Role of TNF ligand and receptor family in the lymphoid organogenesis defined by gene targeting

Mitsuru Matsumoto

Division of Informative Cytology, Institute for Enzyme Research, University of Tokushima, Tokushima, Japan

Abstract : The molecular basis of lymphoid organogenesis has recently been elucidated using gene-targeted mice. Mice deficient in lymphotoxin-α (LTα) lack lymph nodes and Peyer's patches. The action of LTα in lymphoid organogenesis is mediated mostly by the membrane form of LT by a mechanism independent of TNF receptor I (TNFR-I) or II (TNFR-II). Additionally, follicular dendritic cell (FDC) clusters or germinal centers fail to develop in the spleen of LTα-deficient mice. Mice deficient in either TNFR-I or LTβR also fail to develop splenic FDC clusters and germinal centers, indicating that signaling through both TNFR-I and LTβR is required for the development of these structures. The mechanisms underlying the defective lymphoid organogenesis in LTα-deficient mice, together with a natural mutant strain, alymphoplasia (aly) mice, which manifest a quite similar phenotype to LTα-deficient mice, were investigated by generating aggregation chimeras. These studies demonstrate that LTα and the aly gene product together control lymphoid organogenesis with a close mechanistic relationship in their biochemical pathways through governing distinct cellular compartments ; the former acting as a circulating ligand and the latter as a LTβR-signaling molecule expressed by the stroma of the lymphoid organs. J. Med. Invest. 46 : 141-150, 1999

Key words **:** *lymphotoxin, TNF, lymph node, spleen, knockout mice*

INTRODUCTION

Secondary lymphoid organs such as spleen, lymph node (LN) and Peyer's patches (PPs) are the sites where immune cells interact with each other, and a strong immune response against foreign antigens is initiated. Lymphocytes continuously migrate from the blood to secondary lymphoid organs. The mechanisms for lymphocyte migration have been well documented ; homing to LN and PP occurs through the interaction between adhesion molecules expressed on high endothelial venules (HEVs) of the lymphoid tissues and their counter-receptors on lymphocytes (1, 2). Lymphocytes begin to populate LN from the second day after birth, and lymphocyte diapedesis through the HEV becomes readily apparent from day 4 (3). While much is known of the molecular basis for the lymphocyte migration to the developed lymphoid organs, the mechanisms behind the incipient lymphoid organogenesis have been enigmatic. In other word, the molecular basis for the ontogenic development of lymphoid organs is not well understood.

Recent studies with gene-targeted mice manifesting abnormal development of the lymphoid organs have provided new insight into these undefined processes (summarized in Table 1). Mice deficient in lymphotoxin-α (LTα) (previously known as TNFβ) were born with a systemic absence of LN and PP (4, 5) with disturbed spleen architecture (6-9), which

Abbreviations : LT, lymphotoxin ; LN, lymph node ; PP, Peyer's patch ; EF, embryonic fibroblast ; FDC, follicular dendritic cell ; GC, germinal center ; HEV, high endothelial venule ; TNFR-I, type I TNF receptor ; LTβR, lymphotoxin-β receptor ; LTβR-Ig, LTβR-IgG1 fusion protein ; TNFR-I-Ig, TNFR-I-IgG1 fusion protein ; BM, bone marrow ; GFP, green fluorescence protein ; Tg, transgenic mice ; PNA, peanut agglutinin.

Received for publication June 1, 1999 ; accepted June 16, 1999.

Address correspondence and reprint requests to Mitsuru Matsumoto, M.D., Division of Informative Cytology, Institute for Enzyme Research, University of Tokushima, Kuramoto-cho, Tokushima, 770-8503, Japan and FAX : +81-88-633-7434.

Table 1. Gene-targeted mice with defective lymphoid organogenesis.

Disrupted gene	Lymph node	PP	Spleen
LTα LTβ*1 LTβR CXCR 5 (BLR 1)*2 Ikaros Id2	-	-	+
OPGL	-	+	+
IL-2 Rγ (γc) IL-7 Rα Jak 3*3	+	-	+
Hox11	+	+	-

*1 : Mesenteric and cervical lymph nodes are present in LTβ-deficient mice (see Table 2).
*2 : Among all lymph nodes in BLR1-deficient mice, only inguinal nodes are missing and other lymph nodes are normally developed. About half of the BLR 1-deficient mice do not have PPs at all, and the rest possess a few.
*3 : Some of the mice lack peripheral lymph nodes.

will be discussed in detail in this article. Cytokine related genes other than LT have been also recognized to be involved in the lymphoid organogenesis ; mice deficient in either IL-2Rγ chain (10), IL-7Rα or Jak3 (11) have defective PP development. Chemokine receptor CXCR5 (also known as BLR1) has been also demonstrated to be essential for the development of inguinal LN and PP as well as for the formation of B cell follicles in the spleen (12). Recently, it was also demonstrated that mice deficient in osteoprotegerin ligand (OPGL) have defective LN genesis (13). In addition, targeted deletion of transcription factors has caused abnormal development of lymphoid organs and/or lymphoid cells ; Hox11-deficient mice lack spleen (14), Id2-deficient mice lack LN and PP (15), and Ikaros-deficient mice lack cells of all lymphoid lineages, LN and PP (16). Mice deficient in both NF-κB1 (p50) and NF-κB2 (p52) also lack LN with a disorganized spleen architecture (17). Thus, a broader spectrum of factors than had been anticipated influence the lymphoid organogenesis. Because secondary lymphoid organs are essential for the development of immune response, elucidating the genes which control lymphoid organogenesis is critical for a better understanding of the mechanisms for host defence. In this article, I will discuss how the lymphoid organogenesis is controlled at the molecular level focusing on the biology of the LT and tumor necrosis factor (TNF).

I. Ligand and receptor interactions of LT and TNF ; a historical perspective

Because both the LTα and the TNF homotrimers were recognized to bind with similar affinity and to activate in similar fashion both of the defined TNF receptors (p55 or TNFR-I, and p75 or TNFR-II) (18), LTα and TNF were for many years thought to be redundant *in vivo*, differing only in their cellular sources and perhaps the signals that induced their expression. Studies by Browning and Ware have, however, demonstrated that LT can exist in two forms, the initially characterized secreted homotrimeric form and a membrane heterotrimer form which in its most prevalent form consists of one LTα subunit associated with two copies of the type II transmembrane protein LTβ (19). The LTβ gene is located adjacent to the TNF locus within the HLA complex (19, 20). The membrane LT protein (LTα1 LTβ2) is not a ligand for TNFR-I or TNFR-II, but interacts with another member of the TNF receptor family designated the LTβ receptor (LTβR) (21). The cytoplasmic domain of the LTβR shares little homology with the cytoplasmic domains of TNFR-I or TNFR-II, suggesting that activation of the LTβR should induce cellular responses distinct from those mediated by the defined TNF receptors. These observations suggest that LTα acts to signal two distinct sets of cellular responses, one set determined by its homotrimeric form binding to TNFR-I and/or TNFR-II and one set determined by its membrane form binding to the LTβR or related receptors (20). In this model, we expect that many of the functions of LT *in vivo* do not overlap with those of TNF.

In addition, a new TNF ligand and receptor family members closely related to LT and TNF have been recently identified. Herpes simplex virus (HSV) 1 and 2 infect activated T lymphocytes by attachment of the HSV envelope glycoprotein D to the cellular herpesvirus entry mediator (HVEM) (22). It was shown that HVEM binds two cellular ligands, LTα homotrimer (LTα3) and LIGHT (homologous to *l*ymphotoxins, exhibits *i*nducible expression, and competes with HSV *g*lycoprotein D for *H*VEM, a receptor expressed by *T* lymphocytes) ; LIGHT is a 29-kD type II transmembrane protein produced by activated T cells. LIGHT also binds to LTβR as the membrane form of LT does (23). These ligand and receptor interactions are illustrated in Fig. 1.

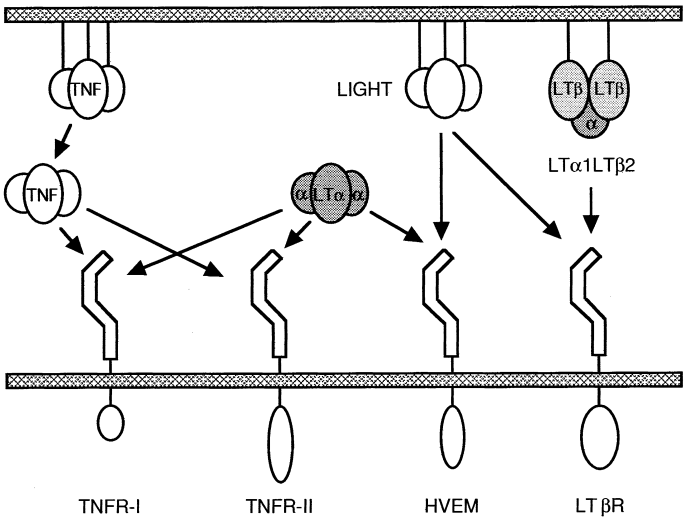

Fig.1. Ligand and receptor interactions of LT and TNF. Three distinct ligand/receptor pathways exist as follows ; 1) TNF/LTα3-TNFR, 2) LTα1LTβ2/LIGHT-LTβR, 3) LTα3/LIGHT-HVEM.

II. LT as an essential factor for the lymphoid organogenesis

LTα, originally discovered as a pro-inflammatory molecule (24), has turned out to be an essential factor that controls the genesis of the secondary lymphoid organs as well as for the organized spleen architecture (25-27). A spectacular phenotype of mice deficient for LTα was systemic absence of LN and PP (4, 5). Subsequent analyses of mice deficient for other LTα-related molecules have provided more detailed information on the action of LT in the lymphoid organogenesis (summarized in Table 2). Although mice deficient for LTβ also lacked PP and most LN, they did possess mesenteric LN (28, 29). These results suggested that LTα 1 LTβ 2 plays major roles of LT in the development of LN and PP, and that there also exists an LTα/β heteromer independent pathway required for the development of mesenteric LN. This hypothesis was later proven by the *in vivo* administration of LTβR-Ig and TNFR-I-Ig fusion protein which block the signals through LTβR and TNFR-I, respectively. Normal mice treated in utero with LTβR-Ig alone lacked systemic LN except for mesenteric LN, whereas concomitant administration of LTβR-Ig and TNFR-I-Ig or anti-TNF blocked the development of all LN including mesenteric LN, indicating that the TNF-TNFR-I axis also contributes to LN genesis (30, 31). Consistent with this model, mice deficient for both LTβ and TNFR-I lacked all LN including mesenteric LN (32). Conflicting data, however, also exist. It was demonstrated that mice deficient for LTβR lack all LN including mesenteric LN, suggesting that LTβR is a primary receptor responsible for the development of all LN (33). Phenotypic difference between

Table 2. Role of TNF ligand and receptor family members in lymphoid organofgenesis and lymphoid structure.

Disrupted gene	LTα	LTβ	LTβR	TNF	TNFR-I	TNFR-II	LTβ/TNFR-I
Lymph node							
Mesenteric	-*1	+	-	+	+	+	-
Other peripheral	-	-*2	-	+	+	+	-
PP	-	-	-	+/-*3	+/-*3	+	-
Spleen structure							
T/B segregation	-	-*4	-	+*5	+*5	+	ND
Marginal zone*6	-	+/-	-	ND	+	+	ND
Germinal center	-	-*7	-	-	-	+	ND
FDC network*8	-	-	-	-	-	ND	-

*1 : About 2% of mice have mesenteric lymph nodes.
*2 : Most of the mice possess cervical lymph nodes.
*3 : Although PPs exist, the development of B cell follicles and germinal center formation is defective.
*4 : Less marked compared with LTα-deficient mice.
*5 : Developement of B cell follicles is poor.
*6 : Revealed by immunohistochemistry using MOMA-1 monoclonal antibody.
*7 : Small numbers of PNA (peanut agglutinin) positive cells exist in both T-cell and B-cell areas.
*8 : Assessed by immunohistochemistry with monoclonal antibody against complement receptors and/or FDC-M1, monoclonal antibody specific for FDC.

LTβ-deficient mice and LTβR-deficient mice also suggested that LTβR binds not only to LTα/β heteromer but to other ligand(s), such as LIGHT (23), which may mediate signals required for the development of mesenteric LN. Thus, the integration of the detailed phenotypic analyses of these gene-targeted mice with current perspectives of LT/TNF biology may illuminate many aspects of the lymphoid organogenesis.

III. LT and TNF as essential factors for the lymphoid organ structure

Studies with knockout mice of LT/TNF-related molecules have also unravelled essential actions of LT and TNF for the organization of lymphoid structure. In mice deficient for either LTα (6, 7), LTβ (28, 29) or TNF (34, 35), organized clusters of follicular dendritic cells (FDCs) and germinal centers (GCs) are absent from the spleen.

GCs are histologically well defined structures that develop in secondary lymphoid organs shortly after challenge with T cell-dependent antigens. B cells within the GC can be clearly identified by their ability to bind to peanut agglutinin (PNA). It has been suggested that it is within GCs that somatic hypermutation and affinity maturation of the antibody response occur (36). Both primary and secondary lymphoid follicles characteristically contain clusters of FDC. FDC trap and can retain antigen-antibody complexes for long periods, apparently by means of receptors for the third complement component (CR) as well as Fcγ receptors (36). We and others have shown that an organized FDC structure, a major morphological characteristic of GCs, is absent in LTα-, LTβ-, TNF-, TNFR-I-, and LTβR-deficient mice (26, 33).

1. Role of LTα and TNFR-I in the development of GC and organized FDC clusters

Interestingly, in LTα-deficient mice, formation of FDC clusters and GCs was restored by transplantation of normal bone marrow (BM), indicating that the LTα-expressing cells required to establish these lymphoid structures are derived from BM (6). Subsequent analyses have identified B cells as an essential source of LT required for this action (37, 38). In contrast to LTα-deficient mice, when TNFR-I-deficient mice were reconstituted with wild-type BM cells, they showed no detectable FDC clusters or GC formation, suggesting that TNFR-I-expression on non-BM-derived cells is necessary for the establishment of these structures (39, 40). An analysis using LTβR-deficient mice has demonstrated similar results ; LTβR-deficient mice reconstituted with wild-type BM cells showed no detectable FDC clusters or GC formation (41). Thus, both BM-derived (expressing LTα, LTβ and TNF) and non-BM-derived (expressing TNFR-I and LTβR) cells contribute to the organization of the lymphoid structure.

To further demonstrate the role of LTα as a signal required to establish an organized FDC structure and to investigate the cell lineage of FDC, we used BM cells from CR 1/2-deficient mice to reconstitute irradiated LTα-deficient mice. In this context, donor CR 1/2-deficient BM-derived cells are LTα wild-type, but can be distinguished from the LTα-deficient recipient cells by their failure to stain with anti-CR1/2 monoclonal antibody. After reconstitution, spleen sections were stained with anti-CR1/2 monoclonal antibody and PNA to assay for the presence of FDC clusters and GC, respectively. Similar to the results obtained after transfer of wild-type BM cells, clustered FDCs were identified with anti-CR1/2 monoclonal antibody (39). These results clearly indicate that the clustered FDCs induced in these BM-chimeric mice are derived from the LTα-deficient recipient, and that LTα provides a signal that supports the development of FDC clusters. Thus, LTα is not absolutely required for the production of the FDC lineage, but rather it is necessary for its maturation or for organization of the mature cells.

2. Dysfunctional antibody responses in the absence of GC

When LTα-deficient mice were immunized with low doses of T cell-dependent antigens such as (4-hydroxy-3-nitrophenyl) acetyl-ovalbumin (NP-OVA) in alum, although a strong IgM anti-NP response developed, no IgG anti-NP was observed (7). The development of an IgM anti-NP response indicated that the failure to produce serum IgG anti-NP antibodies did not represent failure to deliver antigen to the responding B cells in the absence of LN in LTα-deficient mice. Rather, it appeared to indicate an inability to activate productive isotype switching in the setting of a disturbed primary follicle structure.

LTα-deficient mice also showed an impaired IgG response when immunized with sheep red blood cells (SRBCs) without adjuvant (9). When irradiated wild-type mice received splenocytes from LTα-deficient mice, however, a strong IgG anti-SRBC response was

observed, again supporting the idea that LTα-deficient B cells and T cells have no intrinsic defect in ability to generate an IgG response. Rather, the altered microenvironment characteristic of LTα-deficient mice appears to result in an impaired ability to switch to a productive IgG response.

3. Affinity maturation without GC in LTα-deficient mice

Although immunization of LTα-deficient mice with low doses of T cell-dependent antigens elicited an exclusively IgM B cell response as described above, immunization with high doses (100 or 200 μg of heavily haptenated NP-OVA) adsorbed to alum resulted in production of serum IgG anti-NP at levels similar to those detected in wild-type mice (7). This ability to form an IgG response permitted us to use these mice to test the role of the splenic GC in the maturation of the immune response. In previous elegant microdissection studies, the GC compartment has been shown to contain B lymphocytes with somatic mutations of their rearranged heavy and light chain variable regions, suggesting that it is in the GC that the signals for activation of the somatic mutation process are given (36, 42). We have analyzed the quality of the antibody response in LTα-deficient mice after immunization with high doses of NP-OVA adsorbed to alum. Surprisingly, LTα-deficient mice immunized with high doses of NP-OVA in alum, still without GCs, generate high affinity IgG anti-NP antibodies at levels similar to wild-type mice (7). Furthermore, analysis of the expressed V gene repertoire in splenocytes isolated from the LTα-deficient mice showed activation of a somatic mutation with all of the characteristics of that process in wild-type mice. This included mutation of codon 33 in the heavy chain variable region VH186.2 from Trp to Leu in 50% of the amplified VH186.2 sequences. This Trp to Leu mutation has been shown to correlate closely with acquisition of higher affinity for NP and is a reliable marker for the activation of affinity maturation by somatic mutation (42). These results demonstrate that somatic mutation and affinity maturation can occur independent of morphologically defined GCs. They have additional implications regarding the nature of the cells that give signals that regulate the somatic hypermutation response. They suggest that specific cellular elements that are characteristic of GCs are not essential for activation of somatic mutation. For example, clusters of FDCs are absent from the follicles of LTα-deficient mice. It remains possible that the splenic white pulp of these animals contains dispersed FDCs or immature FDC precursors without detectable surface CR1 or immune complex binding activity. Nevertheless, it is clear that the somatic mutation process can occur without the presence of clusters of mature FDCs. The fact that the defect in isotype switching and somatic mutation observed in LTα-deficient mice can be overcome by providing the antigen at high doses together with an adjuvant such as alum (7) or Freund's adjuvant (9) suggests that the physical form and perhaps the density of the antigenic epitopes determines the ability to respond in the absence of a proper follicle structure. This is consistent with the role of the natural follicle organization to facilitate focusing of antigen in the environment of the responding B and T lymphocytes.

VI. Analysis of a natural mutant strain resembling LTα-deficient mice

In addition to the gene-targeted mice described above, alymphoplasia (aly) mice, an autosomal recessive natural mutant strain, have provided a novel and unique model for the abnormal development of lymphoid organs (43). Like LTα-deficient mice, aly mice lack LN and PPs, and the spleen architecture such as the development of GCs and FDC clusters as well as marginal zone formation is disturbed (44, 45). aly mice manifest additional immunodeficiencies including a disorganized thymic architecture, low serum immunoglobulin (Ig) level and impaired allogenic skin rejection, which are not observed in LTα-deficient mice (4, 5, 43). A gene responsible for this mutant strain has just been identified as NF-κB inducing kinase (NIK) (46) by positional cloning (47, 48), but little is known about how the NIK contributes to the lymphoid organogenesis.

Our studies were undertaken to clarify the mechanisms underlying the defective lymphoid organogenesis in both aly mice and LTα-deficient mice. Two issues were specifically addressed. First, although dominant roles of LTβR as well as supportive roles of TNFR-I in the development of secondary lymphoid organs have been demonstrated as already described, exactly how LT control the development of lymphoid organs through these receptors was largely unknown. Second, we had no clue as to the role of the aly gene product (i.e. NIK) in the lymphoid organ development. Consequently, we have generated aggregation chimeras ; *ex vivo* fused

morulae were implanted into pseudo-pregnant host females and allowed to develop to term (49). Chimeric analyses demonstrated that LTα and NIK control lymphoid organogenesis by governing distinct cellular compartments ; LTα, expressed by the BM-derived cells, is essential not only for the organization of spleen architecture but also for lymphoid organogenesis. By contrast, the aly gene product NIK from BM-derived cells, if expressed, has no major role in the development of secondary lymphoid organs. Rather, the lack of LN and PPs in aly mice may be caused by a defect of non-BM-derived cells, possibly through a defective development of the incipient stromal cells of the LN and PP (50). Strategies undertaken to clarify these findings are as follows.

1. Complementation of abnormal LN genesis in aly mice with LTα-deficient mice

Although there is some phenotypic difference between LTα-deficient mice and aly mice, these two strains possess an extremely similar phenotype ; lack of LN and PPs, and a disorganized spleen architecture. It was still possible that the altered function of NIK perturbs the expression of the membrane form of LT to cause the LTα-deficient phenotype in aly mice. We therefore first examined the expression of the membrane form using LTβR-Ig which binds to membrane-associated LTα1LTβ2. LTβR-Ig bound to activated aly spleen cells, demonstrating that expression of the membrane form of LT is retained in aly mice with this *in vitro* system.

If the membrane form of LT is present and functional per se in aly mice, as suggested by the *in vitro* studies, cells from aly mice should reverse the phenotype of LTα-deficient mice, and this was the case ; a chimeric mouse from LTα-deficient mice and aly mice was generated, in which cells from both strains co-exist and interact with each other throughout development. Upon detailed inspection, mesenteric LN and one lumbar LN were observed in this chimera. Thus, lack of LN genesis in aly mice could not be attributed to the lack of functional membrane-associated LT.

2. A novel approach to define the mechanisms underlying the defective lymphoid organogenesis in LTα-deficient mice and aly mice

1) Defective lymphoid organ development in LTα-deficient mice is almost completely restored by the generation of chimeras with normal animals

Lack of FDC clusters as well as the defective GC formation in the spleen from LTα-deficient mice were restored by the transfer of wild-type BM cells, indicating that the LTα-expressing cells required to establish these lymphoid structures are derived from BM (6, 39). In contrast to in the spleen architecture, the development of LN and PP was not restored in the same animals (51). Given that the lack of LN and PP in LTα-deficient mice is developmentally fixed, we speculated that LTα-expressing cells required to generate LN and PP are also BM-derived. This hypothesis, however, has not been formally tested, since BM cells were transferred only into the adult mice in these experiments. We have approached this issue by generating aggregation chimeras from LTα-deficient mouse morulae and LTα-sufficient mouse morulae ; both LTα-deficient BM-derived cells and LTα-sufficient BM-derived cells are expected to circulate in the body and to interact with the incipient stromal cells of the lymphoid organs throughout the development. We used GFP-Tg as wild-type mice in order to monitor the chimeric contribution from each strain by the detection of GFP (52). In particular, detection of GFP from the thymocytes and/or splenocytes by flow cytometry enabled us to focus on the chimerism of BM-derived cells which are the major source of membrane-associated LT.

Upon detailed inspection, all 10 chimeric mice generated showed lymphoid organ development indistinguishable from that seen in wild-type mice. Percentages of the LTα-expressing cells evaluated by the detection of GFP from thymocytes and splenocytes varied among the chimeras (ranging from 8 to 66%), and this variation did not apparently affect the extent of restoration of lymphoid organogenesis in this range. Development of LN and PP occurred irrespective of the status of GFP expression from the body as long as LTα-expressing cells exist in the spleen and thymus. As expected, spleen architecture was also restored in these mice ; organized FDC clusters as well as GCs were present in the spleens from all chimeric mice. Thus, LTα-expressing BM-derived cells, if present throughout the development, could restore the lymphoid organogenesis in LTα-deficient mice, supporting the idea that LTα-dependent interactions must occur during development in order for LN and PP to develop.

2) Defective lymphoid organ development in aly mice is only partially restored by the generation of chimeras with normal animals

To investigate how NIK contributes to the lymphoid organogenesis, chimeric mice from aly mice and GFP-Tg were also generated and evaluated for the restoration of lymphoid organ development.

Out of 11 chimeras generated, two showed no inguinal LN and one had only right inguinal LN. Although 4 mice had more than four PPs, 7 mice had either less than three or none at all. Furthermore, one mouse which lacked inguinal LN and PPs had only one small mesenteric LN. It is particularly important to note that there were many spleen cells derived from GFP-Tg even in the chimeric animals defective in the development of LN and/or PPs. Because BM-derived cells from GFP-Tg should have the normal NIK, if expressed, a lack of LN and/or PP in these chimeras would be independent of the presence of NIK on the BM-derived cells. This limited restoration of lymphoid organ development sharply contrasted to that seen in the chimeras of LTα-deficient mice and GFP-Tg in which relatively small numbers of LTα-expressing cells were sufficient to restore the lymphoid organogenesis, as illustrated in Fig. 2.

Despite the partial restoration of LN and PP development in these chimeras, histological evaluation showed spleen architecture indistinguishable from that seen in wild-type mice ; GC and FDC formation were apparently normal in all 11 chimeras some of which showed abnormal LN and/or PP development. These results demonstrated that an organized spleen architecture can be formed independent of the defective development of LN and/or PP.

3. Possible involvement of LTβR signaling in the abnormal lymphoid organ development in aly mice

The similar phenotypes of the LTα-deficient and aly mouse strains suggested that there might be a close mechanistic relationship in their affected biochemical pathways. The results described above, however, showed no detectable alteration in the expression of the membrane-associated LT ligand in aly mice. We therefore examined downstream elements of the pathway at the level of LTβR signaling using embryonic fibroblast (EF) isolated either from aly mice or from C57BL/6 wild-type mice. We found that the up-regulation of VCAM-1 after stimulation either with agonistic anti-LTβR monoclonal antibody or with recombinant LTα1LTβ2 was defective in aly EF suggesting that signaling through LTβR is impaired in aly mice. Taken together, we concluded that LTα and NIK together control lymphoid organogenesis, governing distinct cellular compartments but with a close mechanistic relationship in their biochemical pathways, ligand-binding and receptor-signaling. Because LTβR is exclusively expressed by non-lymphoid cells (20, 21), a defect in LTβR signaling in aly mice is consistent with the idea that the lack of lymphoid organogenesis in this strain is caused by the defect of non-BM-derived cells as demonstrated by the chimeric analysis.

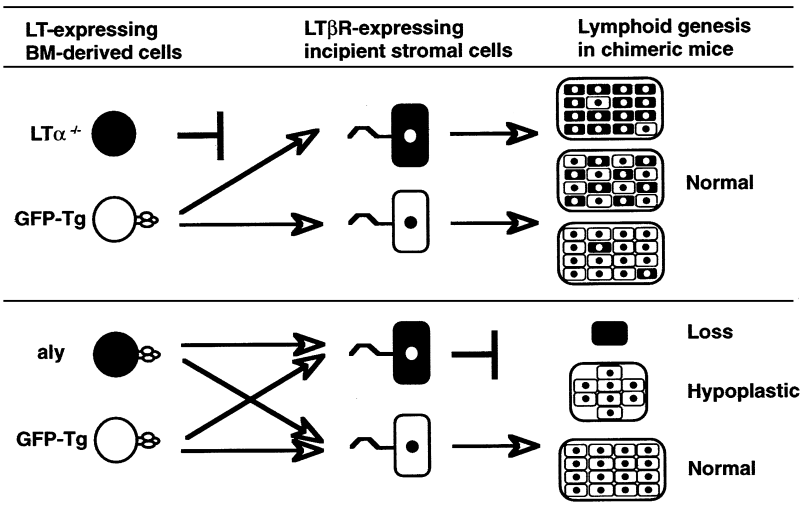

Fig. 2. Presumed mechanisms underlying the defective lymphoid organogenesis in LTα-deficient mice and aly mice revealed by the chimeric analysis. Chimeric mice of LTα-deficient mice and GFP-transgenic mice (GFP-Tg) had restored LN and PPs, because incipient stromal cells derived from both LTα-deficient mice and GFP-Tg can respond to LT provided by the cells from GFP-Tg. By contrast, the development of LN and PP in chimeric mice of aly mice and GFP-Tg showed a different outcome depending on the chimerism of the LTβR-expressing incipient stromal cells ; cells from aly mice cannot contribute to the lymphoid organogenesis because of the defect of LT-LTβR signaling. LT-expressing BM-derived cells and LTβR-expressing incipient stromal cells from GFP-Tg are shown in white, whereas cells from LTα-deficient mice or aly mice are shown in black.

CONCLUDING REMARKS

The various studies described in this review have established that both LT and TNF provide essential signals for the development of a proper peripheral lymphoid organ structure. Together with experiments demonstrating essential roles for CD40 and its ligand (53, 54) and for OX40 and its ligand (55) in the development of a mature antibody response, these studies demonstrate critical roles for multi-

ple members of the TNF ligand and receptor family in controlling organized T cell-dependent B cell responses. LT appears to be important both during ontogeny to establish the architecture within which immunocytes interact and later to maintain the full cellular constituency of the lymphoid follicles. TNF and TNFR-I appear to act in the establishment of clusters of FDCs within the follicles of the splenic white pulp (6, 34, 39, 40). Signals through CD40 and OX40 participate in the direct interactions between T and B cells during the antigen-driven portion of the immune response. Thus, this family of cytokines acts at multiple levels of lymphoid organ morphogenesis and responsiveness. Other components of the signaling pathway undoubtedly remain to be defined.

Identification of essential signaling molecules that act to establish a proper peripheral lymphoid tissue structure provides important information that will ultimately lead to the identification of the specific cell types that must interact for the initial establishment of this structure. It is now important to define the developmental expression patterns of the different members of the TNF ligand and receptor family during ontogeny of the immune system. Once the essential regulatory cells have been identified, then it may become feasible to develop new strategies to interfere with immune responses by changing the lymphoid organ tissue structure.

ACKNOWLEDGEMENTS

I thank David D. Chaplin for encouragement and members of The First Department of Internal Medicine, School of Medicine, Ehime University for support. I wish to thank Editor-in-Chief, Professor Saburo Sone for providing me with the opportunity to write this article.

REFERENCES

1. Springer TA : Traffic signals for lymphocyte recirculation and leukocyte emigration : The multistep paradigm. Cell 76 : 301-314, 1994
2. Butcher EC, Picker LJ : Lymphocyte homing and homeostasis. Science 272 : 60-66, 1996
3. van Deurs B, Ropke C : The postnatal development of high-endothelial venules in lymph nodes of mice. Anat Rec 181 : 659-678, 1974
4. De Togni P, Goellner J, Ruddle NH, Streeter PR, Fick A, Mariathasan S, Smith SC, Carlson R, Shornick LP, Strauss-Schoenberger J, Russell JH, Karr R, Chaplin DD : Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. Science 264 : 703-707, 1994
5. Banks TA, Rouse BT, Kerley MK, Blair PJ, Godfrey VL, Kuklin NA, Bouley DM, Thomas J, Kanangat S, Mucenski ML : Lymphotoxin-α-deficient mice. Effects on secondary lymphoid organ development and humoral immune responsiveness. J Immunol 155 : 1685-1693, 1995
6. Matsumoto M, Mariathasan S, Nahm MH, Baranyay F, Peschon JJ, Chaplin DD : Role of lymphotoxin and the type I TNF receptor in the formation of germinal centers. Science 271 : 1289-1291, 1996
7. Matsumoto M, Lo SF, Carruthers CJL, Min J, Mariathasan S, Huang G, Plas DR, Martin SM, Geha RS, Nahm MH, Chaplin DD : Affinity maturation without germinal centres in lymphotoxin-α-deficient mice. Nature 382 : 462-466, 1996
8. Fu Y-X, Huang G, Matsumoto M, Molina H, Chaplin DD : Independent signals regulate development of primary and secondary follicle structure in spleen and mesenteric lymph node. Proc Natl Acad Sci USA 94 : 5739-5743, 1997
9. Fu Y-X, Molina H, Matsumoto M, Huang G, Min J, Chaplin DD : Lymphotoxin-α (LTα) supports development of splenic follicular structure that is required for IgG responses. J Exp Med 185 : 2111-2120, 1997
10. DiSanto JP, Muller W, Guy-Grand D, Fischer A, Rajewsky K : Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor γ chain. Proc Natl Acad Sci USA 92 : 377-381, 1995
11. Adachi S, Yoshida H, Honda K, Maki K, Saijo K, Ikuta K, Saito T, Nishikawa S-I : Essential role of IL-7 receptor α in the formation of Peyer's patch anlage. Int Immunol 10 : 1-6, 1998
12. Förster R, Mattis AE, Kremmer E, Wolf E, Brem G, Lipp M : A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. Cell 87 : 1037-1047, 1996
13. Kong Y-Y, Yoshida H, Sarosi I, Tan H-L, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ,

Penninger JM : OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 397 : 315-323, 1999
14. Roberts CWM, Shutter JR, Korsmeyer SJ : Hox11 controls the genesis of the spleen. Nature 368 : 747-749, 1994
15. Yokota Y, Mansouri A, Mori S, Sugawara S, Adachi S, Nishikawa S-I, Gruss P : Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. Nature 397 : 702-706, 1999
16. Georgopoulos K, Bigby M, Wang JH, Molnar A, Wu P, Winandy S, Sharpe A : The Ikaros gene is required for the development of all lymphoid lineages. Cell 79 : 143-156, 1994
17. Franzoso G, Carlson L, Xing L, Poljak L, Shores EW, Brown KD, Leonardi A, Tran T, Boyce BF, Siebenlist U : Requirement for NF-κB in osteoclast and B-cell development. Genes Dev 11 : 3482-3496, 1997
18. Tartaglia LA, Goeddel DV : Two TNF receptors. Immunol Today 13 : 151-153, 1992
19. Browning JL, Ngam-ek A, Lawton P, DeMarinis J, Tizard R, Chow EP, Hession C, O Brine-Greco B, Foley SF, Ware CF : Lymphotoxin β, a novel member of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface. Cell 72 : 847-856, 1993
20. Ware CF, VanArsdale TL, Crowe PD, Browning JL : The ligands and receptors of the lymphotoxin system. Curr Top Microbiol Immunol 198 : 175-218, 1995
21. Crowe PD, VanArsdale TL, Walter BN, Ware CF, Hession C, Ehrenfels B, Browning JL, Din WS, Goodwin RG, Smith CA : A lymphotoxin-β-specific receptor. Science 264 : 707-710, 1994
22. Montgomery RI, Warner MS, Lum BJ, Spear PG : Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. Cell 87 : 427-436, 1996
23. Mauri DN, Ebner R, Montgomery RI, Kochel KD, Cheung TC, Yu G-L, Ruben S, Murphy M, Eisenberg RJ, Cohen GH, Spear PG, Ware CF : LIGHT, a new member of the TNF superfamily, and lymphotoxin α are ligands for herpesvirus entry mediator. Immunity 8 : 21-30, 1998
24. Paul NL, Ruddle NH : Lymphotoxin. Annu Rev Immunol 6 : 407-438, 1988
25. Beutler B, C van Huffel : Unraveling function in the TNF ligand and receptor families. Science 264 : 667-668, 1994
26. Matsumoto M, Fu Y-X, Molina H, Chaplin DD : Lymphotoxin-α-deficient and TNF receptor-I-deficient mice define developmental and functional characteristics of germinal centers. Immunol Rev 156 : 137-144, 1997
27. von Boehmer H : Lymphotoxins : from cytotoxicity to lymphoid organogenesis. Proc Natl Acad Sci USA 94 : 8926-8927, 1997
28. Koni PA, Sacca R, Lawton P, Browning JL, Ruddle NH, Flavell RA : Distinct roles in lymphoid organogenesis for lymphotoxins α and β revealed in lymphotoxin β-deficient mice. Immunity 6 : 491-500, 1997
29. Alimzhanov MB, Kuprash DV, Kosco-Vilbois MH, Luz A, Turetskaya RL, Tarakhovsky A, Rajewsky K, Nedospasov SA, Pfeffer K : Abnormal development of secondary lymphoid tissues in lymphotoxin β-deficient mice. Proc Natl Acad Sci USA 94 : 9302-9307, 1997
30. Rennert PD, Browning JL, Mebius R, Mackay F, Hochman PS : Surface lymphotoxin α/β complex is required for the development of peripheral lymphoid organs. J Exp Med 184 : 1999-2006, 1996
31. Rennert PD, James D, Mackay F, Browning JL, Hochman PS : Lymph node genesis is induced by signaling through the lymphotoxin β receptor. Immunity 9 : 71-79, 1998
32. Koni PA, Flavell RA : A role for tumor necrosis factor receptor type 1 in gut-associated lymphoid tissue development : genetic evidence of synergism with lymphotoxin β. J Exp Med 187 : 1977-1983, 1998
33. Fütterer A, Mink K, Luz A, Kosco-Vilbois MH, Pfeffer K : The lymphotoxin β receptor controls organogenesis and affinity maturation in peripheral lymphoid tissues. Immunity 9 : 59-70, 1998
34. Pasparakis M, Alexopoulou L, Episkopou V, Kollias G : Immune and inflammatory responses in TNFα-deficient mice : A critical requirement for TNFα in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. J Exp Med 184 : 1397-1411, 1996
35. Marino MW, Dunn A, Grail D, Inglese M, Noguchi Y, Richards E, Jungbluth A, Wada H, Moore M, Williamson B, Basu S, Old LJ : Characterization of tumor necrosis factor-deficient mice. Proc Natl Acad Sci USA 94 : 8093-8098, 1997

36. Kosco-Vilbois MH, Zentgraf H, Gerdes J, Bonnefoy J-Y : To' B 'or not to' B 'a germinal center? Immunol Today 18 : 225-230, 1997
37. Gonzalez M, Mackay F, Browning JL, Kosco-Vilbois MH, Noelle RJ : The sequential role of lymphotoxin and B cells in the development of splenic follicles. J Exp Med 187 : 997-1007, 1998
38. Fu Y-X, Huang G, Wang Y, Chaplin DD : B lymphocytes induce the formation of follicular dendritic cell clusters in a lymphotoxin α-dependent fashion. J Exp Med 187 : 1009-1018, 1998
39. Matsumoto M, Fu Y-X, Molina H, Huang G, Kim J, Thomas DA, Nahm MH, Chaplin DD : Distinct roles of lymphotoxin-α and the type I TNF receptor in the establishment of follicular dendritic cells from non-bone marrow-derived cells. J Exp Med 186 : 1997-2004, 1997
40. Tkachuk M, Bolliger S, Ryffel B, Pluschke G, Banks TA, Herren S, Gisler RH, Kosco-Vilbois MH : Crucial role of tumor necrosis factor 1 expression on nonhematopoietic cells for B cell localization within the splenic white pulp. J Exp Med 187 : 469-477, 1998
41. Endres R, Alimzhanov MB, Plitz T, Fütterer A, Kosco-Vilbois MH, Nedospasov SA, Rajewsky K, Pfeffer K : Mature follicular dendritic cell networks depend on expression of lymphotoxin β receptor by radioresistant stromal cells and of lymphotoxin β and tumor necrosis factor by B cells. J Exp Med 189 : 159-168, 1999
42. Jacob J, Kelsoe G, Rajewsky K, Weiss U : Intraclonal generation of antibody mutants in germinal centres. Nature 354 : 389-392, 1991
43. Miyawaki S, Nakamura Y, Suzuka H, Koba M, Yasumizu R, Ikehara S, Shibata Y : A new mutation, aly, that induces a generalized lack of lymph nodes accompanied by immunodeficiency in mice. Eur J Immunol 24 : 429-434, 1994
44. Shinkura R, Matsuda F, Sakiyama T, Tsubata T, Hiai H, Paumen M, Miyawaki S, Honjo T : Defects of somatic hypermutation and class switching in alymphoplasia (*aly*) mutant mice. Int Immunol 8 : 1067-1075, 1996
45. Koike R, Nishimura T, Yasumizu R, Tanaka H, Hataba Y, Watanabe T, Miyawaki S, Miyasaka M : The splenic marginal zone is absent in alymphoplastic *aly* mutant mice. Eur J Immunol 26 : 669-675, 1996
46. Malinin NL, Boldin MP, Kovalenko AV, Wallach D : MAP 3 K-related kinase involved in NF-κB induction by TNF, CD95 and IL-1. Nature 385 : 540-544, 1997
47. Kuramoto T, Mashimo T, Koike R, Miyawaki S, Yamada J, Miyasaka M, Serikawa T : The alymphoplasia (*aly*) mutation co-segregates with the intercellular adhesion molecule-2 (*Icam-2*) on mouse chromosome11. Int Immunol 7 : 991-994, 1994
48. Shinkura R, Kitada K, Matsuda F, Tashiro K, Ikuta K, Suzuki M, Kogishi K, Serikawa T, Honjo T : Alymphoplasia is caused by a point mutation in the mouse gene encoding NF-κB-inducing kinase. Nature Genet 22 : 74-77, 1999
49. Otani H, Yokoyama M, Nozawa-Kimura S, Tanaka O, Katsuki M : Pluripotency of homozygous-diploid mouse embryos in chimeras. Develop Growth Differ 29 : 373-380, 1987
50. Matsumoto M, Iwamasa K, Rennert PD, Yamada T, Suzuki R, Matsushima A, Okabe M, Fujita S, Yokoyama M : Involvement of distinct cellular compartments in the abnormal lymphoid organogenesis in lymphotoxin-α-deficient mice and alymphoplasia (aly) mice defined by the chimeric analysis. J Immunol 163 : 1584-1591, 1999
51. Mariathasan S, Matsumoto M, Baranyay F, Nahm MH, Kanagawa O, Chaplin DD : Absence of lymph nodes in lymphotoxin-α(LTα)-deficient mice is due to abnormal organ development, not defective lymphocyte migration. J Inflamm 45 : 72-78, 1995
52. Okabe M, Ikawa M, Kominami K, Nakanishi T, Nishimune Y ! Green mice 'as a source of ubiquitous green cells. FEBS Lett 407 : 313-319, 1997
53. Foy TM, Laman JD, Ledbetter JA, Aruffo A, Claassen E, Noelle RJ : gp39-CD40 interactions are essential for germinal center formation and the development of B cell memory. J Exp Med 180 : 157-163, 1994
54. Kooten CV, Banchereau J : CD40-CD40 ligand : a multifunctional receptor-ligand pair. Adv Immunol 61 : 1-77, 1996
55. Stuber E, Strober W : The T cell-B cell interaction via OX40-OX40L is necessary for the T cell-dependent humoral immune response. J Exp Med 183 : 979-989, 1996