A Role for Tumor Necrosis Factor Receptor Type 1 in Gut-associated Lymphoid Tissue Development: Genetic Evidence of Synergism with Lymphotoxin β

By Pandelakis A. Koni* and Richard A. Flavell‡

Summary
Lymphotoxin α (LTα) signals via tumor necrosis factor receptors (TNFRs) as a homotrimer and via lymphotoxin β receptor (LTβR) as a heterotrimeric LTαβ2 complex. LTα-deficient mice lack all lymph nodes (LN)s and Peyer’s patches (PPs), and yet LTβ-deficient mice and TNFR-deficient mice have cervical and mesenteric LN. We now show that mice made deficient in both LTβ and TNFR type 1 (TNFR1) lack all LN, revealing redundancy or synergism between TNFR1 and LTβ, acting presumably via LTβR. A complete lack of only PPs in mice heterozygous for both LTα and LTβ, but not LTα or LTβ alone, suggests a similar two-ligand phenomenon in PP development and may explain the incomplete lack of PPs seen in tnfr1−/− mice.

Key words: lymphotoxin beta • tumor necrosis factor receptor 1 • knockout mice • mesenteric lymph nodes • Peyer’s patches

Studies on mice genetically deficient in various secondary lymphoid organs are increasing our understanding of the requirement or otherwise for these highly organized structures in immune function, from antiviral immunity (1) to autoimmunity (2). Hox11−/− mice lack a spleen (3), whereas aly/aly mutant mice lack LN and have a disorganized spleen (4, 5). Also, mice made deficient in the putative chemokine receptor BLR1 lack inguinal lymph nodes and fail to form primary B cell follicles in the spleen (6). Our studies have involved members of the TNF receptor and ligand families (7, 8). Studies of TNF family members are not only providing insight into the intricate microarchitecture of immune cell responses in lymphoid organs but also of chronic inflammatory states (9), such as the phenomenon termed lymphoid neogenesis (10).

TNF-α and TNF-β (lymphotoxin α; LTα)1 are the archetypal ligands of a growing family, which includes CD30 ligand (L), CD40L, FasL, TRAIL, and lymphotoxin β (LTβ) (11, 12). LTβ was discovered by virtue of its ability to anchor LTα to the cell surface, without which LTα is secreted as a homotrimer (LTαβ) (13, 14). LTαβ complex itself is a trimmer with a predominant form (LTαβ2) that binds LTβR, and a minor form (LTαββ) that binds TNF receptor type 1 (TNFR1) (15–17). Both forms of LTα are produced by activated lymphocytes and NK cells (12, 18).

Historically, LTαβ is known as a factor that causes cytotoxicity and inflammation, and signals via TNFR1 and TNFR2 (9, 19, 20). Although LTαβ complexes do not appear to mediate inflammation (21), pleiotropic effects of LTβR cross-linking are now emerging, including cytotoxicity (17, 22), chemokine induction (23), and integrin up-regulation (21). Studies with lta−/− mice and ltb−/− mice are beginning to address the in vivo significance of these facets of LTα and LTβ biology (2). However, initial studies of lta−/− mice were dominated by the unexpected observation of a complete lack of LN and Peyer’s patches (PPs), as well as a disorganized spleen lacking follicular dendritic cells and germinal centers (24–27). Since mice deficient in TNFR have LN, it had been assumed that the LTαβ complexes were responsible rather than LTαβ. However, we recently showed that this explanation was not entirely correct (28). Specifically, we determined that ltb−/− mice retain mesenteric LN (MLN)s and to a certain extent, cervical LN, both of which drain mucosal surfaces. It was therefore a paradox that these LN are absent in mice that lack the LTαβ ligand and yet they are present in mice that lack the known receptors TNFR1 and TNFR2.

We now report that mice made deficient in both TNFR1 and LTβ lack MLN. We have thus revealed a redundancy or synergism between TNFR1 and LTβ (presumably signaling via LTβR) that warrants further investi-

1Abbreviations used in this paper: dnf−/−, mice deficient in both TNFR1 and TNFR2; LT, lymphotoxin; MLN, mesenteric lymph nodes; PPs, Peyer’s patches.
gation in other aspects of TNFR1 and LTβ biology. Ltna−/− mice and Ltb−/− mice were derived as littermates by interbreeding, and unambiguously confirmed the lack of MLNs in Ltna−/− mice and their presence in Ltb−/− mice. Surprisingly, the latter studies also revealed a complete and specific lack of only PPs in Ltna+/−/Ltb−/− mice. This presents a unique mouse model for the study of gastrointestinal immunology and suggests that two LTα ligands are involved in PP as well as MLN development, and may explain the incomplete lack of PPs seen in Ltna−/− mice.

**Materials and Methods**

Mice: Ltb−/− and Ltb+/+ wild-type mice (expanded from original littermates of Ltb−/− mice) are those described previously (28). A breeding pair of Ltna−/− mice (24) was obtained from Nancy Rodd (Yale University Department of Epidemiology and Public Health, Yale University), derived originally from David Chaplin (Washington University, St. Louis, MO). Mice deficient in both TNFR1 and TNFR2 were purchased from Clarence Reeder (Frederick Cancer Research Institute, Frederick, MD). All mice were maintained at Yale University in specific pathogen-free conditions. All procedures were conducted in accordance with Yale animal care and use guidelines.

LTβ genotyping was by PCR using three oligonucleotides yielding ~120- and 330-bp products for the Ltb− and Ltb+ alleles, respectively. The oligonucleotide sequences are: LTβfor, 5′-GAGACAGTCACACCTGTTG-3′; LTβrev, 5′-CCTGTAGTCCA-CCATGTCG-3′; and LTβneo, 5′-CTTGGTGAATGCCGA-TCC-3′. TNFR1 and TNFR2 genotyping was by Southern blot analysis as described elsewhere (29).

Bone Marrow Chimeras. Hosts were exposed to 950 rads at 6–8 wk of age and, 1 d later, were given 2 × 10⁶ total nucleated bone marrow cells intravenously in 0.2 ml of PBS. Bone marrow was from sex-matched 8–12-wk-old C57BL/6 Ly5.1 (CD45.1) mice purchased from Charles River Laboratories, South San Francisco, CA. The degree of chimerism was determined by flow cytometry. Both biotin-conjugated anti-CD45.1 and FITC-conjugated anti-CD45.2 were from Pharmingen (San Diego, CA). The degree of chimerism was >95% in all cases. 9–10 wk after irradiation, recipients were challenged intraperitoneally with 0.1 mg of chicken globulin adsorbed to alum in 0.2 ml of PBS. Bone marrow was from sex-matched 8–12-wk-old C57BL/6 Ly5.1 (CD45.1) mice (expanded from original littermates of Ltb−/− mice) that were purchased from Charles River Laboratories, South San Francisco, CA. The degree of chimerism was determined by flow cytometry. Both biotin-conjugated anti-CD45.1 and FITC-conjugated anti-CD45.2 were from Pharmingen (San Diego, CA). The degree of chimerism was >95% in all cases. 9–10 wk after irradiation, recipients were challenged intraperitoneally with 0.1 mg of chicken globulin adsorbed to alum in 0.2 ml of PBS and were culled 12 d later.

Pathology. Visualization of bursal, axillary, inguinal, and popliteal LN sections was aided in some experiments by injecting 50 μl of India ink into each footpad of the mice 3–4 h before culling. The prominence of PPs was greatly increased by immersing the intestine in 10% (vol/vol) acetic acid for 5 min before preservation in 10% neutral-buffered formalin. Hematoxylin and eosin staining was done on paraffin sections using standard procedures.

Immunohistology. Mice were challenged intraperitoneally at 6–8 wk of age with 0.1 mg of chicken γ globulin adsorbed to alum in 0.2 ml of PBS. Spleens and MLNs were harvested 12 d later and frozen in O.C.T. compound using a dry-ice/methylenebath. 5-μm thick sections were cut onto saranized glass slides and fixed in cold acetone for 5 min before storage at −70°C. For staining, sections were allowed to thaw for 10 min and then rehydrated in PBS for 20 min. Endogenous peroxidase was inactivated with 0.3% hydrogen peroxide for 5 min and then washed with PBS for 10 min. Blocking was with PBS/3% BSA/0.1% (vol/vol) Tewen 20 for 30 min. Staining for IgD used anti–mouse IgD (Southern Biotechnology Associates, Birmingham, AL), followed by biotin-conjugated goat anti–rat IgG (Southern Biotechnology Associates) and then β-galactosidase-conjugated avidin (Vector Laboratories, Burlingame, CA). Washing between layers was with PBS/0.1% (vol/vol) Tewen 20 before reblocking as above. Germinal centers were stained using horseradish peroxidase-conjugated peanut agglutinin (EY Laboratories, San Mateo, CA; reference 31). IgM detection was with alkaline phosphatase-conjugated goat anti-mouse IgM (Southern Biotechnology Associates). Follicular dendritic cells were revealed with biotin-conjugated anticomplement receptor 1 (Pharmingen, San Diego, CA; reference 32), followed by alkaline phosphatase-conjugated streptavidin (Zymed, South San Francisco, CA). Substrates for β-galactosidase, horseradish peroxidase, and alkaline phosphatase were HistoMark X-Gal (Kirkegaard and Perry Labs., Inc., Gaithersburg, M D), diaminobenzidine-brown (Zymed), and HistoMark Red (Kirkegaard and Perry Labs., Inc.), respectively.

**Results**

Ltna−/− Littermates of Ltb−/− Mice Lack MLNs. Initial reports of the phenotype of two independently generated Ltna−/− mouse strains differed in that one indicated that MLNs were absent (24), whereas the other indicated that lymphoid structures were present in the mesentry of 4 out of 14 mice (25). Most recently, among ~500 Ltna−/− mice examined for MLNs, only 10 had a single MLN (33). It was thus suggested that the frequency of occurrence of MLNs in Ltna−/− mice may vary depending on how the mice are housed (33). If true, this would perhaps apply equally to Ltb−/− mice, which we described as consistently having MLNs (28). Furthermore, Alimzhanov et al. independently generated Ltb−/− mice and found that only ~75% of these mice have MLNs (34). It was therefore also conceivable that there are effects of background genes, although all mice examined were on a mixed background of 129/Sv and C57BL/6. The studies here were begun to examine these issues and determine why Ltb−/− mice have MLNs despite the fact that Ltna−/− mice mostly do not.

The Ltna and Ltb genes are separated by only ~6 kbp in the MHC locus (12). Thus, we reasoned that it would be possible to generate Ltna−/− mice and Ltb−/− mice as littermates by interbreeding mice which are heterozygous for both Ltna and Ltb (Ltna+/−/Ltb−/− mice). In this way, 137 progeny were generated and genotyped as described in Materials and Methods. Ltna+/−, Ltb−/−, and Ltna+/−/Ltb−/− mice occurred in a relatively normal Mendelian fashion (n = 31, 40, and 66, respectively). Some of these mice were examined at 6–8 wk of age. Ltna−/− mice did not have MLNs (n = 14), whereas almost all of their Ltb−/− littermates did (n = 25). A single Ltb−/− mouse out of 25 appeared to lack MLNs.

Lymphotoxin Gene Dosage Effect in PP Development. Unlike Ltna−/− and Ltb−/− mice, the above heterozygous Ltna+/−/Ltb−/− mice had all MLNs (n = 30), except that two
mice had only one inguinal LN and one mouse had none. Surprisingly, however, Lta<sup>−/−</sup>ltb<sup>−/−</sup> mice showed a complete lack of PPs (n = 30), whereas both Lta<sup>−/−</sup> mice (n = 13) and ltβ<sup>−/−</sup> mice (n = 14) have PPs as well as all LNs. Having made this observation, we examined Lta<sup>−/−</sup>ltb<sup>−/−</sup> mice further. At 6–8 wk of age, the gross spleen architecture was normal by hematoxylin and eosin histology (data not shown). Immunohistology for complement receptor 1 in the spleen (done as previously described; reference 28) revealed the presence of follicular dendritic cells (data not shown). Also, splenic germinal centers were formed in discrete B cell follicles after intraperitoneal challenge, except there

---

**Figure 1.** Lta<sup>−/−</sup>ltb<sup>−/−</sup> mice have relatively normal lymphoid organ architecture. Mice were challenged intraperitoneally with 0.1 mg of chicken γ globulin adsorbed to alum and culled 12 d later. Spleen (A, C, and E) and MLN sections (B, D, and F) were stained for IgM (red), IgD (blue) and peanut agglutinin–binding germinal centers (brown). A and B, wild-type; C and D, ltβ<sup>−/−</sup>; E and F, Lta<sup>−/−</sup>ltb<sup>−/−</sup>. Original magnification, ×65 and ×150 for spleen and MLN, respectively.
appeared to be some disorganization among IgM+/IgDlo/marginal zone B cells (Fig. 1E). Ltα−/− mice (24–28) and Ltβ−/− mice (28, 34) have severe defects in all of these aspects of lymphoid organogenesis.

The organization of the MLNs of Ltα+/-Ltβ+/- mice was also relatively normal (Fig. 1F). As previously noted (28), the organization of the MLN s of Ltβ−/− mice is not normal in that there appears to be a generalized B cell infiltration, but B cell follicles are found around the rim of MLNs and germinal center B cell clusters are formed despite the absence of follicular dendritic cells (reference 28; Fig. 1D).

The lack of PPs in Ltα−/−Ltβ+/- mice was confirmed in progeny from intercrossing Ltα−/− mice with Ltβ−/− mice (n = 4). Bone marrow chimeras were also generated using wild-type bone marrow, to examine whether or not the lack of PPs was reversible. None of the Ltα−/−Ltβ+/- recipients showed any sign of PPs 10–12 wk after irradiation, but they did have LN s (n = 9). None of the Ltα−/− recipients had MLNs (n = 8), but all of the Ltβ−/− recipients did (n = 11). None of the Ltα−/− recipients or Ltβ−/− recipients had PPs. Ltβ+/- wild-type recipients had MLNs and PPs (n = 4).

TNFR1 is Involved in MLN Development. Both Ltβ−/− mice and dtnfr−/− mice have MLNs, and yet Ltα−/− mice do not. This led us to propose that LTα may act without LTβ (i.e., as LTAα3) via an as yet unidentified receptor (28). To test this hypothesis, we generated mice lacking both LTβ and TNFR and examined them for the presence of MLNs. Since TNFR-deficient mice were originally obtained as dtnfr−/− mice, the first mice generated here were Ltβ−/− dtnfr−/− mice. At 6–8 wk of age, Ltβ−/− dtnfr−/− mice showed a complete lack of MLNs (n = 10), whereas Ltβ+/− dtnfr−/− mice still had MLNs (n = 5).

In a similar way to Lt−/− mice, it is conceivable that the apparent absence of MLNs in Ltβ−/− dtnfr−/− mice is due to a possible lack of immune competence and/or lymphocyte homing, and that this might be reversed after reconstitution with wild-type bone marrow. We therefore generated wild-type bone marrow chimera. However, none of the bone marrow chimeras had MLNs 10–12 wk after reconstitution (n = 11).

In the meantime, we also generated Ltβ−/−tnfr1−/− and Ltβ−/−tnfr2−/− mice. The latter had MLNs (n = 4) but Ltβ−/−tnfr1−/− mice clearly did not (n = 5). Most Ltβ−/−tnfr1−/− littersmates (n = 5) had one small MLN (Fig. 2). None Ltβ−/−tnfr1−/− littermate did not appear to have MLNs, whereas another had two small MLNs. This may be explained by the fact that tnfr1 heterozygosity is known to result in a partial phenotype at least in some respects (35), but at the same time Ltβ−/−tnfr1−/− mice had MLNs of a normal size (n = 13).

Discussion

The study reported here extends our knowledge of the roles of TNF ligand/receptor family members in lymphoid organogenesis (Table 1). Based on several observations, we had previously hypothesized that both TNFR1 and LTβR may be involved in PP development (28). First, both Ltα+/− mice (24, 25) and Ltβ−/− mice (28, 34) completely lack PPs. Second, Rennert et al. observed a complete lack of PPs in mice administered recombinant soluble LTβR in utero (36). Third, tnfr1−/− mice lack PPs but have reduced numbers of residual lymphoid aggregates (37). Effective PP development was also reported recently with an independently generated tnfr1−/− mouse strain (29). Others reported that tnfr1−/− mice have PPs but that they appear flattened due to a lack of B cell follicle structures (38). However, even this study noted that tnfr1−/− mice have on average only two to four such PPs compared with six to eight PPs in the wild-type control mice (38).

In this study, we show the existence of a gene dosage effect that is consistent with a role for both TNFR1 and LTβR in PP development. That is, Ltα−/−Ltβ−/− mice specifically lack only PPs, but Ltα−/− mice and Ltβ−/− mice do not. If LTα and LTβ form a single species that signals via a single receptor, it might be expected that either LTα or LTβ would be the limiting factor and that heterozygosity in either Ltα or Ltβ alone should result in the lack of PPs seen in Ltα−/−Ltβ−/− mice. However, this is not the case. O nly when both Ltα and Ltβ are heterozygous does insufficiency become evident. One interpretation would be that two ligands (e.g., LTα3 and LTα1β2 signaling via TNFR1
Table 1. Phenotypes of Mice Made Genetically Deficient in TNF Ligand/Receptor Family Members

| Family M embers   | $\text{lt}\alpha^{-/-}$ | $\text{lt}\beta^{-/-}$ | $\text{tnf}\alpha^{-/-}$ | $\text{tnfr1}^{-/-}$ | $\text{lt}\alpha^{-/-}\text{lt}\beta^{+/-}$ | $\text{lt}\beta^{-/-}\text{tnfr1}^{-/-}$ |
|-------------------|--------------------------|------------------------|--------------------------|------------------------|---------------------------------|---------------------------------|
| Spleen            |                          |                        |                          |                        |                                 |                                 |
| Primary B cell follicles | $-$                    | $-$                    | $-$                       | $-$                    | $+$                             | $\text{N D}$                    |
| Marginal zone     | $-$                       | $+$                     | $+$                       | $+$                    | $+$                             | $\text{N D}$                    |
| Germinal centers  | $-$                       | $-$                     | $-$                       | $-$                    | $+$                             | $\text{N D}$                    |
| Follicular dendritic cells | $-$                    | $-$                    | $-$                       | $-$                    | $+$                             | $\text{N D}$                    |
| M LN s            | $-$                       | $+$                     | $+$                       | $+$                    | $+$                             | $-$                             |
| Germinal centers  | $+$                       | $-$                     | $-$                       | $-$                    | $+$                             | $\text{N D}$                    |
| Follicular dendritic cells | $-$                    | $-$                    | $-$                       | $-$                    | $+$                             | $\text{N D}$                    |
| Cervical LN       | $-$                       | $+$/-/-                 | $+$                       | $+$                    | $+$                             | $-$                             |
| Inguinal LN       | $-$                       | $-$                     | $+$                       | $+$                    | $+$                             | $-$                             |
| Other LN          | $-$                       | $-$                     | $+$                       | $+$                    | $+$                             | $-$                             |
| PPs               | $-$                       | $-$                     | $+$                       | $+$                    | $+$                             | $-$                             |

This table is based on citations in the text and others as summarized elsewhere (8), as well as the findings from this study. Unlike $\text{lt}\alpha^{-/-}$ mice and $\text{lt}\beta^{-/-}$ mice have PPs as well as all LNs. Other features of the phenotype of $\text{lt}\alpha^{-/-}$ mice and $\text{lt}\beta^{-/-}$ mice have not been determined. Also, although $\text{lt}\beta^{-/-}\text{tnfr1}^{-/-}$ mice do not have M LN s, $\text{lt}\beta^{-/-}\text{tnfr2}^{-/-}$ mice do.

and LT βR, respectively) are involved in PP development, and that heterozygosity in either one or the other alone is not enough to cause a complete loss of PP development. This two-receptor model might therefore provide an explanation for the partial defect in PP development seen in $\text{tnfr1}^{-/-}$ mice.

Clearly, our results show that both TNFR 1 and LT β are involved in M LN development, even though both $\text{tnfr1}^{-/-}$ mice and $\text{lt}\beta^{-/-}$ mice have MLNs. TNFR 1 also functions independently of TNFR 2 in this regard, as $\text{lt}\beta^{-/-}\text{tnfr2}^{-/-}$ mice still have MLNs. We have thus revealed a previously unappreciated relationship between TNFR 1 and LT β (presumably acting via LT βR). An explanation for the lack of MLNs in $\text{lt}\alpha^{-/-}$ mice might therefore be that LT α deficiency actually eliminates both ligands of the relationship (i.e., $\text{lt}\alpha_3$ and $\text{lt}\alpha_3\beta_2$ signaling via TNFR 1 and LT βR, respectively). LT α itself is not believed to bind LT β R (16, 17).

However, having said this, it has been indicated that $\text{lt}\beta^{-/-}$ mice lack MLNs (34). Thus, the relationship between TNFR 1 and LT βR may be one of synergism with LT βR as the dominant partner. At the same time, the presence of MLNs in $\text{lt}\beta^{-/-}$ mice would imply that LT βR has a ligand besides the LT α/β complex. Indeed, Mauri et al. have very recently described a new LT βR ligand (LIGHT) as well as a new LT α3 receptor, the herpesvirus entry mediator, expressed by lymphocytes (39).

The molecular basis for the relationship between TNFR 1 and LT βR (presumably via LT βR) remains to be determined. It is conceivable that between TNFR 1 and LT βR signaling in M LN development is simultaneous and that they interact at the level of intracellular signal transducers. Certainly, activation of LT βR has been shown to potentiate TNF-α cytotoxicity, possibly reflecting cross-talk between signaling pathways (17, 22). Ligation of LT βR causes recruitment of TNF-α-associated factor family members (40–42), and activation of NF-κ B and cell death by distinct signaling pathways (42, 43).

Thus far, our studies of $\text{lt}\beta^{-/-}$ mice have evaluated the defects in lymphoid organogenesis (reference 28 and this study). We are now beginning to examine whether or not LT β has roles in vivo in other respects. Certainly, in vitro studies have shown that signaling via LT βR causes cytotoxicity to some cell lines (17, 22), chemokine expression (23), and integrin upregulation (21). It remains to be seen whether or not the relationship between TNFR 1 and LT β (presumably via LT βR) in gut-associated lymphoid tissue development extends to any other facets of biology. With this in mind, caution is advised when interpreting the in vivo role (or rather, apparent lack thereof) of LT βR and TNF 1 based on studies of $\text{lt}\beta^{-/-}$ mice and $\text{tnfr1}^{-/-}$ mice alone.

Finally, $\text{lt}\alpha^{+/-}\text{lt}\beta^{+/-}$ mice may prove to be a useful PP-less mouse model, not only for the study of gastrointestinal infection, but also of oral tolerance, oral vaccination, and chronic disorders such as inflammatory bowel disease (44–46). L $\text{lt}\alpha^{+/-}\text{lt}\beta^{+/-}$ mice are being further characterized, particularly with respect to the subtle defect observed in splenic marginal zone organization. Although it remains possible that $\text{lt}\alpha^{+/-}\text{lt}\beta^{+/-}$ mice have other as yet unidentified defects, unlike any other previously described mouse, these mice specifically and completely lack only PPs and do not appear to have any of the major abnormalities associated with $\text{lt}\alpha^{-/-}$ and $\text{lt}\beta^{-/-}$ mice.
We thank Jacques Peschon (Immunex Corp., Seattle, WA) for tnfr-/- mice; Frank Wilson, Cindy Hughes, and Debbie Butkus for technical assistance; and Fran Manzo for secretarial assistance.

This work was supported by the Howard Hughes Medical Institute (R. A. Flavell) with the aid of grants from the Human Frontiers Science Program (to P.A. Koni) and the American Diabetes Association (to R. A. Flavell). Richard A. Flavell is an investigator of the Howard Hughes Medical Institute.

Address correspondence to R. A. Flavell, Section of Immunobiology and Howard Hughes Medical Institute, Yale University School of Medicine, 310 Cedar Street, FM B 412, New Haven, CT 06520. Phone: 203-737-2216; Fax: 203-785-7561; E-mail: richard.flavell@qm.yale.edu

Received for publication 11 February 1998 and in revised form 8 April 1998.

References

1. Karrer, U., A. Althage, B. Odermatt, C.W.M. Roberts, S.J. Korsmeyer, S. Miyamaki, H. Hengartner, and R.M. Zinkernagel. 1997. Ol n the key role of secondary lymphoid organs in antiviral immune responses studied in allogeneic (aly/aly) and spleenless (H ox11 +/-) mutant mice. J. Exp. Med. 185:2157-2170.

2. Suen, W.E., C.M. Bergman, P. Hjelmström, and N.H. Rudell. 1997. A critical role for lymphotoxin in experimental allergic encephalomyelitis. J. Exp. Med. 186:1233-1240.

3. Roberts, C.W., J.R. Shutter, and S.J. Korsmeyer. 1994. H ox11 controls the genesis of the spleen. Nat. 368:747-749.

4. Miyawaki, S., Y. Nakamura, H. Hori, M. Koba, R. Yasumizu, S. Ikeda, and Y. Shibata. 1994. A new mutation, aly, that induces a generalized lack of lymph nodes accompanied by immunodeficiency in mice. Eur. J. Immunol. 24:429-434.

5. Shinkura, R., F. Matsuda, T. Sakiyama, T. Tsubata, H. Hiai, M. Paumen, S. Miyawaki, and T. Honjo. 1995. The ligands and receptors of the lymphotoxin system. J. Immunol. 154:33-46.

6. Crowe, P.D., T.L. VanArsdale, B.N. Walter, C.F. Ware, C. Hession, B. Ehrenfels, J.L. Browning, W.S. Din, R.G. Goodwin, and C.A. Smith. 1994. A lymphotoxin–β-specific receptor. Science. 264:707-710.

7. Mackay, F., P.R. Bourdon, D.A. Griffiths, P. Lawton, M. Zafari, I.D. Sizing, K. Miatkowski, A. N. ngam-ek, C.D. Benjamin, C. Hession, et al. 1997. Cytotoxic activities of recombinant soluble murine lymphotoxin-α and lymphotoxin-αβ complexes. J. Immunol. 159:3299-3310.

8. Browning, J.L., I.D. Sizing, P. Lawton, P.R. Bourdon, P.D. Rennert, G.R. Majeau, C.M. Ambrose, C. Hession, K. Miatkowski, D.A. Griffiths, et al. 1997. Characterization of lymphotoxin-αβ complexes on the surface of mouse lymphocytes. J. Immunol. 159:3288-3298.

9. Schoenfeld, H.J., B. Piosch, J.R. Frey, H. Loetscher, W. Hunziker, A. Lutzig, and M. Zulauf. 1991. Efficient purification of recombinant human tumor necrosis factor α from Escherichia coli yields biologically active protein with a trimeric structure that binds to both tumor necrosis factor receptors. J. Biol. Chem. 266:3863-3869.

10. Picarella, D., A. Kratz, C.-B. Li, N.H. Rudder, and R.A. Flavell. 1992. Insulitis in transgenic mice expressing TNF-β (lymphotoxin) in the pancreas. Proc. Natl. Acad. Sci. USA. 89:10036-10040.

11. Huchman, P.S., G.R. Majeau, F. Mackay, and J.L. Browning. 1996. Proinflammatory responses are efficiently induced by homotrimeric but not heterotrimeric lymphotoxin ligands. J. Immunol. 46:220-234.

12. Browning, J.L., K. Miatkowski, I. Sizing, D. Griffiths, M. Zafari, C.D. Benjamin, W. Mieir, and F. Mackay. 1996. Signaling through the lymphotoxin β receptor induces the death of some adenocarcinoma tumor lines. J. Exp. Med. 183:867-878.

13. Degli-Esposti, M.A., T. Davis-Smith, W.S. Din, P.J. Smolak, R.G. Goodwin, and C.A. Smith. 1997. Activation of the lymphotoxin β receptor by cross-linking induces chemokine production and growth arrest in A375 melanoma cells. J. Immunol. 158:1756-1762.

14. De Togni, P., J. Goedelner, N.H. Rudder, P.R. Streeter, A. Fick, S. Mattrheisen, S.C. Smith, R. Carlson, L.P. Shornick, J. Strauss-Schoenberger, et al. 1994. Abnormal development of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface. Cell. 72:847-856.
of peripheral lymphoid organs in mice deficient in lymphtoxin. Science. 264:703–707.

25. Banks, T.A., B.T. Rousse, M.K. Kerley, P.J. Blair, V.L. Godfrey, N.A. Kuklin, D.M. Bouley, J. Thomas, S. Kanangat, and M.L. Uscinski. 1995. Lymphotoxin-α-deficient mice: effects on secondary lymphoid organ development and humoral immune responsiveness. J. Immunol. 155:1685–1693.

26. Matsumoto, M., S. Mairathasan, M.H. Nahn, F. Baranyay, J.J. Peschon, and D.D. Chaplin. 1996. Role of lymphotoxin and the type I TNF receptor in the formation of germinal centers. Science. 271:1289–1291.

27. Matsumoto, M., S.F. Lo, C.J.L. Carruthers, J. Min, S. Mairathasan, G. Huang, D.R. Plas, S.M. Martin, R.S. Geha, M.H. Nahn, and D.D. Chaplin. 1996. Affinity maturation without germinal centres in lymphotoxin-α-deficient mice. Nature. 382:462–466.

28. Koni, P.A., R. Sacca, P. Lawton, J.L. Browning, N.H. Ruddie, and R.A. Flavell. 1997. Distinct signals in lymphoid organogenesis for lymphotoxins α and β revealed in lymphotoxin-β-deficient mice. Immunity. 6:491–500.

29. Peschon, J.J., D.S. Torrance, K.L. Stocking, M.B. Glaccum, C. Otten, C.R. Willis, K. Charrier, P.J. Morrissy, C.B. Ware, and K.M. Mohler. 1998. TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. J. Immunol. 160:943–952.

30. Hebel, R., and M.W. Stromberg. 1976. Anatomy of the Laboratory Rat. Williams & Wilkins Co., Baltimore. 112–118.

31. Koni, P., R. Sacca, P. Lawton, J.L. Browning, N.H. Ruddie, and R.A. Flavell. 1997. Distinct signals regulate development of primary and secondary follicle structure in spleen and mesenteric lymph node. Proc. Natl. Acad. Sci. USA. 94:5739–5743.

32. Matsumoto, M., S.C. Birbeck, V.J. Wills, J.A. Forrester, and A.J.S. Davies. 1980. Peanut lectin binding properties of germline in mice. Nature. 284:364–366.

33. Kishimoto, T., J. Takeda, K. Hong, H. Kozono, H. Sakai, and K. Inoue. 1988. Monoclonal antibodies to mouse complement receptor type I (CR1). Their use in a distribution study showing that mouse erythrocytes and platelets are CR1-negative. J. Immunol. 140:3066–3072.

34. Fu, Y.-X., G. Huang, M. Matsumoto, H. Molina, and D.D. Chaplin. 1997. Independent signals regulate development of primary and secondary lymphoid structures in spleen and mesenteric lymph nodes. Proc. Natl. Acad. Sci. USA. 94:5739–5743.

35. Alimzhanov, M.B., D.V. Kuprash, M.H. Kosco-Vilbois, A. Luz, R.L. Turetsky, A. Tarakhovsky, R. Rajecki, S.A. Eddosavos, and K. Pfeffer. 1997. Abnormal development of secondary lymphoid organs in lymphotoxin-β-deficient mice. Proc. Natl. Acad. Sci. USA. 94:9302–9307.

36. Rennert, P.D., J.L. Browning, R. Mebius, F. Mackay, and P.S. Hochman. 1996. Surface lymphotoxin α/β complex is required for the development of peripheral lymphoid organs. J. Exp. Med. 184:1999–2006.

37. Nernst, B., A. Luz, K. Pfeffer, and B. Holzmann. 1996. Defective Peyer's patch organogenesis in mice lacking the 55-kDa receptor for tumor necrosis factor. J. Exp. Med. 184:259–264.

38. Pasparakis, M., L. Alexopoulou, M. Grell, K. Pfizenmaier, H. Bluethmann, and G. Kollias. 1997. Peyer's patch organogenesis is intact yet formation of B lymphocyte follicles is defective in peripheral lymphoid organs of mice deficient for tumor necrosis factor and its 55-kDa receptor. Proc. Natl. Acad. Sci. USA. 94:6319–6323.

39. Mairathasan, G. Huang, D.R. Plas, S.M. Martin, R.S. Geha, M.H. Nahn, and D.D. Chaplin. 1996. Affinity maturation without germinal centres in lymphotoxin-α-deficient mice. Nature. 382:462–466.

40. Peschon, J.J., D.S. Torrance, K.L. Stocking, M.B. Glaccum, C. Otten, C.R. Willis, K. Charrier, P.J. Morrissy, C.B. Ware, and K.M. Mohler. 1998. TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. J. Immunol. 160:943–952.

41. Force, W. R., T.C. Cheung, and C.F. Ware. 1997. Dominant negative mutants of TRAF3 reveal an important role for the coiled coil domains in cell death signaling by the lymphotoxin-β receptor. J. Biol. Chem. 272:30835–30840.

42. VanArsdale, T.L., S.L. VanArsdale, W.R. Force, B.N. Walter, G. Mosalos, E. Kieff, J.C. Reed, and C.F. Ware. 1997. Lymphotoxin-β receptor signaling complex: role of tumor necrosis factor receptor-associated factor 3 recruitment in cell death and activation of nuclear factor κB. Proc. Natl. Acad. Sci. USA. 94:2460–2465.

43. Mackay, F., G. Rameau, P.S. Hochman, and J.L. Browning. 1996. Lymphotoxin-β receptor triggering induces activation of the nuclear factor κB transcription factor in some cell types. J. Biol. Chem. 271:24934–24938.

44. Nierstra, M.R., E. Pringault, and J.-P. Kraehenbuhl. 1996. Antigen sampling across epithelial barriers and induction of mucosal immune responses. Annu. Rev. Immunol. 14:275–300.

45. Mowat, A.M., and J.L. Viney. 1997. The anatomical basis of intestinal immunity. Immunol. Rev. 156:145–166.

46. Mayrhofer, G. 1997. Peyer's patch organogenesis—cytokines rule, OK? Gut. 41:707–709.