Complementation of Lymphotoxin α Knockout Mice with Tumor Necrosis Factor–expressing Transgenes Rectifies Defective Splenic Structure and Function

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Summary
Lymphotoxin (LT)α knockout mice, as well as double LTα/tumor necrosis factor (TNF) knockout mice, show a severe splenic disorganization with nonsegregating T/B cell zones and complete absence of primary B cell follicles, follicular dendritic cell (FDC) networks, and germinal centers. In contrast, as shown previously and confirmed in this study, LTβ-deficient mice show much more conserved T/B cell areas and a reduced but preserved capacity to form germinal centers and FDC networks. We show here that similar to the splenic phenotype of LTβ-deficient mice, complementation of LTα knockout mice with TNF-expressing transgenes leads to a p55 TNF receptor–dependent restoration of B/T cell zone segregation and a partial preservation of primary B cell follicles, FDC networks, and germinal centers. Notably, upon lipopolysaccharide challenge, LTα knockout mice fail to produce physiological levels of TNF both in peritoneal macrophage supernatants and in their serum, indicating a coinciding deficiency in TNF expression. These findings suggest that defective TNF expression contributes to the complex phenotype of the LTα knockout mice, and uncover a predominant role for TNF and its p55 TNF receptor in supporting, even in the absence of LTα, the development and maintenance of splenic B cell follicles, FDC networks, and germinal centers.

Key words: splenic architecture • gene targeting • complementation • follicular dendritic cells • germinal centers

Tumor necrosis factor (TNF), lymphotoxin α (LTα), and LTβ are structurally homologous cytokines, and their genes are closely clustered within the MHC (1, 2). TNF is expressed mainly by macrophages and T cells as either a transmembrane protein or a soluble homotrimeric molecule (3). LTα exists as a soluble homotrimer (LTα3) but also on the membrane of activated lymphocytes in heterotrimeric complexes with LTβ (1). TNF and LTα3 use the same cell surface receptors, the p55 and p75 TNFRs, which are expressed on a wide variety of cells (4), whereas the predominant surface LTα1β2 heterotrimer binds to the LTβR, which is expressed on cells of nonhematopoietic origin (5).

Recent studies in gene-targeted mice have revealed essential roles for TNF, LTα, and LTβ in secondary lymphoid organ structure and function. TNF was found to be essential for the formation of primary B cell follicles, follicular dendritic cell (FDC) networks, and germinal centers in all secondary lymphoid organs (6, 7). LTα knockout mice lack lymph nodes and Peyer's patches and show a severe disorganization of splenic architecture where B/T cell areas do not segregate, marginal zones are absent, and FDC networks and germinal centers do not form (8, 9). Interestingly, mice deficient in both the LTα and TNF genes show a phenotype identical to the LTα knockout phenotype (10). Remarkably, however, these phenotypes were not fully reproduced in LTβ knockout mice, in which mesenteric and cervical lymph nodes do develop, splenic white pulp lymphocytes segregate into B/T cell zones (11, 12), and some capacity to form FDC networks and germinal centers is preserved (12). These discrepancies led to the hypothesis that LTα should have additional functions independent of LTβ in the organogenesis of mesenteric and cervical lymph nodes and in the formation of distinct B/T cell areas, FDC networks, and germinal centers in the spleen.

The apparently similar phenotypes of LTα and double TNF/LTα (10) knockout mice and the differences these mice show when compared with LTβ knockout mice (11–13) have led us to search for functional redundancies in the TNF/LT system by complementing LTα knockout mice with TNF-expressing transgenes. Surprisingly, although the lack of mesenteric and peripheral lymph nodes and
Peyer's patches could not be rescued by transgenic expression of TNF, TNF-complemented LTα knockout mice displayed intact T/B cell segregation and retained a suboptimal capacity to develop primary B cell follicles that contained FDC networks and could support the formation of germinal centers. TNF-mediated restoration of LTα knockout splenic architecture was dependent on the presence of the p55 TNFR. These observations suggested that altered TNF expression may contribute to the complex splenic phenotype of the LTα knockout mice. Indeed, defective TNF production in the LTα knockout mice could be documented by measuring TNF accumulation in LPS-stimulated sera and in the supernatants of macrophages from LTα knockout mice. Our studies support a model where LTα's function to provide the optimal reticular/stromal splenic architecture for the efficient formation of B cell follicles and FDC networks, phenomena primarily dependent on TNF/p55 TNFR interactions.

Materials and Methods

Mice. LTα knockout mice (8) were obtained from The Jackson Laboratory (Bar Harbor, ME); LTβ knockout (12) mice were provided by Dr. K. Pfeffer (Technical University of Munich, Munich, Germany); and p55TNFR-deficient (14) mice were provided by Dr. H. Bluethmann (Hoffmann-LaRoche Inc., Basel, Switzerland). Transgenic (Tg)1278 mice carrying ~3 copies of a genomic 3.6-kb DNA fragment containing the entire wild-type human TNF gene together with 0.6 and 0.8 kb of 5' and 3' flanking sequences, respectively (15), and TgA86 mice carrying ~50 copies of a 3.2-kb hybrid TNF/globin transgene construct containing the promoter of the murine TNF gene, the coding sequence of the murine TNFα, -β, and -γ genes (16), and the 3'-untranslated region (UTR) and polyadenylation site of the human β-globin gene (16) have been previously generated in our laboratory. Tg1278 and TgA86 mice were always heterozygous for the TNF transgenes. All mice used in this study were maintained under specific pathogen-free barriers in the animal facilities of the transgene. LTα knockout mice (8) have been previously generated in our laboratory. Tg1278 and TgA86 mice were always heterozygous for the TNF transgenes. All mice used in this study were maintained under specific pathogen-free barriers in the animal facilities of the transgene. LTα knockout mice (10) were provided by Dr. H. Bluethmann (Hoffmann-LaRoche Inc., Basel, Switzerland). Transgenic (Tg1278 and TgA86 mice were always heterozygous for the TNF gene (16) have been previously generated in our laboratory. LTα knockout (12) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). LTα knockout (10) mice were always heterozygous for the TNF gene (16). TgA86 mice carrying ~50 copies of a 3.2-kb hybrid TNF/globin transgene construct containing the promoter of the murine TNF gene, the coding sequence of the murine TNFα, -β, and -γ genes (16), and the 3'-untranslated region (UTR) and polyadenylation site of the human β-globin gene (16) have been previously generated in our laboratory. TgA86 mice were always heterozygous for the TNF transgenes (16). All mice used in this study were maintained under specific pathogen-free barriers in the animal facilities of the transgene. LTα knockout mice (10) were obtained from The Jackson Laboratory (Bar Harbor, ME). LTα knockout (10) mice were always heterozygous for the TNF gene (16). For double immunostaining of B cells and FDC networks or germinal centers, sections were incubated with rat anti-B220 (PharMingen), followed by peroxidase-conjugated goat anti-rat IgG. After blocking with 20% normal rat serum in PBS, sections were incubated with biotinylated anti-mouse complement receptor (CR) antibody (8C12; PharMingen) or biotinylated peanut agglutinin (PNA; Sigma Chemical Co., St. Louis, MO), followed by avidin–alkaline phosphatase. In all of the above stainings, bound peroxidase activity was developed with diaminobenzidine (DAB; Sigma Chemical Co.), and alkaline phosphatase activity was visualized with naphthol AS-MX phosphate (Sigma Chemical Co.) and fast blue BB salt (Sigma Chemical Co.). For double staining with ER-Tr9 and antisialoadhesin, sections were incubated with ER-Tr9 (reference 19; provided by Dr. P. Leenen, Erasmus University, Rotterdam, The Netherlands) followed by alkaline phosphatase–conjugated streptavidin. Bound peroxidase activity was detected by staining with aminoethylcarbazol (Sigma Chemical Co.), and alkaline phosphatase activity was visualized with naphthol AS-MX phosphate and fast blue BB salt. All incubations were carried out under humidified conditions, and slides were washed in PBS between steps.

Immunization Protocol. Indicated groups of mice were immunized intraperitoneally with 10⁶ SRBC in PBS on days 0 and 21. Mice were bled on day 28 for measurement of anti-SRBC serum antibodies.

ELISA for SRBC-specific Serum Antibodies. Sera from immunized mice were assayed using SRBC-specific ELISA for IgG1 antibodies as described previously (6). In brief, 96-well ImmunoMaxisorp plates (Nunc, Roskilde, Denmark) were coated with a solubilized extract from SRBC (100 μl at 5 μg/ml [21]) suspended in carbonate buffer, pH 9.6. Plates were washed with 0.05% Tween 20 in PBS and blocked with 1% BSA in PBS. Serum samples diluted in PBS containing 0.05% Tween 20, 0.1% BSA, and 1 M NaCl were incubated overnight at 4°C. Horseradish peroxidase-conjugated goat anti-mouse IgG1 (Southern Biotechnology Associates, Inc.) was diluted 1:5,000 in PBS containing 1% BSA, and 100 μl/well was added and incubated for 1 h at room temperature. ELISAs were developed with 0.4 mg/ml o-phenylenediamine dihydrochloride (Sigma Chemical Co.) in 0.05 M phosphate-citrate buffer, pH 5.0, containing 0.03% H₂O₂, stopped with 2 M H₂SO₄, and OD₅₉₅ from duplicate wells was measured using a microplate reader (MRX; Dynatech Laboratories, Inc., Chantilly, VA).

Measurement of TNF Production after LPS Stimulation. Levels of TNF in macrophage supernatants and sera were determined as described previously (16). In brief, thioglycollate-elicited peritoneal macrophages were seeded at 5 x 10⁶ cells/ml in culture medium containing the presence of 1 μg/ml LPS (Salmonella enteritis, L601; Sigma Chemical Co.) at 37°C, 5% CO₂ for 24 h. Mice were injected intraperitoneally with 100 μg LPS in 0.5 ml PBS, and 90 min later blood samples were collected by cardiac puncture. The ELISA assay for murine TNF was provided by Dr. W. M. Buurman (University of Limburg, Maastricht, The Netherlands) and performed as described previously (22).

Results

TNF-complemented LTα knockout mice. LTα knockout mice (8, 9) as well as double LTα/TNF knockout mice (10)
show a more severe disorganization of their splenic architecture compared with TNF (6) or LTβ knockout mice (11, 12). To determine whether the additional lymphoid abnormalities seen in the LTα knockout mice may be rescued by TNF-specific signals, we complemented LTα knockout mice with TNF-expressing transgenics (TgTNF/LTα−/−). Two previously characterized TNF transgenic lines were used: Tg1278 mice expressing a human wild-type TNF transgene (15) and Ta86 mice expressing a mutant transmembrane form of murine TNF from a TNFα−/−/globin hybrid gene construct (reference 16, and see Materials and Methods). Tg1278 mice are free of pathology and express a low level of wild-type human TNF mRNA in several tissues, including thioglycollate-elicited peritoneal macrophages, thymus, lung, spleen, kidney, brain, skin, and joints. Similar to endogenous murine TNF, low-level mRNA expression of the wild-type human TNF transgene does not result in a detectable level of TNF protein secretion in either sera or supernatants from thioglycollate-elicited peritoneal macrophages as assessed by ELISA or cytotoxicity assays (not shown). However, after LPS stimulation of ex vivo peritoneal macrophages, correct upregulation of transgene mRNA (15) and of protein production (not shown) could be demonstrated. On the other hand, Ta86 mice express a constitutively high level of the TNFα−/−/globin mRNA in several tissues, including thioglycollate-elicited peritoneal macrophages, thymus, lung, spleen, mesenteric lymph nodes, kidney, heart, brain, skin, and joints (16). Constitutive overproduction of a bioactive transmembrane TNF protein is suggested by the development of chronic inflammatory arthritis in these mice (16), indicating aberrant regulation probably resulting from the absence of the putatively suppressive TNF 3'-UTR from the mRNA of this specific transgene.

Macroscopic examination of TNF-complemented LTα knockout mice (n = 7 per transgenic line) showed that they lack mesenteric and peripheral lymph nodes and Peyer’s patches, confirming a TNF-independent role for LTαs in the organogenesis of these lymphoid tissues. However, in both TgTNF/LTα−/− and Ta86/LTα−/− mice, a substantial preservation of splenic structure could be observed (see below), indicating a composite nature of the LTα null mutation.

Rescued Splenic T/B Cell Segregation in TNF-complemented LTα knockout mice was assessed using double immunostaining with anti-IgM and anti-IgD antibodies. By this staining, B cell follicles containing IgM+/IgD− B cells can be clearly discriminated from IgMhigh/IgDlow marginal zone B cell populations in spleens from wild-type mice (Fig. 1). Consistent with previous studies (23), double IgM/IgD staining revealed the absence of follicular organization in spleens from LTα knockout mice (Fig. 1). However, similar analyses in spleens from Tg1278/LTα−/−, Ta86/LTα−/−, and Ltβ−/− mice (Fig. 1) revealed the presence of organized primary B cell follicles although at reduced size and numbers compared with wild-type controls. Double immunostaining with anti-B220 as a marker for B cells, and an antibody to CR1 (mAb 8C12) as a marker for FDCs (18), showed that the follicular structures observed in TgTNF/LTα−/− and LTβ−/− mice contain networks of CR1+ FDCs (Fig. 2). These FDC networks were greatly diminished in number and size; however, similar to wild-type FDCs, they showed a typical network organization and follicular localization. Thus, even in the absence of LTα, TNF has the capacity to support, albeit suboptimally, the development and maintenance of organized B cell follicles and FDC networks.

To examine the structure of the splenic marginal zone in TgTNF/LTα−/− mice, we performed immunocytochemical analysis of splenic sections using markers specific for the specialized macrophage populations of the marginal zone. Double immunostaining with ER-TR9, an mAb recognizing marginal zone macrophages (19), and the 1C2 mAb against mouse sialoadhesin (20), which stains specifically the metallophilic macrophages in the spleen (21), revealed the characteristic concentric organization of these macrophage subsets peripheral to the white pulp in spleen sections from wild-type mice (Fig. 2). However, similar to spleen sections from LTα knockout mice (Fig. 2), double ER-TR9/anti-sialoadhesin staining of sections from TgTNF/LTα−/− and LTβ−/− mice showed the absence of these macrophage populations of the marginal zone (Fig. 2). Absence of metallophilic macrophages was also shown using the MOMA-1 mAb (data not shown; provided by G. Kraal, Vrije University, Amsterdam, The Netherlands), and staining using the R3-3C12C7 anti-mucosal addressin cell adhesion molecule 1 mAb (provided by B. Holzmann, Technical University of M unich, M unich, Germany) demonstrated the complete absence of marginal zone mucosal addressin cell...
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adhesion molecule 1 expression from the spleens of LT$^{\alpha-/-}$, Tg1278/LT$^{\alpha-/-}$, Tgα86/LT$^{\alpha-/-}$, and LTβ$^{\alpha-/-}$ mice (not shown). Deficient marginal zone formation in these mice was also confirmed by the absence of the characteristic IgM$^{\text{high}}$/IgD$^{\text{low}}$ marginal zone B cell population as assessed by double IgM/IgD staining (Fig. 1). Taken together, these data confirm the requirement for LT function in the development of the splenic marginal zone.

Germinal Center Formation and IgG Antibody Responses in TgTNF/LT$^{\alpha-/-}$ Mice. To investigate whether the rectified follicular organization in TgTNF/LT$^{\alpha-/-}$ mice could support development of germinal centers, we analyzed germinal center formation in the spleens of wild-type, LT$^{\alpha-/-}$, LTβ$^{\alpha-/-}$, and TgTNF/LT$^{\alpha-/-}$ mice 10 d after immunization with the T cell–dependent (TD) antigen SRBC. Double immunocytochemical analysis using anti-B220 antibodies as a B cell marker and PNA as a marker for germinal center B cells demonstrated the presence of typical germinal centers forming within B cell follicles in wild-type mice (Fig. 2). Consistent with previous studies (9), control LT$^{\alpha-/-}$ mice did not form typical germinal centers, although rare aggregates of PNA+ cells could be detected around central arterioles (reference 12, and Fig. 2). Similar analyses of spleen sections from immunized Tg1278/LT$^{\alpha-/-}$, Tgα86/LT$^{\alpha-/-}$, and LTβ$^{\alpha-/-}$ mice revealed the presence of PNA+ germinal centers forming within B cell follicles (Fig. 2). Although the number and size of these germinal centers are reduced compared with wild-type mice, their formation within B cell follicles, their typical appearance as B220+ IgD− areas surrounded by IgD+ follicular mantle B cells (not shown), and the finding that they contain FDC networks distinguish them from the PNA+ patches often observed to form around central arterioles in LT$^{\alpha-/-}$ (12) and TNF knockout mice (6).

To investigate the ability of the TNF-complemented LT$^{\alpha}$ knockout mouse to respond to a TD immunization, we tested the secondary antibody responses of TgTNF/LT$^{\alpha-/-}$ mice to the TD antigen SRBC. Wild-type, Tg1278/LT$^{\alpha-/-}$, and LT$^{\alpha-/-}$ mice were immunized intraperitoneally with SR BC on days 0 and 21, and anti-SR BC IgG1 antibody responses were measured on day 28 using an antigen-specific ELISA. Tg1278/LT$^{\alpha-/-}$ mice showed increased levels of IgG1 anti-SR BC antibodies compared with LT$^{\alpha-/-}$ mice, although they could not reach the levels produced by wild-type mice (Fig. 3). Similar results were observed using Tgα86/LT$^{\alpha-/-}$ mice (data not shown). Thus, the observed germinal centers in the TNF-complemented LT$^{\alpha-/-}$ mice appear functional and contribute positively, albeit suboptimally, to the development of a TD humoral immune response.

A Reduced Level of TNF Production in LT$^{\alpha}$ Knockout Mice. Given the surprising result of partial reconstitution of the splenic phenotype of the LT$^{\alpha}$ knockout mice with TNF-expressing transgenes, and the striking similarities in the splenic architecture of TgTNF/LT$^{\alpha-/-}$ and LTβ$^{\alpha-/-}$ mice, it would seem likely that the splenic phenotype of the LT$^{\alpha}$ knockout mice could actually result from a coexistent deficiency in TNF production in these mice. To address this question, we measured levels of TNF protein production in LPS-stimulated LT$^{\alpha}$ knockout mouse sera or ex vivo peritoneal macrophage supernatants. Compared with background-matched wild-type control mice, LT$^{\alpha}$ knockout mouse sera showed severely reduced accumulation of TNF protein in both sera and macrophage exudates (Fig. 4), indicating that in addition to the LT$^{\alpha}$ null mutation, this strain of mice may show further complications due to inherent defects in the neighboring TNF gene expression. Further analyses should address the underlying mechanism producing the additional defect on TNF expression and assess the impact of this phenomenon in the interpretation of different phenotypes occurring in the LT$^{\alpha}$ knockout mouse strain.

Discussion

Targeted disruption of genes encoding ligands and receptors in the TNF/LT family have clearly established the important roles these molecules play in regulating the development and function of secondary lymphoid tissues (13, 25, 26). From these studies, a role has been proposed for LTβ heterotrimers, presumably signaling through the LTβR, in the organogenesis of lymph nodes and Peyers patches and in the regulation of splenic structural organization. However, apparent differences between the lymphoid phenotypes of LT$^{\alpha}$ and LT$^{\beta}$ knockout mice suggested that LT$^{\alpha}$ should have additional biological activities independent of LT$^{\beta}$ (11–13). For example, defects in splenic T/B cell organization and complete absence of FDC networks and typical germinal centers in LT$^{\alpha}$ or double LT$^{\alpha}$/TNF knockout mice (8–10) could not be fully reproduced in LT$^{\beta}$-deficient mice (12), which show more conserved B/T cell organization and retain some capacity to form FDC networks and germinal centers (Figs. 1 and 2). Using complementation analysis, we show in this study that many of the phenotypic complications of LT$^{\alpha}$ knockout mice in the spleen may be compensated by the reintroduction of functional TNF transgenes. Complementation of LT$^{\alpha}$ knockout mice with TNF-encoding transgenes leads to a p55TNFR-dependent restoration of B/T cell zone segregation and to the partial preservation of B cell follicles.

![Figure 1](image-url)
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FDC networks, and germinal centers. Interestingly, the TNF-complemented LTα-deficient phenotype is strikingly similar to the splenic phenotype of LTβ-deficient mice, as demonstrated in this study (Figs. 1 and 2). These observations suggest that defective TNF expression may contribute to the complex phenotype of the LTα knockout mice. Alternatively, it may be suggested that complementation of the LTα knockout phenotype by TNF is due to a substitution for LT function rather than restoration of defective TNF expression, for example by transgenic overexpression of TNF. However, this seems unlikely, since phenotypic complementation by TNF occurs similarly with three independent transgenic lines carrying either correctly regulated or overexpressed TNF transgenes (i.e., Tg1278 mice expressing correctly regulated levels of human wild-type TNF, TgA86 mice overexpressing a bioactive murine transmembrane TNF, or Tg6079 mice [our unpublished data and not shown here] expressing wild-type murine TNF). In addition, transgenic expression of LTα in LTα knockout mice was reported recently as not sufficient to rescue defective splenic architecture, although it could restore lymph node organogenesis (27). Interestingly, the observed splenic phenotype of TgLTα/LTα-/- mice seems to be similar to the phenotype of TNF-deficient mice (6), with B cells organized in ring-like structures around the periarteriolar lymphoid sheath, and PNA+ patches forming around central arterioles (see Fig. 4C in reference 27).

Most importantly, decreased expression of the endogenous TNF gene in LTα knockout mice could be documented in this study by TNF-specific quantitative assays (Fig. 4). It is not clear, however, whether this defect in TNF expression occurs at the level of gene transcription, mRNA translation, or protein processing. Although this certainly awaits further detailed characterization, it is tempting to speculate that defective expression of the TNF gene in the LTα knockout mouse strain is due to transcriptional interference caused by retention of a phosphoglyceraldehyde kinase (PGK)-neo selection cassette within the targeted LTα locus. This is now well documented in several other cases where retention of the PGK-neo cassette in targeted loci has yielded unexpected phenotypes due to the altered expression of neighboring genes (28, 29). In light of the evidence presented here, interpretation of the different phenotypes occurring in the LTα knockout mice should be carefully readdressed.

Interestingly, although expression of TNF is sufficient, even in the absence of LTα, to drive white pulp organization into distinct B and T cell areas and to partially support follicular structure and germinal center formation, the function of LTs is clearly required for the development of the marginal zone, as documented in this study but also as suggested previously by the complete absence of marginal zone structures in LTβ-deficient mice (11, 12). In this context, it is perhaps not very surprising that humoral responses to SRBC, as measured in the TNF-complemented LTα knockout system, although partially restored could...
not reach normal levels. This may be due to the presence of a suboptimal number of germinal centers in the spleen of these mice, but it may also be due to the documented complete absence of marginal zones in this system. Indeed, marginal zones are thought to play an important role in processing particulate antigens such as SRBC (30).

Taken together with the presence of mesenteric and cervical lymph nodes in LTβ-deficient mice (11, 12), our evidence that TNF-complemented LTα knockout mice still lack their mesenteric and peripheral lymphoid organs supports previous suggestions that LTα should have additional lymphoid organogenetic functions, independent of LTβ (11). It is perhaps interesting to note that correct B/T cell segregation, and also primary follicular organization and germinal center reactivity, appear in this study to be tightly regulated phenomena occurring in the absence of preserved marginal zone structures, and seemingly uninfluenced by a coinciding complete absence of secondary lymphoid organs. Therefore, it seems that they represent primary phenomena directly dependent on the local functioning of TNF and LTs.

Inhibition of LTα/β signaling either in transgenic mice expressing a soluble LTβR-IgG1 fusion protein (31), or by administration of soluble LTβR-Ig fusion proteins in normal adult mice (32), was shown to have profound effects on splenic organization. Similar effects were not observed when a p55TNFR-Ig fusion protein was administered to adult mice, suggesting that at least within the 3-4 wk time frame of inhibition, the TNF/p55TNFR system is not required for the maintenance of splenic architecture (32). We and others have previously suggested that a function for TNF/p55TNFR in splenic organization may be in the development and differentiation of FDC networks which would then support follicular organization and germinal center reactivity. If this is true, then given the long-living character of the FDCs, one would expect that only long-term inhibition of TNF signaling would be revealing for a role in the maintenance of splenic organization in the adult. The role of the TNF/p55TNFR system may also seem dispensable for correct B/T cell segregation, since this phenomenon is not affected in either TNF (6) or p55TNFR knockout mice (14). However, our present evidence suggests that even in the absence of LTs, TNF is sufficient to suboptimally support development and maintenance of follicular organization, indicating that the LT system shares a redundant role with TNF in regulating the conserved appearance of splenic white pulp areas. In addition to the splenic defects, administration of LTβR-Ig during gestation disrupted lymph node development (33), suggesting a basic organogenetic role for LTα/β in these processes. However, a differential role for TNF/p55TNFR in these phenomena is suggested by the presence of secondary lymphoid organs with clear B/T cell zone segregation in the TNF−/− (6) or p55TNFR−/− deficient strains of mice (14). As discussed above, a function for TNF/p55TNFR in splenic organization may be in the development and differentiation of FDC networks which would then support follicular organization and germinal center reactivity (6, 7, 34). In support of this hypothesis, recent bone marrow transplantation experiments in LTα−/− and p55TNFR−/− deficient mice showed that FDC clustering induced by wild-type bone marrow transfers is dependent on the presence of the p55TNFR on nonhematopoietic cells (35, 36). On the other hand, the LT/LTβR system is expected to function on hematopoietic lineage cell interactions with nonlymphocytic stroma elements (37, 38), and it may be that such interactions control the basic architecture, which appears essential for the organogenesis of the lymph nodes but also for the fine structural organization of the spleen. Formation of FDC networks should be a composite phenomenon requiring an optimal splenic infrastructure, perhaps provided by LTs, and a TNF/p55TNFR-specific signal which leads to the maturation/differentiation and/or follicular localization of FDCs. The capacity to form FDC networks and germinal centers even in the absence of LTs, as documented in both TNF-complemented LTα knockout mice and LTβ knockout mice, supports this hypothesis. Therefore, it is likely that the role of LTs is functionally distinct from that of TNF, and that both pathways need to cooperate for the optimal development and maintenance of splenic structure.

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