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LETTER

Colostrum is required for the postnatal ontogeny of small intestine innate lymphoid type 2 cells and successful anti-helminth defences

To the Editor,

Colostrum is the physiological food for the first 72 h of a newborn.[1] Its window of intake and high content in microbiota-shaping and growth factors[1] suggest that colostrum is critical in guiding gut immune development. To address this hypothesis, we developed a mouse model of colostrum deprivation (Figure 1A). Like humans, mice have different lactation stages.[2] We compared pups nursed immediately after birth by dams that no longer produced colostrum (Day 9 of lactation, a well-defined lactation stage in mice that is distinct from colostrum[2]) with control pups. This allowed us to assess the causal role of colostrum in the perinatal expansion of two cell types important in gut immune regulation, namely ILCs and CD4+ T cells.

While we found a major increase in small intestine ILC2 frequency and numbers between Days 7 and 14 in control mice, ILC2 expansion was severely compromised when mice were deprived of colostrum (Figure 1B). Colostrum deprivation did not impact gut ILC, ILC1, ILC3, CD4+ T cells, Th1, Th2, Th17 and Treg cells representation in 2-week-old mice (Figure S1), suggesting a selective effect of colostrum on ILC2 ontogeny. The low numbers of Th1, Th2 and Th17 cells are consistent with the predominantly naïve T cell compartment at this time point of life.[3] A more detailed analysis of CD4 T-cell phenotype including T-cell activation and their response to inflammatory signals remains to be performed to fully elucidate the role of colostrum in T-cell ontogeny.

To verify that the decreased representation of ILC2 in 14-day-old mice was due to the imprinting of a different trajectory due to the absence of colostrum at birth, we performed two additional experiments. First, we investigated whether the impact of the intervention on ILC2 at Day 14 was due to changes in diet at birth versus at later time points. Therefore, pups were cross-fostered at Day 10, instead of Day 0, to dams that gave birth 9 days earlier than their biological mothers. As shown in Figure S2A, their percentage and number of ILC2 at Day 14 were similar to ctrl mice demonstrating that ILC2 ontogeny is not affected by exposure to 'old' milk at the time when ILC2 massively expands. We then investigated whether the reduced ILC2 expansion in mice nursed from birth by dams at Day 9 of lactation was due to colostrum deprivation versus exposure to mature milk at birth. We cross-fostered pups at birth to dams that had delivered only 3 days earlier. Similar to mice nursed by mothers at Day 9 of lactation, we found a major decrease in the representation of ILC2 compared to control mice (Figure S2B), supporting the hypothesis that colostrum at birth is required for ILC2 ontogeny.

Given the importance of the microbiota in gut immune ontogeny, we next evaluated whether colostrum shaped the gut microbiota.[3] Both the alpha and beta diversity of the gut microbiota significantly differed between control and colostrum-deprived 2-week-old mice (Figure S3A,B). To address whether this difference played a causal role in decreased ILC2 expansion in colostrum-deprived mice, experiments were repeated in germ-free mice. As observed in specific pathogen-free mice, we found that colostrum deprivation resulted in a 30% decrease in small intestine ILC2 compared to control germ-free mice (Figure S3C), indicating that the role of colostrum in ILC2 ontogeny is microbiota independent.

The alarmins, IL-33, IL-25 and TSLP, play a major role in ILC2 proliferation and/or activation.[4] During the first days of lung alveolarization, transient high levels of IL-33 were found to promote ILC2 accumulation.[5] We also observed a transient increase in IL-33 secretion in the gut of 4-day-old control mice, which was two-fold lower in colostrum-deprived mice (Figure 1C). In addition to IL-33, IL-25, which is known to be important for gut ILC2 expansion/activation was significantly reduced in colostrum-deprived mice at Day 4 (Figure 1C). TSLP has a synergistic effect on the proliferation and Type 2 cytokine production of ILC2 and may be particularly important in early life where IL-33 alone is not sufficient to activate cytokine secretion.[4] We also found a trend towards reduced TSLP secretion in colostrum-deprived mice (Figure 1C). Altogether, these data strongly suggest an important role for colostrum in alarmins-driven perinatal ILC2 expansion.

Whereas we found that colostrum intake affected neither the circulating pool of ILC2 nor the expression of gut-homing molecules CCR9 and α4β7 on small intestine ILC2 nor their proliferation (Figure S4A–C), we found a threefold increase in apoptotic ILC2 in colostrum-deprived 2-week-old mice compared to controls (Figure 1D). The reduction in alarmins secretion in

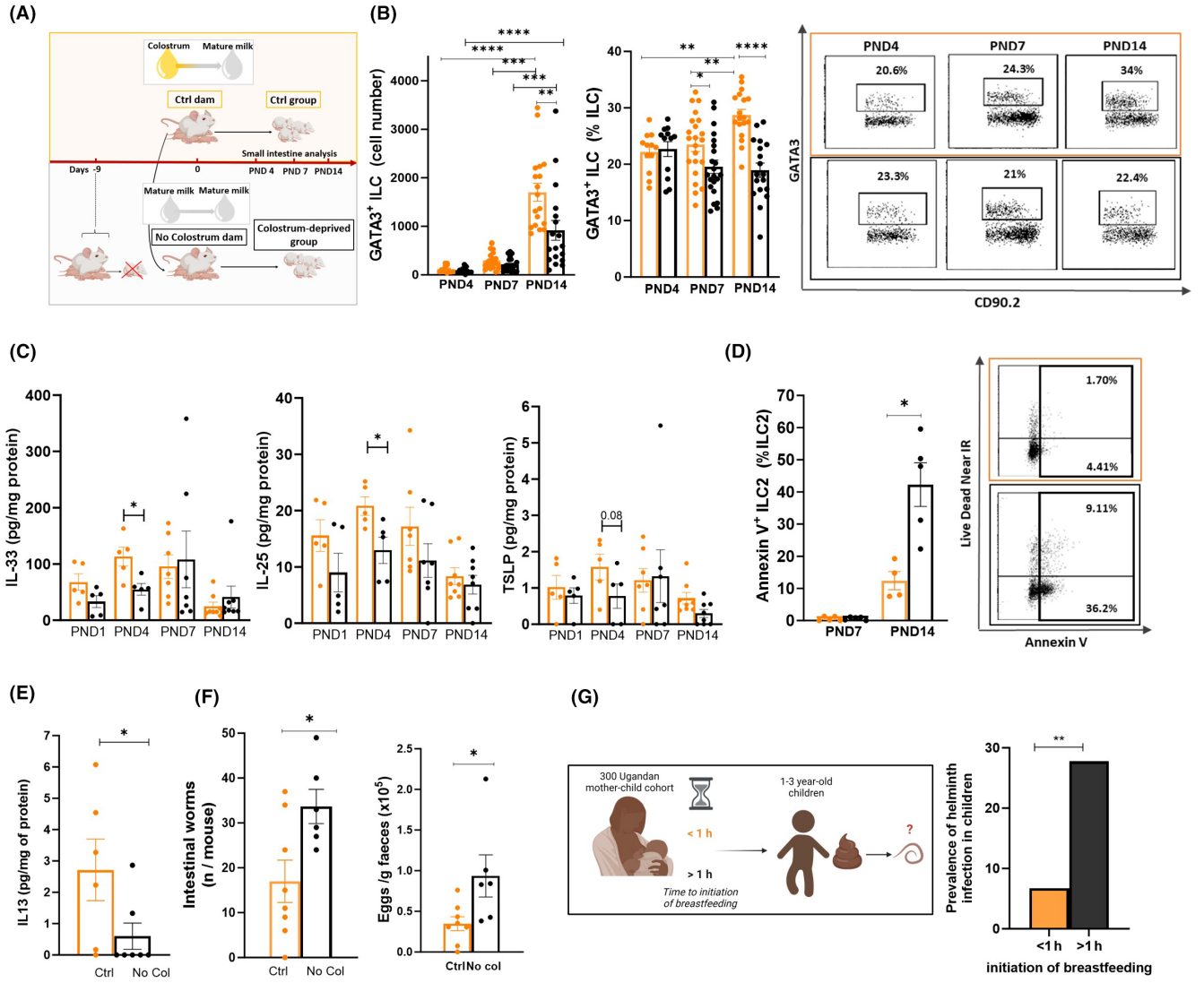

FIGURE 1 (A) Mouse model of colostrum deprivation. Colostrum-deprived pups were cross-fostered at birth by dams that were no longer providing colostrum ((postnatal day (PND) = 9); no colostrum dam, orange). Control groups were cross-fostered at birth by dams that just delivered (Day 0) (Ctrl dam, black). (B–E) Gut ontogeny. (B) Flow cytometry analysis of small intestine ILC2 on PND 4, 7 and 14. Left panel. Individual data on ILC2 number frequency among total ILC and their number assessed by flow cytometry. Right panel. Dot plot showing concatenated event-number controlled averages of ILC2 of all animals from within each condition from a single experiment (C) Kinetic of gut tissue IL-33, IL-25 and TSLP content (D) Flow cytometry analysis of AnnexinV$^+$ apoptotic gut ILC2 . Left panel. Individual data. Right panel. Dot plot showing concatenated event-number controlled averages of AnnexinV$^+$ ILC2 (E) Gut Tissue IL-13 content at Day 14 (F, G) Susceptibility to helminth infection (F) Number of worms in small intestine and eggs in the faeces 21 days post *Heligmosomoides polygyrus* infection in 3-week-old mice. (G) Prevalence of helminths in faeces of 1-3-year-old Ugandan children who were breastfed within or after 1 h after delivery; (A–E) Data represent mean +/− sem and individual values; $n = 5–8$ mice per group, four experiments (B), one experiment (C–F). Statistical significance of the differences between ctrl and No colostrum groups was analyzed using Mann–Whitney test and between different time points using Kruskal–Wallis test. (*$p < .05$; **$p < .01$; ***$p < .001$, ****$p < .0001$). (F) Statistical significance determined using Fisher test.

colostrum-deprived mice may contribute to their increased apoptotic death of ILC2.[4]

Finally, we evaluate the functional consequences of a decreased representation of small intestine ILC2 in colostrum-deprived mice by measuring their gut content in IL-13 and their ability to clear helminth infection, which is known to involve ILC2.[5] IL-13 levels in gut tissues form colostrum-deprived mice were significantly reduced compared to control mice (Figure 1E). When 3-week-old colostrum-deprived mice were infected with Heligmosomoides polygyrus, twice as many worms in the intestine and threefold more eggs in the faeces were found 21 days later, compared to control mice (Figure 1F), showing the decreased ability of colostrum-deprived mice to efficiently control helminth infection later in life. Our data suggest that the reduced representation of ILC2 underlies this increased susceptibility to helminth infection. Future studies will establish whether other characteristics of colostrum-deprived mice may explain this

TABLE 1 Maternal and children characteristics.

	Full cohort	Early breastfeeding initiation	Delayed breastfeeding initiation	
	(*N* = 300)	(*N* = 282)	(*N* = 18)	*p*-value
Maternal age [years; mean (SD)]	27.3 (6.7)	27.6 (6.7)	22.6 (5.3)	.002**
Child age [years; mean (SD)]	2.26 (0.7)	2.28 (0.7)	1.92 (0.7)	.03*
Vaginal delivery [*n* (%)]	296 (99)	279 (99)	17 (95)	.17
Birthing location [*n* (%)]				.96
Institution	218 (73)	205 (73)	13 (72)	
Home	82 (27)	77 (27)	5 (28)	
Infant Sex (female) [*n* (%)]	152 (51)	144 (51)	8 (44)	.58
Gestation age term (>37 weeks) [*n* (%)]	286 (95)	270 (96)	16 (89)	.2
Breastfeeding parameters				
Early initiation of breastfeeding	282 (94)			
Exclusive Breastfeeding for first 6 months [*n* (%)]	93 (31)	87 (30)	6 (33)	.8
Duration of any Breastfeeding [months; Median (IQR)]	14 (12,18)	14 (12,18)	14 (12,18)	
Children with helminth infection [n (%)]	24 (8)	19 (6)	5 (28)	**.009****

Note: Unpaired t-tests were used to compare continuous variables and Pearson's Chi square or Fisher's exact tests to compare categorical variables.
*$p < .05$; **$p < .01$.

observation. As a first step in translating our findings to humans, we analyzed the association between delayed initiation of breastfeeding (based on WHO guidelines recommending initiation within 1 h[6]), a practice that deprives the newborn of the full dose of colostrum, and the susceptibility to helminth infection in young children. Three-hundred mothers and their children (aged 1–3 years) were recruited in Uganda, and data on early feeding practices were collected retrospectively (Table 1). Among mothers who initiated breastfeeding after 1 h, 78% initiated breastfeeding on the first day of life, 17% on Day 2 and 5% after 1 week. Delayed initiation of breastfeeding was strongly associated with an increased risk for helminth infection [OR (95% CI): 5.3 (1.9–15.06, $p = .009$)] (Figure 1G). Data remained significant after adjustment for maternal and child age, which differed between the two groups [aOR (95% CI): 6.6 (2–22)]. A limitation of this proof-of-concept study is the recall bias on early feeding practice. To fully establish the importance of colostrum in the prevention of helminth infections, prospective studies specifically addressing the relationship between the amount of colostrum feeding and helminth infections will be required.

Mouse and human data show there is a massive infiltration of ILC2 in the infant small intestine[7,8]; however, factors involved in this process remained unknown. This work uncovers a critical role for colostrum in gut ILC2 ontogeny and reveals its importance for anti-helminth defence. Given the importance of ILC2 in the regulation of allergic responses,[5] future research will need to address the impact of colostrum deprivation at birth on allergy risk. Despite WHO guidelines, more than half of newborns globally are non-optimally colostrum-fed,[6] which deprives the newborn of colostrum bioactives at a time of both high vulnerability and critical developmental change. There is strong evidence that optimal colostrum feeding has a major impact on the prevention of neonatal mortality, especially in low-and middle-income countries.[9] Our data provide evidence that colostrum may also be fundamental in imprinting healthy immune development. Expanding the knowledge on colostrum bioactives responsible for ILC2 expansion should lead to a major impact on child health.

AUTHOR CONTRIBUTIONS

Project design and supervision: VV. Conceptualization: VV (whole project), AR (whole project), ML (GF exp), DL (GF exp), RL (ILC2 ontogeny), RM (helminth mice), TE (helminth human) and RB (microbiota). Mice experiments, data analysis and interpretation: AR, LvdE, CI, SM, ND, CT, ML, NS, TY and VV. Human data collection and analysis: MB, CR and TE. Microbiota data analysis: FS, RB and VV.

Writing—original draft: AR and VV. Writing—review and editing: All.

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CONFLICT OF INTEREST STATEMENT

Florence Servant declares she is an employee of the "Vaiomer SAS" company. All the other authors declare they have no conflict of interest related to this publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.