Letter: GATA binding protein 3(+) group 2 innate lymphoid cells are present in cord blood and in higher proportions in male than in female neonates

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To the Editor:

Innate lymphoid cells (ILCs) have recently gained much attention as important mediators of tissue homeostasis and inflammation. In contrast to other members of the ILC family, including ILC1 and ILC3, group 2 ILCs (ILC2) produce IL-5 and IL-13 in response to IL-25, IL-33, and thymic stromal lymphopoietin, cytokines that may be released after epithelial damage. In spite of their association with type 2 mediated inflammation in both humans and mice, it is not known whether ILC2 are present in cord blood or whether they are involved in subsequent allergy development. Early life events occurring during critical windows of immune development can have a long-term impact on immune-mediated diseases, and immune status at birth, in part influenced by maternal immunity, may be an intrinsic factor predisposing to allergy development. The aim of this study was to assess whether ILC2 are present in cord blood and whether their proportions are associated with allergy development and sex.

We report here that ILC2 are present in human cord blood (for gating strategies, see this article’s Methods section and Fig E1 in the Online Repository at www.jacionline.org). Thus, we identified a population of lineage negative (Lin−) cells lacking the expression of cell surface markers associated with T cells (CD3, CD4, T-cell receptor (TCR)αβ, and TCRγδ), B cells (CD19), dendritic cells (CD11c, CD123, CD303, CD1a), macrophages/monocytes (CD14), mast cells and basophils (FcεRIα), and hematopoietic progenitor cells (CD34). The cells expressed CD161, CD127, and CRTH2 and lacked expression of CD56 (Fig 1, A), while CD117 was heterogeneously expressed (data not shown), as previously described in adult blood ILC2. It was recently discovered that human ILC2 are dependent on the expression of transcription factor GATA-3, which is important for IL-5 and IL-13 cytokine production from these cells. Accordingly, we found that ILC2 in peripheral blood of adults (n = 7) and neonates (n = 8) expressed GATA-3 in a similar way as Th2 cells, while natural killer cells (CD56dim) had low GATA-3 expression (Fig 1, A). The GATA-3 expression was higher in neonate than in adult ILC2 (P = .009), expressed as a ratio between ILC2 and natural killer cells (Fig 1, D). Speculatively, the higher GATA-3 expression could be related to the function and cytokine-producing capacity of ILC2 in cord blood. Unfortunately, no functional assays could be performed because of insufficient amounts of blood for cell isolation and culturing. However, previous studies have demonstrated the crucial function of GATA-3 in ILC2 since ectopic expression of GATA-3 in human Lin−CD127+(+)CD117−RTH2−) cells resulted in induction of CRTH2 and the capacity to produce high amounts of type 2 cytokines in response to thymic stromal lymphopoietin plus IL-33.
Because a more pronounced T\(_{H2}\) deviation is suggested to precede the development of allergic disease\(^6\) and ILC2 have been implicated to be involved in allergic responses,\(^1\)\(^2\) we investigated whether high ILC2 proportions in cord blood could predict the development of allergic disease. However, no differences were detected in cord blood between children who later developed allergic diseases and those who remained nonallergic up to the age of 6 years (Table I) (percentages of ILC2 among lymphocytes: mean, 0.09 \pm 0.03, \(n = 7\), and mean 0.09 \pm 0.02, \(n = 7\), respectively). Neither did maternal atopy affect the ILC2 proportions (percentages of ILC2 among lymphocytes: mean, 0.09 \pm 0.02, \(n = 12\), and mean 0.07 \pm 0.01, \(n = 15\), in children of atopic and nonatopic mothers, respectively). Our observations suggest that cord blood ILC2 proportions are not related to allergy development, although this should be confirmed in a larger study. The increased ILC2 proportions in male neonates could be associated with the heightened T\(_{H2}\) responses and susceptibility to T\(_{H1}\)-dependent infections in boys than in girls during childhood.\(^6\)\(^8\)

In conclusion, we demonstrated that ILC2 are present in cord blood and display a higher GATA-3 expression than in adult ILC2. The increased ILC2 proportions in male neonates could be associated with the heightened T\(_{H2}\) responses and susceptibility to T\(_{H1}\)-dependent infections in boys than in girls during childhood.

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REFERENCES


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METHODS

Study group
Volunteer pregnant women were recruited from the maternal health care unit in Linköping. The children, 14 males and 13 females, were born in a period from August 2000 to March 2003. Only 1 of the children was delivered by cesarean section. Both parents signed an informed consent before the children’s inclusion. The Regional Ethics Committee for Human Research at the University Hospital of Linköping approved the study (Dnr 99184 and 99323).

Seven of the children developed allergic symptoms and sensitization (a positive SPT result and/or detectable IgE to allergens) during the first 6 years of life (Table I) and 7 children remained healthy without sensitization. The remaining children developed either allergic symptoms without sensitization (n = 4) or sensitization without allergic symptoms (n = 4), while 5 children were not followed up because of various reasons. Because these 13 children cannot be definitely classified, they were not included in the allergy comparisons.

The children were monitored by research nurses at 6 and 12 months and follow-ups were done at 2 and 6 years by a pediatric allergologist. The parents answered questionnaires about environmental factors and allergic symptoms at 3, 6, 12, and 18 months and at 2 and 6 years.

Symptomatic diagnoses were set depending on predefined criteria. Atopic dermatitis was defined as chronic, pruritic, noninfectious dermatitis with typical appearance and anatomical localization. Urticaria was defined as an immediate skin reaction caused by the same allergen within an hour at least 2 times. Asthma was defined as bronchial obstruction with wheezing at least 3 times in total, at least 1 of these times diagnosed by a physician. Allergic rhinoconjunctivitis was defined as rhinitis and conjunctivitis appearing at least twice after exposure to an inhalant allergen and not related to infection. Food allergy was defined as vomiting and/or diarrhea on at least 2 separate occasions after the intake of a certain offending food. Of the 7 allergic children, 6 had atopic dermatitis, 3 had asthma, and 3 had rhinoconjunctivitis.

Sensitization was also measured through the analysis of circulating IgE antibodies to allergens. Levels of IgE antibodies to food antigens including egg, milk, fish, wheat, peanut, and soybean were tested with the Phadiato- pllnfant test (Phadia, Uppsala, Sweden) at ages 6, 12, and 24 months and 6 years. The Phadiatop test (Phadia) was used at 6 years to detect IgE antibodies to inhalant antigens birch, mugwort, timothy, cat, dog, horse, house-dust mite, and Cladosporium.

Volunteer adult individuals were recruited from the maternal health care unit in Linköping. The children, 14 males and 13 females, were born in a period from August 2000 to March 2003. Only 1 of the children was delivered by cesarean section. Both parents signed an informed consent before the children’s inclusion. The Regional Ethics Committee for Human Research at the University Hospital of Linköping approved the study (Dnr 99184 and 99323).

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Volunteer adult individuals were recruited (9 men and 8 women, mean age, 34.9 and 35.1 years, respectively).

Sample preparations
Cord and adult peripheral blood was collected into heparinized vacutainers. Cord and adult PBMCs were obtained by ficoll gradient centrifugation. Briefly, blood was layered on a ficoll gradient, centrifuged, and the PBMC layer was collected with subsequently washing and centrifugation steps. Cells were resuspended in freezing media consisting of 40% Iscove’s modified Dulbecco’s medium, 10% dimethyl sulfoxide, and 50% FCS. Cells were then placed in a freezing container at −70°C for 24 hours and thereafter stored in liquid nitrogen, pending analysis.

Identification and characterization of cord and peripheral blood ILC2
To explore the presence of ILC2, flow cytometry was used to analyze peripheral and cord blood. To obtain a reliable number of cells for analysis, 3 million cells were used for staining (cord blood mononuclear cell/PBMC) and 1 million lymphocytes were collected on the flow cytometer using forward scatter/side scatter. A lineage-negative population was identified; the antibody cocktail included the following antibodies (clone name within parentheses): fluorescein isothiocyanate–conjugated anti-CD1a (HI149), CD3 (OKT3), CD11c (3.9), CD123 (6H6), FcεRIα (AER-37), TCRαβ (IP26) (all from BioLegend, San Diego, Calif); CD4 (RPA-T4), CD14 (MφP9), CD19 (HB19), CD34 (581), and TCRγδ (B1) (all from Beckton Dickson, Franklin Lakes, NJ); and CD303 (AC144, Miltenyi, Bergisch Gladbach, Germany). The low side scatter population expressed PECy7-conjugated anti-CD127 (B34.34, Beckman Coulter, Brea, Calif), phycoerythrin-conjugated anti-CD161 (HP-3G10, BioLegend), and allophycocyanin-conjugated anti-CD294 (BM16, BD Pharmingen, Franklin Lakes, NJ), and was partially positive for PerCpCy5.5-conjugated anti-CD117 (104D2, BioLegend) as compared with natural killer cells (APC Cy7-conjugated anti-CD56 [HC55], BioLegend). Cells were also stained with phycoerythrin-conjugated anti-GATA3 (TWA1, ebioscience, San Diego, Calif) according to the manufacturer’s instructions. Data were acquired on a BD FACS CANTO II and analyzed using Kaluza 1.2 (Beckman Coulter).

The effect of freezing was evaluated on peripheral blood from 6 individuals. The proportion of ILC2 was not affected by the freezing procedure (data not shown). However, the CD117 expression was significantly decreased after freezing and thawing (P = .003, data not shown). Because the proportion of ILC2 was unaffected by the freeze-thawing procedure, we used freeze-thawed samples from the birth cohort throughout this study. Also, the PBMCs from adults were frozen to limit variations between comparisons with CBMCs.

Statistics
Data are means ± SD unless indicated otherwise. Statistical significance was examined by unpaired Student t test. Statistical analyses were performed with GraphPad Prism software v5.0.
FIG E1. Gating strategy for ILC2 in human adult peripheral blood. To obtain a reliable number of cells for analysis, 3 million PBMCs were used for flow cytometry staining and 1 million lymphocytes were collected on the flow cytometer using forward scatter/side scatter. A lineage-negative population expressing CD161 was identified that also expressed CD127 and CRTH2 but was negative for CD56.