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Lymphotoxin α/β and Tumor Necrosis Factor Are Required for Stromal Cell Expression of Homing Chemokines in B and T Cell Areas of the Spleen

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Summary

Mice deficient in the cytokines tumor necrosis factor (TNF) or lymphotoxin (LT) α/β lack polarized B cell follicles in the spleen. Deficiency in CXC chemokine receptor 5 (CXCR5), a receptor for B lymphocyte chemoattractant (BLC), also causes loss of splenic follicles. Here we report that BLC expression by follicular stromal cells is defective in TNF−, TNF receptor 1 (TNFR1)−, LTα− and LTβ−deficient mice. Treatment of adult mice with antagonists of LTαβ2 also leads to decreased BLC expression. These findings indicate that LTαβ2 and TNF have a role upstream of BLC/CXCR5 in the process of follicle formation. In addition to disrupted follicles, LT−deficient animals have disorganized T zones. Expression of the T cell attractant, secondary lymphoid tissue chemokine (SLC), by T zone stromal cells is found to be markedly depressed in LTα−, and LTβ−deficient mice. Expression of the SLC-related chemokine, Epstein Barr virus–induced molecule 1 ligand chemokine (ELC), is also reduced. Exploring the basis for the reduced SLC expression led to identification of further disruptions in T zone stromal cells. Together these findings indicate that LTαβ2 and TNF are required for the development and function of B and T zone stromal cells that make chemokines necessary for lymphocyte compartmentalization in the spleen.

Key words: lymphoid tissue • follicle • lymphocyte • follicular dendritic cell • dendritic cell

Genetic studies in mice have established that the cytokines TNF, lymphotoxin α (LTα),1 and LTβ, and the receptors TNFR1 and LTβR, are required for normal compartmentalization of lymphocytes in the spleen (1, 2). In TNF− and TNFR1-deficient mice, follicular dendritic cells (FDCs) are lacking and B cells fail to form follicular clusters, instead appearing in a ring at the T zone periphery (3–6). Mice deficient in LTα, which are deficient in membrane LTα1β2 heterotrimers and soluble LTα3 complexes (7), show a distinct phenotype that includes absence of lymph nodes and Peyer’s patches, and loss of marginal zone cells, FDCs, and normal B/T segregation in white pulp cords of the spleen (6, 8–10). LTβ−/− mice, which cannot produce membrane forms of LT that bind LTβR but continue to express LTα, exhibit a similar phenotype except that some lymph nodes are retained and splenic architecture is somewhat less disturbed than in LTα−/− mice (11, 12; Korner, H., and J.D. Sedgwick, unpublished observations).

In parallel studies an understanding has begun to develop of the role played by chemokine receptors and chemokines in controlling cell movements within lymphoid tissues. Mice lacking CXCR5 (formerly Burkitt’s lymphoma receptor 1 [BLR1]), a chemokine receptor expressed by ma-
ture B cells (13, 14), lack polarized follicles in the spleen and B cells appear as a ring at the boundary of the T zone (5). Recently, a CXCR5 ligand, termed B lymphocyte chemoattractant (BLC) or B cell attracting chemokine (BAC)-1 (15, 16), has been found constitutively expressed by stromal cells in lymphoid follicles and has been proposed to act as a B cell homing chemokine (15). Three other chemokines have been identified that are constitutively expressed in lymphoid tissues and that are efficacious attractants of resting lymphocytes: stromal cell–derived factor 1 (SDF1) (17–19), secondary lymphoid tissue chemokine (SLC)/ICAM-3 (20–24), and EBV-induced molecule 1 ligand chemokine (ELC)/macrophage inflammatory protein (MIP)-3α (25–28). SDF1 is an efficacious attractant of mature lymphocytes (19), although its pattern of expression in lymphoid tissues is not well characterized. SLC and ELC are related chemokines that strongly attract naive T cells and more weakly attract B cells. SLC is expressed by high endothelial venules (HEVs) in lymph nodes and Peyer’s patches and by stromal cells in the T zone of spleen, lymph nodes, and Peyer’s patches (21, 22, 24), whereas ELC is expressed by T zone DCs (27). The strong chemotactic activity of SLC and ELC combined with their compartmentalized expression pattern has led to the suggestion that these chemokines function in lymphocyte homing to the T zone of peripheral lymphoid tissues (21, 27).

The strikingly similar disruption of splenic B cell distribution in TNF- and CXCR5-deficient mice suggested that these molecules act in a common pathway to maintain follicular organization (29). The more severe splenic disruptions in LTαβ-deficient mice suggest LT may function upstream of molecules that help organize cells into both follicles and T zones. Here we report that expression of the CXCR5 ligand, BLC, is substantially reduced in TNF-, TNFR1-, LTα, and LTβ-deficient mice. Expression of SLC and ELC is also reduced, whereas SDF1 is unaffected. Antagonism of LTαβ2 function in the adult by treatment with soluble LTβR-Ig or anti-LTβ antibody caused reductions in BLC and SLC expression. We also observed that in addition to defects in follicular stromal cells, the LT- and TNF-deficient mice had disruptions in T zone stromal cells that appeared to affect follicular development. The LTαβ and TNF required for upregulating BLC expression, mice lacking subpopulations of hematopoietic cells were studied. Mice deficient in B cells, which also lack follicular stromal cells, had reduced BLC expression, whereas T cell and marginal zone macrophage (MZM)-deficient mice were unaffected. These findings suggest that TNF and membrane LTαβ heterotrimeric protein signals are required for development and function of stromal cells that produce chemokines essential for normal organization of lymphoid tissue compartments.

Materials and Methods

Animals. TNF-/-, TNF/LTαβ-/-, and LTαβ-/- mice were generated using C57BL/6 embryonic stem (ES) cells and maintained on a pure C57BL/6 background as described (6, 30). A further strain, generated by targeting of the LTβ gene in Bruce 4 C57BL/6 ES cells (31), was produced. The LTβ gene was disrupted by insertion of the neomycin cassette in reverse orientation in exon I, leading to complete gene inactivation and typical LTβR-/- phenotype (10, 11). 129 mice were used for all experiments. 129 mice were from a C57BL/6 colony maintained at the University of California San Francisco. Treatment was with 100 μg of fusion protein or 200 μg of antibody intraperitoneally once per week as described previously (35–37). As a control for the LTβR-/- fusion protein, which contains human IgG1 hinge, C2 and C3 regions, mice were treated with a human LFA3-IgG1 hinge, C2 and C3 region fusion protein (100 μg/wk, i.p.) as in previous studies (35, 36). Human LFA3 does not bind to mouse C2 (8). The control group for the hamster anti-LTβ mAb-treated mice were injected with hamster anti-KLH mAb (37).

Northern Blot Analysis. 10–15 μg of total RNA from mouse spleens was subjected to gel electrophoresis, transferred to Hybond N+ membranes (Amersham Pharmacia Biotech), and probed using randomly primed 32P-labeled mouse cDNA probes of the following types: BCL, bases 10–532 (15); SDF1, bases 1–848 (21); ELC, bases 1–755 (27); and SDF1α, bases 30–370 (18). To control for loading and RNA integrity, membranes were probed with a mouse elongation factor 1α (EF-1α) probe. For quantitation, Northern blots were exposed to a phospho screen for 6 h to 3 d and images were developed using a Storm860 PhosphorImager (Molecular Dynamics). Data were analyzed using ImageQuant software (Molecular Dynamics). Densitometric analysis was performed using the ImageQuant software. The mean corrected signal for the wild-type or control treated samples, as appropriate, that were included on each of the Northern blots was used as the control signal for the same sample. Relative expression levels were calculated by dividing the corrected signal for each mutant or treated sample with the mean corrected signal for the wild-type or control treated samples, as appropriate, that were included on each of the Northern blots.

In Situ Hybridization. For in situ hybridizations, frozen sections (6 μm) were treated as described (15). In brief, sections were fixed in 4% paraformaldehyde, washed in PBS, prehybridized for 1–3 h, and hybridized overnight at 60°C with sense or antisense digoxigenin-labeled riboprobes. After washing at high stringency, sections were incubated with sheep antidigoxigenin antibody (Boehringer Mannheim). Rat IgG antibodies were detected with goat anti-rat-conjugated horseradish peroxidase or alkaline phosphatase (Jackson Immunoresearch Laboratories) and developed with NBT (Bio-Rad) and BCIP (Sigma).

Immunohistochemistry. Cryostat sections (6–7 μm) were fixed and stained as described previously (27) using the following mAbs: rat anti-B220 (RA3-6B2); rat anti-CD4 and -CD8 (Caltag); rat anti-CD35 (8C12; Pharmingen); rat anti-MOMA1 (provided by Georg Kraal, Free University, Amsterdam, The Netherlands); and biotinylated mouse anti–BPR-3 (38). Rat IgG antibodies were detected with goat anti–rat-conjugated horseradish peroxidase or alkaline phosphatase (Sigma Chemical Co.). Enzyme reactions were developed with conventional substrates for peroxidases (diaminobenzidine/H2O2, 3,3′-diaminobenzidine/H2O2).
Reduced Chemokine Expression in TNF-α-, LTα-, and LTβ-deficient Mice

To explore whether TNF/TNF-R1 and CXCR5 function in a common pathway of follicular organization, we measured CXCR5 expression in TNF-α- and TNF-deficient mice by flow cytometry. Splenic B cells from TNF-α- and TNF-α-deficient mice expressed levels of CXCR5 that were slightly elevated compared with wild-type controls (39; Ansel, K.M., and J.G. Cyster, data not shown). Increased CXCR5 expression seemed unlikely to account for the disrupted organization of B cells in TNF-α- or TNF-1-deficient animals, but could result from reduced expression of ligands that normally engage and downregulate CXCR5. Therefore, we tested whether TNF/TNF-F1 regulated CXCR5 ligand expression by measuring BLC RNA levels in TNF-α- and TNF-1-deficient mouse spleens (Fig. 1, A and B). BLC expression was reduced approximately threefold in both types of mutant mice compared with wild-type littermates. In situ hybridization analysis of TNF-F1-deficient spleen confirmed the reduced expression of BLC by follicular stromal cells (Fig. 1 C). Animals deficient in LTα or LTβ also lack follicles and follicular stromal cells, although the absence of MZMs and the severely disrupted B/T boundary make the splenic phenotype of these mice distinct. BLC expression was reduced even more severely in spleens from LTα- and LTβ-deficient animals than from TNF-α-deficient mice (Fig. 1, A and B), and the residual expression was too low to be detected in in situ hybridization analysis (Fig. 1 C). In mice deficient in both LTα and TNF, BLC expression was reduced to an extent similar to LTα single mutants (Fig. 1, A and B), consistent with the possibility that these cytokines function in a common pathway leading to BLC expression.
Splenic T zone organization is also disrupted in the cytokine- and cytokine receptor-deficient animals, ranging from subtle changes in TNF- and TNFR1-deficient mice to almost complete loss of T zones in LTα, LTβ, and LTα/TNF double mutant mice. To determine whether LTα/β and TNF also functioned in a pathway leading to T zone chemokine expression, we measured the expression of SLC and ELC, related T cell attracting chemokines that are made in the T zone. We also measured expression of the more broadly distributed chemokine, SDF1, which is an efficacious attractant of both B and T cells. SLC was reduced in expression ~2-fold in TNFR1- and TNF-deficient animals and >20-fold in LTα, LTβ, and LTα/TNF double mutant animals (Fig. 1, A and B). By in situ hybridization analysis, the network of SLC expressing stromal cells remained visible in the TNFR1 mutant mice but could not be detected in LTα-deficient animals (Fig. 1 C). Expression of ELC was also reduced in all of the mutant strains, although less severely than SLC (Fig. 1, A and B). By contrast, SDF1 expression was not significantly reduced in any of the mutant animals (Fig. 1, A and B), indicating that the reductions in BLC, SLC, and ELC are physiologically relevant and not the result of an overall decrease in chemokine gene expression. It should be emphasized that the TNFR1 mutant and the four cytokine mutants used in this study (6, 30, 32; Korner, H., D.S. Riminton, F.A. Lemckert, and J.D. Sedgwick, unpublished) were all generated using C57BL/6 ES cells and maintained on a C57BL/6 background, making it unlikely that any of the differences we observed in chemokine expression are due to linked genetic differences. Therefore, these experiments demonstrate that TNF/TNFR1 and LTα/β are required for normal expression of BLC, SLC, and ELC in the spleen.

To determine whether the requirement for LTα and LTβ in the expression of BLC and SLC was developmental or constitutive, we treated adult mice for various time periods with soluble LTβR-Ig fusion protein (8, 40), an antagonist of LTα1pβ2, and a related molecule, LIGHT (41). Control mice were treated for equal periods of time with an LFA3-Ig fusion protein (8). After 1 wk of LTβR-Ig treatment, splenic BLC expression was reduced twofold compared with the controls (Fig. 2 A). A further reduction in BLC expression occurred after 2 wk of treatment and did not become more severe after 3 or 4 wk of treatment (Fig. 2 A). 2 wk of treatment also lead to decreased BLC levels in mesenteric lymph nodes (Fig. 2 B). Expression of SLC was reduced in spleen and mesenteric lymph nodes of mice given LTβR-Ig, although the degree of inhibition was variable and less severe than the reduction in BLC (Fig. 2, A and B). To distinguish the possible contribution of LIGHT from that of LTα1pβ2, mice were treated for 1 or 2 wk with an anti-LTβ mAb that specifically blocks LTα/β heterotrimer function (37, 40). Analysis of splenic RNA showed that BLC and SLC expression were both reduced after 2 wk of anti-LTβ mAb treatment (Fig. 2 A). These results establish a key role for LTα1pβ2 in maintaining normal chemokine expression. Although the lesser effect of the antibody treatment compared with LTβR-Ig treatment (Fig. 2 A) suggests that LIGHT might also contribute, the results may equally be explained by the mAb causing less complete inhibition of LTα1pβ2 function, as has been observed in in vitro studies (40). To explore

![Figure 2](image-url)
further the relationship noted in the mutant mice between chemokine deficiency and loss of follicular organization, spleen sections from LTβR- and LTβ-deficient mice were stained for B cell markers as well as FDCs and marginal zone markers. As observed previously (36), expression of mucosal addressin cell adhesion molecule (MAdCAM)-1 and FDC markers were reduced after 1 wk of treatment and were undetectable by 2 wk, whereas loss of the marginal metallophilic macrophage (MOMA) marker MOMA1 was more gradual (data not shown). Changes in B cell follicular organization were also observed after 1 wk of treatment (Fig. 2 C) and were maximal after 2 wk of treatment (Fig. 2 C), paralleling the decrease in BLC expression. These results establish a constitutive requirement for LTα1β2 in maintaining normal levels of BLC and SLC. The more modest decrease in BLC and SLC expression in LTβR- and LTβ-deficient mice compared with LTα- or LTβ-deficient mice could reflect incomplete blocking of LTα1β2 function but is also consistent with a role for LTα1β2 in development that does not continue in the adult mouse.

TNF- and LTα1β2-dependent Stromal Cells in Follicles and T Zone. The above experiments demonstrated that TNF, LTα, and LTβ are required for normal expression of the chemokines BLC and SLC by stromal cells in the spleen. Several studies have established that the organization of FDCs is disrupted in TNF-, LTα-, and LTβ-deficient mice (1), indicating that the cell type normally producing BLC might be disrupted. However, disruption of FDC organization could not account for the decreased SLC expression, since FDCs do not extend into the T zone. To test whether changes in addition to reduced SLC expression could be detected in T zone stromal cells, we examined expression of BP-3, a marker for an extensive network of stromal cells in both the T zone and follicles (38, 42). Strikingly, the number of BP-3-staining cells was greatly reduced in both the B and T zones of TNF- and TNFR1-deficient mice (Fig. 3, and data not shown) and was undetectable in LTα- and LTβ-deficient mice except for a small number of cells with altered morphology that were occasionally observed (Fig. 3). The disruption of BP-3-staining stromal cells in both TNF- and LTα- and LTβ-deficient spleens appeared more severe than in lymphocyte-deficient (RAG-1-/-) spleens (Fig. 3). BP-3 expression in T zone and follicles was also markedly disrupted after 1 wk of treatment with LTβR-Ig or anti-LTβ antibody and was almost undetectable after 2 wk of treatment (Fig. 3, and data not shown). This period of treatment is also sufficient to disrupt staining for MAdCAM-1 and FDC markers (36, 43). To determine the relationship between BP-3 expressing cells in follicles and the cell types previously defined as TNF- and LTα and LTβ-dependent, sections from wild-type mice were double stained for MAdCAM-1 or CR1 (CD35) and BP-3 (Fig. 4 A). BP-3-expressing cells in the outer follicle appeared to line the marginal sinus, and in some cases these cells co-stained for MAdCAM-1 (Fig. 4 A). Many of the BP-3+ cells located in the center of the follicle co-stained with CD35 (Fig. 4 A), whereas BP-3+ cells in other parts of the follicle, especially cells near the marginal sinus, were CD35-low or -negative (Fig. 4 A). Therefore, the BP-3-expressing stromal cell population includes T zone stromal cells (Fig. 3 and Fig. 4 A), FDCs, marginal sinus lining cells, and follicular stromal cells that are low or negative for FDC markers (Fig. 4 A). The severe disruption of BP-3 expression in the mutant mice, together with the reduced BLC and SLC expression and the loss of FDCs, indicates that TNF, LTα, and LTβ have a broad role in inducing and maintaining stromal cell integrity in T zones and B zones of lymphoid tissues.

MZMs Are Not Required for BLC Production. In addition to defects in FDCs, MAdCAM-1+ cells, and BP-3+ cells, LTα- and LTβ-deficient mice also lack MZMs and MMMs (1, 11, 12). To test the possibility that the deficiency in these macrophage populations in LTα-/- and LTβ-/- mice contributed to the greatly reduced BLC ex-
expression and loss of follicular organization, we characterized spleens from op/op mice, a strain that is deficient in M MMs and M ZMs due to a mutation in the colony stimulating factor 1 gene (44, 45). Organization of B cell follicles appeared normal in op/op spleen (Fig. 4 B), and BLC expression was not reduced (Fig. 5). Expression of BP-3, MAdCAM-1, and CD35 was also not disrupted (Fig. 4 B, and data not shown). These findings demonstrate that M ZMs and M MMs do not make a significant contribution to the constitutive production of BLC, and also establish that these cells are not required as a source of TNF or LTα1β2 to maintain BLC expression or follicular organization.

Normal expression of BLC is dependent on B cells. Recent studies have demonstrated that B lymphocytes are an essential source of membrane LTα1β2 for establishing FDC networks and follicular organization (46, 47). However, mice congenitally deficient in LTα have a more severe disruption of lymphoid compartmentalization than mice lacking only in lymphocyte LTα expression, indicating that there is also a nonlymphocyte source of LTα (47, 48). To determine whether either or both sources of LTα were required for induction of BLC, chemokine expression levels in RAG-1−/−, B cell receptor (BCR)−/−, and TCR−/− mice were compared with levels in LTα−/− animals. BLC expression was reduced approximately fivefold in spleens from lymphocyte-deficient (RAG-1−/−) and B cell-deficient (μMT) mice, but were not reduced in T cell-deficient (TCR−/−) mice (Fig. 5), demonstrating that B cells are important for induction of BLC expression, presumably providing LTα1β2 and possibly also TNF. However, BLC levels in RAG-1−/− and BCR−/− mice were not reduced to the extent of LTα−/− or LTβ−/− mice (Fig. 5, and see Fig. 1), indicating that some BLC expression in the spleen is induced by LTα/β-expressing cells other than B and T lymphocytes.

**Discussion**

These studies provide new insight into the mechanism by which TNF and LTα/β promote normal compartmentalization of lymphocytes in the white pulp cords of the spleen. The findings extend the previously defined requirement for TNF and LTα/β in the development and function of follicular stromal cells to also include stromal cells in the splenic T zone. The results demonstrate that a key function of the LTα/β- and TNF-dependent stromal cells is constitutive production of chemokines that strongly attract resting lymphocytes, and they suggest that these chemokines function with other properties of the stroma to compartmentalize cells into follicles and T zones.

The chemokine receptor CXCR5 is expressed by all mature B cells and is the only known receptor for BLC, an efficacious attractant of resting B cells (13, 15, 16). Since loss of CXCR5 is sufficient to disrupt organization of splenic follicles (5), it is reasonable to suggest that the
greatly reduced expression of BLC in TNF-<sup>a</sup>, TNFR1-<sup>a</sup>, LT-<sup>α</sup>-, and LTβ-deficient mice directly contributes to the disrupted organization of splenic follicles in these animals. Polarized follicles also fail to form in lymph nodes of TNF-deficient mice and in the nodes that develop under some conditions in LT-deficient mice (6, 11, 12, 37, 49). The finding that BLC expression is reduced in mesenteric lymph nodes of LTβR-<sup>-Ig</sup>-treated mice indicates that LT<sup>α/β</sup> plays a role in directing BLC expression in lymph nodes. However, whether BLC is likely to contribute to the organization of B cells into lymph node follicles is presently unclear, since CXCR5 does not appear to be required (5).

SLC and ELC both stimulate cells through CCR7, a receptor expressed by T and B lymphocytes, and these chemokines are the most efficacious attractants of T cells so far described (21, 25, 27, 50). We propose that the severe reduction in T zone SLC expression in LT<sup>α/β</sup>-deficient mice directly contributes to the loss of normal T cell compartmentalization in these animals. Maturing DCs upregulate CCR7 and have been suggested to migrate to lymphoid tissues in response to CCR7 ligands (51), making it possible that the decrease in SLC also leads to reduced accumulation of mature DCs in the T zone. Consistent with this possibility, lymph nodes developing in mice with reduced LT<sup>α</sup> levels have threefold fewer DCs than controls (52), and we have observed a similar decrease in DC frequency in LT<sup>α</sup>-deficient mouse spleens (our unpublished observations). Reduced DC accumulation may be at least partially responsible for the decreased expression of ELC, a chemokine made by T zone DCs (27). The lowered ELC levels are likely to exacerbate the effect of SLC deficiency and contribute to the loss of T zone organization. TNF and TNFR1 are also required for maximal SLC and ELC expression. However, mice deficient in TNF or TNFR1 do continue to express significant amounts of SLC and ELC, and this is consistent with the relatively unaffected T zone organization in these mutant animals (3, 4, 6, 53). The generally greater reduction of chemokine expression in TNF-deficient compared with TNFR1-deficient mice should not be due to background gene effects, since all the animals were generated on the C57BL/6 background; a more likely possibility is that TNFR2 transmits some of the TNF signals necessary for chemokine expression. In support of this possibility is the finding that Langerhans cell migration to lymph nodes is depressed in TNFR2-deficient mice (54). Interestingly, during the analysis of several TNFR1-deficient mice that had been housed in a conventional animal facility, we found that whereas BLC and SLC levels remained depressed, ELC expression was equal to the wild-type controls (our unpublished observations). These observations are similar to other findings indicating that some of the nonredundant functions of TNF in the resting state can be overcome during an immune response (43).

The deficiency of DCs in LT- and TNF-deficient mice has been well characterized (1). Ultrastructural studies have demonstrated that DCs are part of a broader network of follicular stromal cells (55), and using the molecule BP-3 as a marker it has been possible to show that this more extensive stromal cell network is also LT and TNF dependent (see Fig. 3). Elegant bone marrow chimera and adoptive transfer experiments have established that DC development requires TNFR1 and LTβR expression by the follicular stroma and cytokine (LT and TNF) expression by hematopoietic cells, in particular B cells (39, 46-48). The necessity for B cells in the maximal expression of BLC (see Fig. 5) is consistent with these results and suggests that a feedback loop exists which helps to keep the number of BLC-producing follicular stromal cells in proportion to the number of B cells. The more depressed BLC expression in LT<sup>α</sup>- and LTβ-deficient mice than in B cell-deficient animals is also in agreement with studies showing that B cells cannot be the sole source of LT<sup>α/β</sup> for follicle formation (47, 48). Perhaps the LT<sup>α/β</sup>-expressing CD4<sup>+</sup>CD3<sup>-</sup> cells that enter lymphoid tissues early in development (56) induce stromal cells to express BLC. Requirements for development of T zone stromal cells have been less well characterized than for DCs, but our results indicate they are similar in being TNF and LT<sup>α/β</sup> dependent. Experiments are ongoing to address whether T cells, B cells, or other cell types must express TNF or LT<sup>α/β</sup> for induction of normal SLC expression. At this stage, it has not been possible to determine whether LT and TNF work directly to induce chemokine expression or whether they function further upstream, inducing and maintaining the development and viability of chemokine-expressing stromal cells. Although treatment of adult mice with soluble LTβR-Ig leads within 1 wk to decreased expression of BLC and SLC, the treatment also leads to rapid disruption of stromal cells as defined by a variety of markers (36; and see Fig. 3). Future studies must define in more detail the subpopulations of LT- and TNF-dependent stromal cells that express BLC and SLC and characterize the signaling pathways that control chemokine expression.

The studies in this report have established a major role for LT<sup>α/β</sup>, and a lesser but significant role for TNF, in promoting the function of chemokine-expressing stromal cells in lymphoid areas of the spleen. Analysis of lymph nodes from LTβR-<sup>-Ig</sup>-treated mice has provided initial evidence that LT<sup>α/β</sup> is also required for normal chemokine expression in lymph nodes. Given the requirement for LT<sup>α/β</sup> and TNF in normal organization of all peripheral lymphoid tissues, as well as the ability of ectopically expressed LT<sup>α</sup> to promote accumulation of B and T lymphocytes in lymphoid aggregates (57), it appears likely that LT<sup>α/β</sup> and TNF function broadly in regulating lymphoid tissue chemokine expression. Accumulation at nonlymphoid sites of cells in follicle- and T zone-like structures also typifies several human diseases, including rheumatoid arthritis and type I diabetes, and the possibility that locally produced LT and TNF induce the development of BLC- and SLC-expressing stromal cells deserves investigation.
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References


19. Bleul, C.C., R.C. Fuhlbrigge, J.M. Caasnovas, A. Aiuti, and J.D. Sedgwick’s present address is DNAX Research Institute, 901 California Ave., Palo Alto, CA 94304-1104.

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56. Mebius, R.E., P. Rennert, and I.L. Weissman. 1997. Developing lymph nodes collect CD4\(^+\)CD3\(^-\) LT\(\beta\)+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. Immunity. 7:493–504.