzling issue to be debated regards the physiological maturation of $CD8^+$TEMs in the newborn's gut via either DLE or whole colostrum.

Actually, we should take into consideration that due to the initial increased intestinal permeability of the newborn gut mucosa, many dialyzable components, even from bovine colostrum, may better allow penetration of immune factors and possibly contributing in accelerating the more correct tightening of the cell junctions of the intestinal barrier in a fully sequential manner, after tolerizing and sensitizing antigens and immune factors are allowed to penetrate the gut mucosa. This consideration should still be further elucidated.

Our hypothesis about the similarity between colostrum immunity and gut immunity must not be undertaken as such, obviously, due to the clear differences between those compartments but from an immune perspective. Colostrum or breast milk cannot be simply considered nourishment for infants. Breast-feeding might have a major role in developing gut microbiome and gut immunity in newborns, taking into account the close relationship with mother's immunity. Therefore, while it could be suggested that DLE can improve intestinal barrier with soluble factors and $CD8^+$TEMs, colostrum might have a more outstanding role in shaping gut microbiome and hence gut immunity in newborns, besides improving the development of the intestinal barrier.

The research study by Myles and Datta deserves, therefore, further consideration.

First, it indirectly contributed in putting into the spotlight the role of colostrum, as it could account for the development of proper gut immunity in the newborn. This could occur with the role of crucial CD8+ subpopulations in tailoring the optimal immune microenvironment for the expression of intestinal $CD8^+$TEMs. Second, it focused on DLE as being an opportunity to replace breastfeeding, once the effect of the former on the newborn's gut immunity is fully elucidated.

We hope to expand the debate about how newborns build up their immunity via breastfeeding and how to characterize those factors in the extra-lymphoid compartment of colostrum able to be supplemented or enriched in formulas.

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REFERENCES

1. Myles JA, Datta SK. Breast milk confers passive cellular immunity via CD8-depenent mechanisms. *J Leuk Biol*. 2020. In press.
2. Samji T, Khanna KM. Understanding memory CD8$^+$ T cells. *Immunol Lett*. 2017;185:32-39.
3. Peroni DG, Chirumbolo S, Veneri D, et al. Colostrum-derived B and T cells as an extra-lymphoid compartment of effector cell populations in humans. *J Matern Fetal Neonatal Med*. 2013;26(2):137-142.
4. Brink LR, Mercer KE, Piccolo BD, et al. Neonatal diet alters fecal microbiota and metabolome profiles at different ages in infants fed breast milk or formula. *Am J Clin Nutr*. 2020;111(6):1190-1202.
5. Luu M, Weigand K, Wedi F, et al. Regulation of the effector function of CD8$^+$ T cells by gut microbiota-derived metabolite butyrate. *Sci Rep*. 2018;8(1):14430.
6. Dawod B, Marshall JS. Cytokines and soluble receptors in breast milk as enhancers of oral tolerance development. *Front Immunol*. 2019;10. https://doi.org/10.3389/fimmu.2019.00016.
7. Bardanzellu F, Peroni DG, Fanos V. Human breast milk: bioactive components, from stem cells to health outcomes. *Curr Nutr Rep*. 2020;9(1):1-13.
8. Dzidic M, Boix-Amorós A, Selma-Royo M, Mira A, Collado MC. Gut microbiota and mucosal immunity in the neonate. *Med Sci*. 2018;6(3). https://doi.org/10.3390/medsci6030056.
9. Jansen MA, van den Heuvel D, van Zelm MC, et al. Decreased memory B cells and increased CD8 memory T cells in blood of breastfed children: the generation R study. *PLoS One*. 2015;10(5):e0126019.
10. Huang Y, Park Y, Wang-Zhu Y, et al. Mucosal memory CD8$^+$ T cells are selected in the periphery by an MHC class I molecule. *Nat Immunol*. 2011;12(11):1086-1095.