Initiation of Cellular Organization in Lymph Nodes Is Regulated by Non-B Cell-Derived Signals and Is Not Dependent on CXC Chemokine Ligand 13


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The molecular and cellular events that initiate the formation of T and B cell areas in developing lymph nodes are poorly understood. In this study we show that formation of the lymphoid architecture in murine neonatal lymph nodes evolves through a series of distinct stages. The initial segregation of T and B cells is regulated in a CXCL13-independent manner, characterized by the localization of B cells in a ring-like pattern in the outer cortex on day 4. However, during this CXCL13-independent phase of lymph node modeling, CXCL13 is expressed and regulated in a lymphotoxin-αβ2 (LTαβ2)-dependent manner. Surprisingly, neonatal B cells are unable to respond to this chemokine and also lack surface LTαβ2 expression. At this time, CD45+CD4+CD3− cells are the predominant LTαβ2-expressing cells and are also capable of responding to CXCL13. From day 4 on, architectural changes become CXCL13 dependent, and B cells become fully CXCL13 responsive, express LTαβ2, and cluster in anatomically distinct follicles. Because the initial induction of CXCL13 is dependent on LTαβ2, a role for CD45+CD4+CD3− cells in inducing chemokine expression in the developing lymph nodes is proposed and, as such, a role in initiation of the shaping of the microenvironment. The Journal of Immunology, 2004, 173: 4889–4896.

One of the hallmarks of the adaptive immune system is the presence of secondary lymphoid organs, which provide the appropriate environment for activation and expansion of naive lymphocytes in response to signals delivered by APCs. Lymph nodes (LNs),2 Peyer’s patches (PPs), and spleen contain anatomically distinct, B cell-rich microenvironments in which an environment permissive for affinity maturation and the generation of immunological memory is created. As such, the correct formation of lymphoid follicles is important for the ability to mount adaptive immune responses (1–5). The generation of gene-targeted mice provided insights into the cellular and molecular pathways governing the establishment of B cell follicles, implicating several members of the TNF superfamily as pivotal regulators of this process (6–13). Mice with deficient TNF receptor I (TNF-R1) or lymphotoxin-β receptor (LTβ-R) signaling lack follicle formation in the spleen, whereas impaired LTβ-R signaling in addition leads to a block in the development of LNs and PPs (8, 9, 12, 14–18). The cellular source of LTαβ2 and TNF-α was determined in a series of splenocyte and bone marrow transfer studies (6, 19, 20). B cells have been shown to deliver the signals necessary for both the induction of follicular dendritic cells (FDCs) as well as the maintenance of lymphoid follicles in adult animals. In spleen, engagement of the LTβ-R by B cells results in increased production of the chemokine CXCL13 by stromal cells. Binding of CXCL13 to its receptor, CXCR5, reciprocally induces signaling events, leading to the additional up-regulation of surface LTαβ2 on B cells and subsequent clustering of these cells, effectively establishing a positive feedback loop important for the integrity of B cell follicles. This is illustrated by the fact that in the absence of CXCL13, follicles in both spleen and LNs fail to form (21). The role of B cells in this process was further dissected by preventing B cells from expressing LTαβ2, by selectively ablating the LTβ gene in B cells. In the spleen, this resulted in the absence of FDCs and disruption of the B cell follicles. However, LNs were much less affected, and follicles and FDCs could still be detected (22). Therefore, although B cell-derived signals appear important in the formation and maintenance of lymphoid follicles in the spleen, the requirements for follicle formation in LNs are less clear.

In this study we analyzed the cellular and molecular mechanisms governing the initial segregation of T and B cells in neonatal LNs. The initial migration of B cells to the outer cortex of the LNs occurs in a CXCL13-independent manner, because these processes are indistinguishable in C56BL/6 and CXCL13−/− mice. Non-B cells regulate the induction of CXCL13, which depends on LTαβ2 expression. We propose a role for LTαβ2+CD45+CD4+CD3− cells in inducing CXCL13 and possibly additional chemokines responsible for the earliest B cell migration and, therefore, in the molding of the LN architecture.
Materials and Methods

Animals

C57BL/6 mice were purchased from Harlan (Horst, The Netherlands) and were kept under routine laboratory conditions. LTα/− and SCID mice were bred and kept at the in-house animal facilities of the Vrije Universiteit Medical Center. CXCL13+/− mice were described previously (21) and were bred and kept at the in-house animal facilities of Trudeau Institute.

Antibodies

For flow cytometry and immunohistology, the following Abs were used: 6B2 (anti-B220), GK-1.5 (anti-CD4), 2.4G2 (anti-CD16/CD32; all were affinity-purified from hybridoma cell culture supernatants with protein G-Sepharose (Pharmacia Biotech, Uppsala, Sweden) and labeled in our laboratory), anti-IgM F(ab)2 (Cappel, West Chester, PA), anti-IgD-PE (Dako-Sepharose (Pharmacia Biotech, Uppsala, Sweden) and labeled in our laboratory), and anti-CD19-PE (Coulter, Fullerton, CA), 1D3 (anti-CD19; BD Pharmingen), and 2G8 (anti-CXCXR5; BD Pharmingen). Biotin-labeled anti-CXCL13 and anti-CCL21 were purchased from R&D Systems Europe (Oxon, U.K.). Recombinant murine CXCL13 (1000 U) were used as second-step conjugate. Splenocytes from adult LTα−/− mice were used as negative controls.

Flow cytometric analysis was performed on a FACS Calibur (BD Biosciences, San Jose, CA) using CellQuest software (BD Biosciences). 7-Aminoactinomycin D (Molecular Probes, Eugene, OR) was used to exclude dead cells.

Chemotaxis

Chemotactic activity was determined using a Transwell system with 5-μm pores (Costar, Corning, NY). Recombinant murine CXCL13 (1 μg/ml; R&D Systems Europe) was used in the lower compartment, whereas 2.5 x 10^5 to 1 x 10^6 cells were placed in the upper compartment. Cells were allowed to migrate for 3 h at 37°C, then migrated cells were analyzed by flow cytometry.

Immunofluorescence microscopy

Six-micron cryosections were fixed in dehydrated acetone for 5 min and air-dried for an additional 10 min. Sections were incubated with primary Ab for 1 h at room temperature, followed by a 30-min incubation with a Fluor-Alexa-labeled conjugate (Molecular Probes) when needed. Sections were embedded in Fluorostab (ECN Biomedicals, Aurora, OH) and analyzed on an Eclipse E800 microscope (Nikon Europe, Haarlem, The Netherlands). For visualization of chemokines, sections were incubated overnight at 4°C with chemokine-specific Abs. The Alexa-Fluor 594 tyramide amplification system was subsequently used to enhance the signal according to the manufacturer’s protocol (Molecular Probes).

CXCL13 mRNA expression

RNA was extracted on the day of birth from whole mesenteric lymph nodes (C57BL/6) or from mesenteric lymph node rudiments (LTα/−) using TRIzol (Invitrogen Life Technologies, Gaithersburg, MD) and reverse transcribed with oligo(dT)12-18 (Invitrogen Life Technologies) and random hexamer primers (Invitrogen Life Technologies) using standard protocols. cDNA was analyzed for the expression of CXCL13 and LTβ-R by TaqMan PCR assay using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA).

In situ hybridization was performed on 6-μm cryostat sections using a digoxigenin-labeled CXCL13 probe as described previously (23).

Results

B cell follicles develop during the first week of life

To gain insight into the cellular and molecular processes underlying the initial shaping of the LN microdomains, immunohistochemical analysis of inguinal, axillary, brachial, and mesenteric LNs was performed on the day of birth and 4 and 7 days after birth. Three distinct phases could be distinguished in the segregation of T and B lymphocytes and the formation of B cell follicles. On the day of birth (day 0) the developing LNs showed no apparent organization, with B cells scattered throughout the LN, and T cells being virtually absent (stage I; Fig. 1A). Starting on approximately day 2, the first wave of αβTCR+ thymic emigrants colonized the LNs, and B cells started to localize to the outer cortex (24, 25). On day 4, the first phase of B cell migration was completed, and B cells were organized in a ring-like structure in the outer cortex of the LNs, the paracortex being occupied by numerous T lymphocytes. However, no follicles were detectable at this time (stage II; Fig. 1B). Between days 4 and 7, B cells localized in cortical clusters, and the first clearly distinguishable B cell follicles became evident on day 7 (stage III, Fig. 1, C and D). The generation of these follicles was accompanied by the emergence of FDCs, as detected by the FDC-M2 Ab (Fig. 1D) as well as positive staining for complement receptor 1 (data not shown). Because FDCs are dependent on TNF-α and LTαβ for their development (6, 19), this suggests that insufficient levels of these signals are available before day 7.

LTαβ-dependent expression of CXCL13 in developing LNs

Development and maintenance of splenic B cell follicles depends on local production of CXCL13, regulated largely by LTαβ expression (6, 19–22). To determine the role of CXCL13 in the organization of developing LNs, expression of CXCL13 mRNA in newborn LNs was analyzed by RT-PCR (Fig. 2A). Mesenteric LNs (MLNs) were dissected on the day of birth, day 2, and day 8, and total RNA was extracted. By means of this method, CXCL13 mRNA was already detected within the developing LNs on the day of birth. To address whether this early expression of CXCL13 was dependent on LTαβ signaling, as described for the adult spleen, CXCL13 expression was compared between C57BL/6 MLNs isolated at birth and rudimentary MLNs (rMLNs) from LTα−/− mice (Fig. 2B). To control for possible differences in stromal cell populations between these two mouse strains, FIGURE 1. Formation of B cell follicles during the first week after birth. Peripheral LNs (PLNs) and MLNs were dissected on the day of birth, day 4, and day 7. A–C, B cells were detected with anti-B220 Abs (green), and T cells with anti-CD3ε (red). A, On the day of birth, virtually no T cells are present. B cells are scattered throughout the organ, with no apparent organization. B, On day 4, B cells have migrated to the outer cortex. T cells now occupy the paracortical region. B cells appear as a ring-like structure in which no follicles can be distinguished. C, From day 7 on, organized B cell follicles become apparent; D, now containing FDCs (FDC-M2; red). At least 10 animals were analyzed per time point; representative PLNs are shown.
CXCL13 levels were normalized to the expression of the LTβ-R, expressed on stromal cells. Quantitative analysis of LTα−/− and C57BL/6 mice revealed a 5-fold decrease in CXCL13 message in the absence of LTα,β2 signaling in total LNs as well as in sorted CD45− stromal cells. Popliteal LNs (PLNs) were dissected on the day of birth and on days 2, 4, and 7 after birth, and CXCL13 mRNA was visualized by in situ hybridization. At birth, no CXCL13 message could be detected; the first signal became apparent on day 2. CXCL13 was expressed in the outer cortex, although not in a follicular pattern. On day 4, CXCL13 is organized in a follicle-like expression pattern, which was even more obvious on day 10.

FIGURE 2. Lymphotoxin-dependent CXCL13 expression in developing lymph nodes. A, Newborn MLNs were analyzed by RT-PCR on the day of birth and days 2 and 8 after birth. Already at birth, CXCL13 mRNA was readily detectable by RT-PCR. B, Newborn MLNs were isolated from C57BL/6 mice, and rudimentary MLNs from LTα−/− mice; mRNA was isolated from the total organ or from sorted CD45− stromal cells. CXCL13 message was normalized to LTβ-R message using TaqMan analysis. In the absence of a LTα,β2 signal, an average 5-fold reduction in CXCL13 message was observed, both in total LN as well as in sorted CD45− stromal cells. C, Popliteal LNs (PLNs) were dissected on the day of birth and on days 2, 4, and 7 after birth, and CXCL13 mRNA was visualized by in situ hybridization. At birth, no CXCL13 message could be detected; the first signal became apparent on day 2. CXCL13 was expressed in the outer cortex, although not in a follicular pattern. On day 4, CXCL13 is organized in a follicle-like expression pattern, which was even more obvious on day 10.
4, and adult B cells from MLNs were allowed to migrate in a Transwell assay. To determine the CXCL13 responsiveness of neonatal B cells, day 0, day 4, and adult MLNs isolated from day of birth, on day 4, and from adult mice were analyzed for the expression of LTA2B2 (Fig. 4C). Spleen cells from LTA2−/− mice were used as negative controls. In conjunction with their hampered migratory response to CXCL13, day 0 MLN B cells were also surface LTA2B2 negative. The expression of LTA2B2 became apparent from day 2 (data not shown) and reached adult levels on day 4 (Fig. 4C). This shows that although newborn B cells appear phenotypically mature, their low levels of CXCR5 lead to CXCL13 unresponsiveness as well as lack of surface LTA2B2.

CXCL13 responsiveness of neonatal B cells

The findings that B cells only start organizing in a CXCL13-dependent manner after day 4 prompted us to determine the capacity of neonatal B cells to migrate to response to CXCL13. B cells were isolated from MLNs on the day of birth, on day 4, and from adult mice and tested for their CXCL13 responsiveness in an in vitro Transwell assay (Costar, Corning, NY) (Fig. 3B). Shown are specific migrations relative to migration of B cells from adult MLNs. Surprisingly, on the day of birth, B cells were virtually CXCL13 unresponsive (Fig. 3B). The ability to respond to CXCL13 was acquired during the first days of life, reaching levels comparable to those in adults on day 4. However, at this time the B cell pool was still highly heterogeneous with regard to CXCL13-induced migration. To find an explanation for this unexpected unresponsiveness of newborn B cells, neonatal and adult MLNs were dissected, and expression levels of CXCR5, the receptor for CXCL13 (26), were compared between newborn and adult B cells. In agreement with their unresponsiveness to CXCL13 in Transwell assays, neonatal MLN-derived B cells showed lower expression of CXCR5 compared with adult B cells, explaining the inability of these cells to respond to CXCL13. The acquisition of normal levels of expression and full CXCL13 responsiveness represents an additional, previously unappreciated, developmental step in B cell maturation.

Neonatal B cells are LTα2β2 negative

Because neonatal LN-derived B cells still have to acquire a distinct feature of mature B cells (CXCL13 responsiveness), an additional phenotypic analysis was performed to assess the degree of maturity of the B cell pool in the neonatal LNs. As shown in Fig. 4A, phenotypically mature IgM+IgD+ B cells were found on the day of birth in MLNs (Fig. 4A). These data were confirmed by the fact that ~40% of CD19+ cells at birth expressed the λ chain (Fig. 4B), indicative of progression beyond the pre-B cell stage. Therefore, a large part of the neonatal MLN B cells appeared phenotypically mature despite their low expression of CXCR5.

In the spleen, induction of surface LTα2β2 on B cells is the second feature attributed to CXCR5 signaling. To test whether the newborn CXCR5low B cells from MLNs had initiated expression of LTα2β2, MLNs from mice on the day of birth and those from adult mice were analyzed for the expression of LTα2β2 (Fig. 4C). Splenic LTα2β2 cells from LTα2−/− mice were used as negative controls. In conjunction with their hampered migratory response to CXCL13, day 0 MLN B cells were also surface LTα2β2 negative. The expression of LTα2β2 became apparent from day 2 (data not shown) and reached adult levels on day 4 (Fig. 4C). This shows that although newborn B cells appear phenotypically mature, their low levels of CXCR5 lead to CXCL13 unresponsiveness as well as lack of surface LTα2β2.
of all hemopoietic cells, and upon adoptive transfer, these cells home to the B cell follicles (32, 33). These features make LTα1β2+CD45+CD4+CD3− cells likely candidates for inducing LTα1β2-dependent chemokines in the developing LN. SCID mice contain a normal population of LTα1β2+CD45+CD4+CD3− cells (32), and in Fig. 5, A and B, the predominant localization of LTα1β2+CD45+CD4+CD3− cells at the outer cortex of the SCID lymph nodes could clearly be seen due to the absence of CD4+TCR+ lymphocytes. This cortical distribution was apparent around day 2 after birth in both SCID and C57BL/6 mice (32) and coincided with the migration of B cells to this area during stage II of follicle formation. Furthermore, similar to the migration of B lymphocytes, this migration of CD45+CD4+CD3− cells occurred independently of CXCL13, because a predominant cortical localization of these cells was seen in CXCL13−/− mice (Fig. 6B). However, in contrast to neonatal B cells, CD45+CD4+CD3− cells showed a very potent migratory response to CXCL13 in vitro (Fig. 6C) (34). Therefore, LTα1β2+CD45+CD4+CD3− cells display many of the features attributed to B cells in the adult spleen for induction of the LTα1β2-CXCL13 feedback loop, indicating that LTα1β2+CD45+CD4+CD3− cells may very well be responsible for the induction of homeostatic chemokines during postnatal development and initiation of the lymphoid microdomains.

Discussion

The shaping of the cellular architecture within developing LNs requires an intricate interplay among several cell types and soluble factors. Postnatal segregation of lymphocytes and arrangement into functionally and anatomically distinct microdomains is a multistep process, in which several distinct stages can be discerned (Figs. 1 and 7). During the first stage (stage I), from day of birth until day 2, LNs are still devoid of αβTCR+ T cells, and the main hemopoietic cell types present are B cells, LTα1β2+CD45+CD4+CD3− cells, and γδT cells (25). During this time, no cellular organization is apparent. The transition from stage I to stage II is marked by an influx of the first αβTCR+ T cells. In conjunction with the influx of T cells, which accumulate in the paracortex of the LN, B cells and CD45+CD4+CD3− cells start to migrate toward the outer cortex. In this study CXCL13 expression was up-regulated, in an LTα1β2-dependent manner, most likely induced by LTα1β2+CD45+CD4+CD3− cells. During stage II, the pattern of CXCL13 mRNA expression indicates the creation of a microenvironment permissive for follicle formation, culminating on day 4 in the organized follicular expression of this chemokine. Stage III is initiated when B cells become responsive to CXCL13, and CXCL13-dependent migration can occur, leading to the clustering of B cells in the outer cortex and the expression of surface LTα1β2.

FIGURE 4. Newborn LNs contain phenotypically mature, but LTα1β2-negative, B cells. On the day of birth, B cells from MLNs were analyzed for the expression of surface Igs. A, Gated on CD19+ cells, ~33% of the B cells in newborn LNs express both IgM and IgD; B, roughly the same percentage of CD19+ B cells expresses the κ-L chain, indicative of maturation beyond the pre-B cell stage. C and D, Surface expression of LTα1β2 was evaluated by staining with a soluble LTβ-R-IgG fusion protein. Splenic B cells from LTα1β2− mice were used as a negative control, and B cells from either day 0 MLNs or day 4 MLNs were compared with B cells from adult C57BL/6 MLNs. At birth, no surface LTα1β2 could be detected on B cells. Expression was instigated on approximately day 2 (data not shown) and reached levels comparable to adult values on day 4. The percentage LTα1β2-expressing B cells of the total B cells is shown.
The latter enables B cells to contribute to the sustained production of CXCL13 in a similar way as described for the spleen (21). The LN architecture is completed on day 7, when FDCs become apparent, and B cells are clustered in anatomically distinct, tightly packed follicles (Fig. 7).

The observed discrepancy between CXCL13 mRNA and protein levels indicates that low levels of CXCL13 protein are likely to be present before day 10. However, because development of the lymphoid architecture in C57BL/6 mice and that in CXCL13−/− mice are completely identical until day 4, this low level of expression is obviously not essential for the early phases of T/B segregation. Furthermore, differences in the sensitivity of the CCL21 and CXCL13 Abs might exist, which could hamper determination of the exact onset of protein expression.

Recently, the importance of B cells in maintenance of the architecture in spleen and LNs of adult mice was questioned (22). Tumanov et al. (22) showed that mice with a B cell-specific LTβ deletion display normal chemokine levels, FDC, and follicular structures in the LN. Furthermore, it was shown that T cell-derived LTβ1/2 is sufficient to maintain the lymphoid architecture. This

**FIGURE 5.** Production of homeostatic chemokines in SCID mice. LNs from SCID and C57BL/6 mice were analyzed for the expression of CXCL13 and CCL21 in combination with CD4 on days 3 and 7 after birth and in adult animals. Chemokines were stained in red, and CD4 in green. A, Three days after birth, LNs from SCID mice showed no expression of CXCL13, but showed a strong production of CCL21. This was similar to the chemokine expression patterns seen in C57BL/6 LNs using this method. B, At 10 days after birth, SCID lymph nodes showed the first CXCL13 expression in addition to the already present CCL21, again in agreement with C57BL/6 LNs. C, At 37 days after birth, CXCL13 was abundantly expressed in the cortical region of the SCID lymph nodes, colocalizing with the CD4+ cells. CCL21 expression was comparable to that at earlier time points. In C57BL/6 mice, CXCL13 was localized in the follicular areas, with CCL21 being expressed in the paracortex.

**FIGURE 6.** Properties of CD45+CD4+CD3− cells in newborn LNs. Newborn MLNs were dissected and analyzed for surface LTα1β2 expression using a LTβ-R-IgG fusion protein. A, The only cells expressing LTα1β2 at birth are CD45+CD4+CD3− cells. B, LTα1β2+CD45+CD4+CD3− cells localize to the outer cortex in a CXCL13-independent manner, as shown in MLN from day 4 CXCL13−/− mice. T cells appear red (anti-CD3ε) or yellow (anti-CD3ε together with anti-CD4), and CD45+CD4+CD3− cells appear green (anti-CD4). C, In contrast to newborn B cells, CD45+CD4+CD3− cells show a vigorous response to CXCL13 in an in vitro Transwell assay. Shown are specific migrations within one experiment. A representative example of three independent experiments is shown.
CXCL13
Stage I
Day 0-2
Organization
CXCL13 independent
B cell independent

CXCL13
Stage II
Day 4
CXCL13 dependent
B cell dependent

CXCL13
Stage III
Day 7
CXCL13 dependent

FIGURE 7. Model for the initiation of lymphoid segregation and follicle formation in LNs. During the segregation of T and B cells and the formation of lymphoid follicles, three distinct stages can be perceived. At birth, no αβTCR⁺ cells are present, and B cells and CD45⁻CD4⁺CD3⁻ cells make up the main hemopoietic cell types (stage I). During stage I, CXCL13 is expressed in the outer cortex (left panel). Organization is initiated on approximately day 2, when the first T cells enter the LN. B cells and CD45⁻CD4⁺CD3⁻ cells now start to localize to the outer cortex, forming a ring-like pattern on day 4 (stage II). This migration is regulated in a CXCL13-independent manner, whereas expression of CXCL13 is organized in an follicular constellation on day 4. From day 4 on, B cells become CXCL13-responsive and start to cluster in follicular structures. At 7 days after birth, the first anatomically distinct B cell follicles can be detected as well as the first FDCs (stage III). The migrational events during stage III are CXCL13-dependent, and abundant CXCL13 expression can be found within the follicles.

indicates that during adult life the lymphoid architecture is preserved by a combination of T and B cell-derived signals. However, it has to be noted that all these observations were made 8–10 days after immunization with sheep RBC. Because LTαβ₁β₂ levels on T cells are dependent on activation state, it is conceivable that high levels of LTαβ₁β₂ on activated T cells leads to the generation of a normal lymphoid architecture through reorganization of the already present CXCL13 expression into a follicular pattern.

During the first days after birth (stages I and II), the neonatal B cell pool needs to undergo additional maturation steps before being able to contribute to the LTαβ₂-CXCL13 feedback loop. Complete CXCL13 responsiveness is not reached until approximately day 4, when B cells show CXCL13-dependent migration and express LTαβ₂. The CXCL13-LTαβ₂ positive feedback loop, which was shown to be instrumental in generating tightly packed B cell follicles and maintaining these structures in spleens of adult mice, is therefore in all probability in LNs initiated by non-B cell-derived LTαβ₂. The only cells in neonatal LNs that express LTαβ₂ are CD45⁺CD4⁺CD3⁻ cells; therefore, these cells are the most likely candidate to induce the expression of homeostatic chemokines.

Before the CXCL13-mediated migration leading to follicle formation in stage III, the initiation of T/B cell segregation as well as migration of CD45⁺CD4⁺CD3⁻ cells during stage II are regulated independently of CXCL13. This initial segregation of T and B cells is therefore mediated by alternative chemokines. These chemokines are not dependent on T cells for their induction, because in athymic nude mice, B cell migration to the outer cortex occurs via the same kinetics as in C57BL/6 mice (data not shown). Moreover, induction is most likely LTαβ₂-independent, because in solitary MLNs, which occasionally develop in LTα-deficient animals, normal T/B segregation was found (35). It is conceivable that CD45⁺CD4⁺CD3⁻ cells are also essential for inducing these unidentified chemokines. To establish the involvement of LTαβ₂, CD45⁺CD4⁺CD3⁻ cells in this process, we have extensively tried to eliminate these cells in vivo using depleting Abs; however, this proved infeasible, probably due to a combination of low complement levels in neonates and the high degree of apoptosis resistance of CD45⁺CD4⁺CD3⁻ cells (our unpublished observations). Identification of the signals and chemokines that mediate this CXCL13-independent migration provides novel challenges for future research.

This study provides new insights into the complex interactions underlying the generation of distinct microdomains in which T and B lymphocytes are allowed to efficiently sample Ags presented by APCs. This is a phenomenon seen in secondary lymphoid organs as well as in ectopic tertiary lymphoid structures commonly found in several chronic inflammatory conditions (17, 36–42). There is accumulating evidence for a high degree of homology between the generation of inflammatory lymphoid structures and the normal development of the lymphoid architecture (36, 43, 44). This indicates that understanding the factors that shape the developing LNs will probably lead to new insights into the formation of ectopic lymphoid structures and will eventually open new therapeutic avenues leading to the targeted disruption or prevention of formation of these structures during chronic inflammation.

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References


