MINIREVIEWS

Role of Lymphotoxin in Experimental Models of Infectious Diseases: Potential Benefits and Risks of a Therapeutic Inhibition of the Lymphotoxin-β Receptor Pathway

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The immune system provides different mechanisms to protect organisms against pathogens, most of which are infectious agents. Simultaneously, immune activation secondary to genetic factors and/or environmental signals can induce detrimental autoimmunity. The effector pathways in host defense and autoimmunity use similar cytokines and chemokines. Accordingly, tumor necrosis factor alpha (TNF-α) and autoimmunity use similar cytokines and chemokines. The effector pathways in host defense and autoimmunity use similar cytokines and chemokines. Accordingly, tumor necrosis factor alpha (TNF-α) and autoimmunity use similar cytokines and chemokines. The effector pathways in host defense and autoimmunity use similar cytokines and chemokines.

Hallmarks of the adaptive immune system are antigen-specific cellular and humoral immune responses. Secondary lymphoid organs serve as sites of contact between antigen-presenting cells (APCs) and immune effector T and B lymphocytes. Chemokines and cytokines serve as messengers determining the type of immune response to a given antigen. The TNF family cytokine lymphotoxin (LT) plays a pivotal role in the development of secondary lymphoid organs.

The chronic and relapsing course of many autoimmune diseases calls for new biological agents capable of suppressing the underlying inflammatory disorders. Recent studies indicate that inhibition of LTβ receptor (LTβR)-mediated signaling in adult animals suppresses autoimmunity by modulating the cellular structure of secondary lymphoid organs (reviewed in reference 22). Because of the wide range of autoimmune diseases positively influenced by this treatment, blockade of the LTβR might serve as a new treatment principle for human autoimmune diseases. However, immune responses to infectious pathogens are also altered in mice with disrupted LTβR signaling. While the course of virus- and lipopolysaccharide (LPS)-induced shock, experimental Trypanosoma brucei infection, cerebral malaria, and experimental prion disease are less severe, inhibition of the LTβR is also associated with exacerbation of mycobacterial infection and infectious colitis. This review summarizes the findings of studies using mice with disrupted LTβR signaling in models of infectious diseases and discusses the relevance of these observations in considering LTβR blockade as a potential treatment for human autoimmune diseases.

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Deletion of the lta gene in LTA−/− mice is associated with the loss of all lymph nodes (LNs) and Peyer's patches (PPs) and changes to the lymphoid architecture of the spleen (3, 11). Targeted disruption of the ltb gene results in loss of peripheral LNs, whereas the mucosa draining cervical and mesenteric LNs (MLNs) are retained (29). LTBR−/− mice have a pheno-
type very similar to that of LTα−/− mice (19). LIGHT, the second ligand of the LTβR, is predominantly expressed on T cells and serves as a costimulatory molecule (reviewed in reference 66). In contrast to LTα−/− mice, LIGHT−/− mice develop intact lymphoid organs, indicating a predominant role for the LTαβ-LTβR interaction in the formation of secondary lymphoid organs (61). Conversely, transgenic expression of LIGHT in LTα−/− mice restores splenic T- and B-cell segregation, FDCs, and germinal-center (GC) formation but not marginal-zone (MZ) formation, suggesting that LIGHT can compensate for the loss of certain LTαβ functions in the presence of the LTβR (66). CD8+ cells from LIGHT−/− mice show decreased proliferative responses, while the IL-2 secretion is decreased in CD4+ cells from these mice (61), indicating a role for LIGHT as a costimulatory molecule. Conversely, transgenic expression of LIGHT on T cells is associated with a hyperactivated enlarged T-cell compartment and spontaneous autoimmunity, including inflammatory bowel disease (67).

**Gestational and postgestational inhibition of LTβR-mediated signaling.** Treatment with soluble LTβR-immunoglobulin G (IgG) fusion protein (LTβRlgG) similarly blocks the interaction of LTαβ and LIGHT with the LTβR. Therefore, the effects observed in LTβRlgG-treated animals are a result of the inhibition of both ligands. Gestational treatment with LTβRlgG prevents the formation of PPs and LNs (56). Postgestational LTβRlgG signaling during the first 6 weeks after birth is critical for the development of intestinal lamina propria B cells, IgA secretion, and isolated lymphoid follicles of the intestine (38, 48).

**Mode of action of LTβR inhibition in adult mice.** The lymphoid microenvironment is defined as the local interplay between mobile lymphocytes and the fixed reticular/stromal cells and includes cell adhesion, trafficking, chemokine function, and cellular positioning (22). Secondary lymphoid organs are structures with a high degree of plasticity. Inhibition of LTβR signaling in adult mice alters the lymphoid microenvironment. As shown in Fig. 1, bottom, FDC networks and GCs disintegrate and B-cell follicles disappear in the absence of LTβR signaling. Figure 2 depicts the lymphoid microarchitecture of a murine wild-type (wt) spleen and describes the changes observed in mice with blocked LTβR signaling. Table 2 and Fig. 3 summarize the changes to the lymphoid microenvironment observed in mice with disrupted LTαβ-LTβR signaling. FDC networks consist of a scaffold of specialized reticular fibroblasts that retain and present intact antigen to B cells. Memory B cells develop during the GC reaction. Permanent LTαβ-LTβR interaction is required to maintain a CXCL13 chemokine gradient, which attracts CXCR5+ B cells to the follicle and is also required to maintain the differentiation status of the recruited B cells and the FDCs in the network. Similarly, LTβR engagement is required for continued expression of the vascular-cell adhesion molecule 1 (VCAM1) by the FDC network (23, 25).

The expression of VCAM1 is similarly reduced in FDC networks and splenic MZs following LTβRlgG treatment. Splenic MZs are located between the red and white pulp of the spleen and consist of a reticular matrix harboring B cells, macrophages, and dendritic cells (DCs). Blood-borne antigen is captured in the MZ and presented to B cells. Marginal zones are critical for immunity against T-cell-independent bacterial antigens (24). MZ markers disappear following LTβRlgG treatment.

Inhibition of the LTβR also blocks the migration or maturation of the cysteine-rich domain of the mannose receptor (CR-Fc)-positive DCs (42, 71). Figure 3 describes three potential mechanisms by which inhibition of the LTβR alters autoimmunity and host defense.

PPs, isolated lymphoid follicles, cryptopatches, and colonic patches are organized lymphoid aggregates of the intestine. The number and cellular contents of these aggregates are reduced in adult mice undergoing anti-LTβR treatment (13).

The potential mode of action in anti-LTβR treatment is to impair immune function by preventing proper placement of T cells, B cells, and APCs in secondary lymphoid organs, thus preventing the induction of appropriate antigen-specific immune responses. In contrast, selective inhibition of LIGHT signaling results in a loss of LIGHT-mediated costimulatory stimuli. Soluble HVEM-Fc fusion protein selectively blocks LIGHT-HVEM interactions, inhibits CD3-induced T-cell proliferation, and reduces the frequency of spontaneous diabetes in nonobese diabetic mice (67).

**Ectopic “tertiary” lymphoid organs and inflammatory diseases.** A number of human inflammatory and autoimmune disorders are associated with the formation of ectopic lymphoid structures at the site of the inflamed organ which resemble secondary lymphoid organs (69; reviewed in reference 60). It is likely that immune responses to self antigens expand in these de novo lymphoid organs, as they allow colocalization of

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**TABLE 1. Mice with defects in organized GALT development induced by gene defects or by gestational or postgestational treatment†**

<table>
<thead>
<tr>
<th>Gene-deficient/treated mice</th>
<th>PPb</th>
<th>MLNb</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTα−/−, LTβR−/−</td>
<td>−</td>
<td>−</td>
<td>3, 11, 19</td>
</tr>
<tr>
<td>LTβ−/−</td>
<td>−</td>
<td>+</td>
<td>2, 29, 33</td>
</tr>
<tr>
<td>Light−/− × LTβ−/−</td>
<td>−</td>
<td>+</td>
<td>61</td>
</tr>
<tr>
<td>TNF−/−</td>
<td>Reduced number</td>
<td>+</td>
<td>30, 52</td>
</tr>
<tr>
<td>TNFR-I (55 kDa)−/−</td>
<td>Reduced number</td>
<td>+</td>
<td>47, 52</td>
</tr>
<tr>
<td>LTα−/− × LTβ−/−</td>
<td>−</td>
<td>+</td>
<td>28</td>
</tr>
<tr>
<td>Gestational LTβRlgG treatment</td>
<td>−</td>
<td>+</td>
<td>56</td>
</tr>
<tr>
<td>Gestational LTβRlgG and TNFRlgG treatment</td>
<td>−</td>
<td>− or few</td>
<td>57</td>
</tr>
<tr>
<td>Postgestational LTβRlgG treatment</td>
<td>Fewer cells lost after long-term treatment</td>
<td>+</td>
<td>13</td>
</tr>
</tbody>
</table>

† Modified from Reference 43a. GALT, gut-associated lymphoid tissue.

b +, organ(s) present; −, organ(s) not detectable.
FIG. 2. Splenic lymphoid architecture and effects of inhibition of LTβR-mediated signaling. The spleen consists of a red and a white pulp, which are segregated by marginal zones that contain macrophages. Marginal zones are not detectable in LTα−/− and LTβR−/− mice. Within the white pulp (inset with magnification), T and B cell areas are clearly separated in mice with physiological LTβR-mediated signaling. The borders between T- and B-cell zones are ill defined in LTα−/−, LTβR−/−, and LTβ−/− mice. B-cell follicles are absent in these gene-deficient mice. FDCs can be detected within B-cell follicles of wild-type mice. Within days after inhibition of LTβR-mediated signaling, FDCs disappear in adult mice. Similarly, a proportion of CD11c+ dendritic cells disappear in the spleens of mice with blocked LTβR-mediated signal transduction.

**TABLE 2. Changes to lymphoid microenvironment secondary to inhibition of TNFR/LTβR during pregnancy or after gestation**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Developmenta with:</th>
<th>LTβR IgG</th>
<th>Gestationalb</th>
<th>Postgestationalb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF/LTα−/−, LTα−/−</td>
<td>LTβR−/−</td>
<td>TNFα−/−</td>
<td>LTβ−/−</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary B-cell follicles</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Marginal zone</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Germinal centers</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Follicular dendritic cells</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>Reduced</td>
<td>Reduced</td>
<td>NDd</td>
<td>Reduced</td>
</tr>
<tr>
<td>Mesenteric LN</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Primary B-cell follicles</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Germinal centers</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Follicular dendritic cells</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Lamina propria B cells</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a +, structure present; −, structure not detectable.
b Treatment with 200 μg LTβRIgG on gestational days 16 and 18.
c As described in references 42 and 49.
d ND, not determined.
antigen-specific T and B cells with APCs. In mice, ectopic lymphoid structures can be induced by transgenic overexpression of LTαβ (31), and they are called tertiary lymphoid tissues. Data from animal models of autoimmune diseases associated with the formation of ectopic lymphoid tissue indicate that autoimmunity is less severe, cured, or prevented if the LTαβ is blocked in these conditions (reviewed in reference 22). A potential role for microbial pathogens in the pathogenesis of inflammatory disorders, such as rheumatoid arthritis or inflammatory bowel disease, has been discussed (18). Granulomas are common histological hallmark of Crohn’s disease and mycobacterial infections and serve as sites where T cells, APCs, and antigens collocate (44). Granulomas resemble tertiary lymphoid tissues. The recruitment of T cells to granulomas was impaired in LTαβ−/− (donor) → (transfer) wt (recipient) bone marrow chimeras infected with Mycobacterium tuberculosis (58). It is therefore possible that anti-LTβR targeted therapy might also shut down common pathways of host defense and inflammatory responses that might lead to autoimmunity in genetically predisposed persons.

Lymphotoxin and LIGHT contribute to central immune tolerance in mice. While a stimulatory role for LTβR signaling in the induction of peripheral autoimmune disease has been demonstrated by effective treatment of such conditions by anti-LTβR therapy, the thymic expression of LTβR and LTβR ligands contributes to central tolerance. Lymphotoxin signaling is required for the expression of Aire, which is a key mediator of central tolerance for peripherally restricted antigens. Similarly, LTβR ligand expression on thymic epithelial cells is required for proper differentiation of thymic medullary epithelial cells. LTαβ−/− and LTβR−/− mice show infiltration of liver, lung, pancreatic islands, and kidney with activated lymphocytes, and autoantibodies can be detected in these mice (7, 10). Thus, LTβR signaling plays different roles in the peripheral and central control of immune tolerance.

THE ROLE OF LTβR-MEDIATED SIGNALING IN INFECTIOUS DISEASES

Bacterial infections. Both types of animal models predominantly requiring cellular immunity and T-cell-mediated humoral immunity for clearance of bacteria have been investigated. Host defense against intracellular bacteria eliminated by cellular immune mechanisms in mice with disrupted LTβR-mediated signaling was severely impaired in most studies. The immune response against bacterial pathogens inducing combined T- and B-cell immunity varied in mice with blocked LTβR activation with the different models tested, suggesting that certain bacterial antigens (Citrobacter rodentium) required LTβR-mediated signaling while others (Salmonella enterica) were cleared in an LTβR-independent fashion.
(i) Intracellular mycobacterial infections: BCG and M. tuberculosis. While a central role for TNF-α in immunity against mycobacterial infections has been well characterized (1, 20, 27, 64), the contribution of soluble LTα3 as the second TNFR-I ligand in antimycobacterial host defense was unknown. Therefore LTα−/− mice and TNF-α and LTα double-gene-deficient (TNF/LTα−/−) mice were infected with Mycobacterium bovis bacillus Calmette-Guérin (BCG) (8, 26, 51) or Mycobacterium tuberculosis (14). Studies investigating the role of LT in experimental mycobacterial disease are of clinical relevance considering the reactivation of tuberculosis observed in patients treated with TNF-α antagonists (21). Table 3 summarizes studies investigating bacterial infections in mice with blocked LTβR signaling. The course of mycobacterial infection was lethal in TNF/LTα−/− and LTα−/− mice. Survival was longer in BCG-infected LTα−/− mice (182 days) and in TNF-α−/− mice (56 days) than in TNF/LTα−/− mice (35 days), indicating that the absence of TNF-α in this infection leads to broader immunodeficiency than the absence of LTα. The impaired antimycobacterial immune response in mice without TNF-α was aggravated by the simultaneous lack of LTα (8). Similarly, the bacterial loads of the lung on day 28 of the infection were 1,000-, 40-, and 1.4-fold higher in TNF/LTα−/−, TNF-α−/−, and LTα−/− mice than in the respective wt mice. Introduction of an LTα transgene in TNF/LTα−/− mice delayed disease onset but failed to restore resistance to BCG infection, suggesting a transient protective effect exerted by LTα in this disease model. Roach et al. generated LTα−/− → wt bone marrow chimeras in order to investigate the role of soluble LTα3 in antimycobacterial immunity (58). There was lethal disease in wt mice with lymph nodes and LTα−/− bone marrow, indicating a critical role for soluble LTα in antimycobacterial immunity.

The proinflammatory cytokine TNF-α is secreted as a soluble TNF-α3 molecule and is also tethered to the cell membrane. Olleros et al. investigated the role of membrane-bound noncleavable TNF-α by creating membrane TNF-α transgenic mice on the TNF/LTα−/− background, thus specifically studying the role of membrane TNF-α in the absence of soluble TNF-α and LTα. Following inoculation with BCG, the infection was controlled in membrane TNF/LTα−/− transgenic TNF/LTα−/− mice, though the bacterial load was higher in these

### Table 3. Course of experimental bacterial infections in mice with disrupted LTβR signaling

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>LT/TNF ligand/receptor−/− mouse/inhibitor</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium bovis</td>
<td>BALB/c wt treated with LTβRlgG/human IgG</td>
<td>Reduced number of splenic granulomas, splenic eosinophil infiltrate, higher bacterial burden in LTβRlgG-treated mice</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>TNF/LTα−/−</td>
<td>Increased bacterial burden, lethal course of infection, impaired and delayed granuloma formation</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>TNF/LTα−/−, TNF/LTα−/−, TNF/LTα−/− transgenic, LTα−/−</td>
<td>Lethal course of disease with pulmonary necrosis and increased bacterial burden in TNF/LTα−/− mice; prolonged survival in LTα−/− transgenic TNF/LTα−/− mice; resistance to LPS-induced shock in TNF/LTα−/− mice</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Transgenic transmembrane TNF+/+ expression in TNF/LTα−/− mice, TNF/LTα−/−, TNF-α−/−, LTα−/−</td>
<td>TNF/LTα−/− mice with transgenic membrane TNF expression control mycobacterial infection with higher bacterial burden than in wt mice; TNF/LTα−/−, TNF-α−/−, LTα−/− mice succumb</td>
<td>51</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>LTα−/− → RAG1−/− bone marrow chimera</td>
<td>Lethal course of infection, formation of enlarged granulomas</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>LTβ−/− → RAG1−/− bone marrow chimera</td>
<td>Clearing of infection at the same rate as wt mice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LTα−/−, LTβ−/−, LTβR−/−, LIGHT−/−</td>
<td>LTx−/−, LTβ−/−, higher bacterial loads in lungs and livers; LTβR−/−, lethal course of infection, formation of enlarged granuloma; LIGHT−/−, clearing of infection at the same rate as wt mice</td>
<td>14</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>TNF/LTα−/−</td>
<td>Lethal course of infection, higher bacterial burden in liver and spleen, hepatic necrosis</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>LTβR−/−</td>
<td>Lethal course of infection</td>
<td>14</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>TNF/LTα−/−</td>
<td>Resistance to lethal LPS challenge, lethal course of infection, higher organ bacterial load</td>
<td>12</td>
</tr>
<tr>
<td>serovar Typhimurium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>LTβR−/−</td>
<td>Course of colitis induced following streptomycin or water treatment is similar in wt and LTβR−/− mice</td>
<td>4</td>
</tr>
<tr>
<td>Citrobacter rodentium</td>
<td>LTα−/−, LTβ−/−, LTβR−/−, LTβRlgG-treated</td>
<td>Lethal course of infection in all LT ligand/receptor gene-deficient mice with spread of bacteria to liver and spleen; more severe course in LTβRlgG-treated wt mice</td>
<td>65</td>
</tr>
</tbody>
</table>

- BCG: bacillus Calmette-Guérin
- TNF: tumor necrosis factor
- LT: lymphocyte activating factor
- LTβR: lymphocyte activating factor receptor
- LTα: lymphocyte activating factor alpha
- TNFR-I: tumor necrosis factor receptor type I
- LTβRlgG: lymphocyte activating factor receptor gamma
- TNF-α: tumor necrosis factor alpha
- LTα3: lymphocyte activating factor alpha 3
- LTβ: lymphocyte activating factor beta
- LIGHT: lymphocyte activating factor with helical carboxyl terminus
- LPS: lipopolysaccharide
- TNF/LTα−/−: tumor necrosis factor and lymphocyte activating factor double-gene-deficient mice
- LTα−/−: lymphocyte activating factor single-gene-deficient mice
- LTβ−/−: lymphocyte activating factor beta single-gene-deficient mice
- LTβR−/−: lymphocyte activating factor receptor beta single-gene-deficient mice
- LIGHT−/−: lymphocyte activating factor with helical carboxyl terminus single-gene-deficient mice
- TNF-α−/−: tumor necrosis factor alpha single-gene-deficient mice
- LTα−/−, LTβ−/−: lymphocyte activating factor alpha and beta double-gene-deficient mice
- LTα3: lymphocyte activating factor alpha 3
- LTβRlgG: lymphocyte activating factor receptor gamma
- TNF/LTα−/− transgenic: tumor necrosis factor and lymphocyte activating factor double-gene transgenic mice
mice than in wt animals. Thus, membrane TNF alone is capable of controlling BCG infections (51). As TNF-α−/− mice also succumb to BCG infection, the expression of LTα in the absence of TNF is not sufficient to control this mycobacterial infection (8).

Causes for the impaired antimycobacterial immunity in LTα and TNF/LTα gene-deficient mice varied in the different models studied. The granulomatous responses to BCG infection were similarly delayed and impaired in TNF/LTα−/− and TNF-α−/− mice (8, 26). There were fewer macrophages with reduced inducible nitrite oxide synthase (iNOS) and acid phosphatase expression. Fewer T cells could be detected in these lesions. These observations indicate a central role for TNF in the recruitment of T cells and macrophages to granulomatous lesions, which cannot be compensated for by the presence of LTα. Conversely, transgenic expression of noncleavable membrane TNF-α in TNF/LTα−/− mice resulted in a two- to fourfold increase in the number of hepatic granulomas, which were of smaller size and predominantly consisted of macrophages (51).

In LTα−/− → wt bone marrow chimeras infected with M. tuberculosis, there was normal recruitment of T cells to the lungs (58). However, pulmonary T cells remained in the perivascular and peribronchial areas and failed to collocate with the macrophages in granulomas.

There are controversial findings generated in different systems regarding the role of LTα, β2-LTβR interaction in the control of mycobacterial infections (14, 39, 58). Wild-type mice infected with BCG and undergoing LTβRIgG treatment and LTβR−/− mice infected with M. tuberculosis suffered a more severe course of disease (14, 39). Similarly, the bacterial loads in livers and lungs of LTβ−/− mice infected with M. tuberculosis were elevated (14). LTβR−/− → wt bone marrow chimeras failed to control M. tuberculosis infection (14). The impaired immune response against mycobacteria in mice with disrupted LTβR was associated with decreased iNOS activity in the lung and spleen (14, 39). In contrast, Roach reported normal clearance of mycobacterial infections in LTβ−/− → wt bone marrow chimeras. The reasons for these discrepant observations are unknown and might be related to a different lymphoid microenvironment in LTβ−/− → wt and LTβR−/− → wt bone marrow chimeras. LIGHT, a second ligand of the LTβR, is not involved in the control of disease, as LIGHT−/− mice cleared M. tuberculosis infections at the same rate as wt mice did (14).

The course of experimental murine listeriosis was more severe in TNF/LTα−/− and LTβR−/− mice, suggesting that in addition to TNF-α, LTα and engagement of the LTβR are critical for control of this intracellular pathogen (27, 53, 59).

Most studies indicate that interaction of LTα, β2-LTβR and of LTβ with the LTβR is required for control of infections with the intracellular pathogens Mycobacterium and Listeria. The elimination of these pathogens depends strongly on cellular immunity. Only one study utilized LTβRIgG in adult mice (39), a situation comparable to human treatment of autoimmune diseases. This study showed a significant but moderate increase in the number of acid-fast bacilli (three- to fourfold) in LTβRIgG-treated mice compared to 10- to 1,000-fold increases observed in the studies utilizing gene-deficient mice. However, the biological relevance of this observation in terms of disease-related mortality was not investigated in this study, as all mice were sacrificed for in vitro analysis 4 weeks after infection, while most gene-deficient mice used in other studies died after day 30 following mycobacterial infection.

Experimental infectious colitis. (i) Salmonella enterica serovar Typhimurium and Salmonella enterica. Infection of LT family gene-deficient mice with Salmonella enterica serovar Typhimurium has been utilized to investigate the roles of LTα and TNF-α in the regulation of anti-Salmonella immunity.

Oral infection of TNF/LTα−/− mice with S. enterica serovar Typhimurium results in a lethal course of infection compared to mild disease in wt mice. This difference was most likely due to reduced recruitment of neutrophils to the site of infection, as well as reduced intracellular killing of S. enterica serovar Typhimurium by granulocytes (12).

Mice undergoing oral pretreatment with streptomycin develop infectious colitis, which closely resembles human S. enterica-induced colitis, following oral infection with S. enterica serovar Typhimurium. The development of S. enterica-induced colitis was not affected by the presence of PP, MLN, or the LTβR, as the courses of the infection in wt and LTβR−/− mice without PP and MLN were similar. Infection of mice with S. enterica without antibiotic treatment induced a typhoid type of disease with bacterial expansion in PP and MLN. Interestingly, the typhoid type of S. enterica infection was also similar in wt and LTβR−/− mice, indicating that while S. enterica might home to intestinal lymphoid organs, PP, MLN, and LTβR are not required for antibacterial immunity against this invasive pathogen (4).

(ii) Citrobacter rodentium. We have recently investigated the role of LTα, β2-LTβR interactions in the course of infectious colitis induced by Citrobacter rodentium (65). Infection of mice with the gram-negative bacterium C. rodentium serves as an animal model of human infection with enteropathogenic and enterohemorrhagic Escherichia coli (36). In adult and immune-competent mice, there is only mild transient colitis with hyperplasia of infected colonic epithelial cells. The course of C. rodentium-induced colitis was more severe in LTβRIgG-treated mice, with increased disease-related mortality (65). Similarly, there was nearly 100% disease-related mortality in C. rodentium-infected LTα−/−, LTβ−/−, and LTβR−/− mice, suggesting a critical role for LTα, β2-LTβR interactions in anti-Citrobacter immunity. In mice with disrupted LTβR signaling, there were fewer splenic CD11c+ dendritic cells following oral infection. FDCs were absent in the spleens of LTβRIgG-treated mice. Similarly, there were fewer colonic lymphoid follicles in LTβRIgG-treated mice and in the gene-deficient mice used. In LTβR−/− mice, anti-Citrobacter IgG2a antibody titers were reduced while IgG1 titers were increased. Similarly, there was increased Citrobacter-induced secretion of IL-4 in LTβR−/− mice. These observations indicate that the loss of local intestinal lymphoid organs and changes to antigen-presenting functions of the spleen are associated with impaired immunity against this noninvasive pathogen.

(iii) LPS-induced systemic shock. A number of studies showed resistance of TNF/LTα−/− mice against lethal endotoxemia induced by intravenous LPS injection (12, 17), depending on the bacterial origin of the LPS. Eugster described resistance to shock induced by coadministration of β-galactosamine and E. coli-derived LPS (17). Netea et al. demon-
stratified increased resistance of TNF/LTα−/− mice to lethal endotoxia induced by E. coli and K. pneumoniae LPS compared to S. enterica serovar Typhimurium LPS (46). These differences were associated with increased IL-1 and gamma interferon secretion following injection of the lethal S. enterica serovar Typhimurium LPS. BCG-sensitized TNF/LTα−/− and TNF-α−/− mice were completely resistant to E. coli LPS-induced shock, whereas LTα−/− mice showed prolonged survival compared to wt mice (8). Thus, LTα contributes to septic shock, although TNF-α appears to be more potent in the induction of LPS shock than LTα.

**Viral infections.** A number of studies have investigated the role of LT in viral infections, most of them studying influenza virus, herpesvirus, and lymphocytic choriomeningitis virus infections in gene-deficient mice with anatomical defects. Table 4 summarizes studies of experimental viral infections in mice. Except for two studies (37, 55), all of them utilized mice with genetic defects of the LT ligands. Similar to LPS-induced shock models, virus-induced systemic shock was less severe in mice with impaired LTβR, most likely due to a depletion of virus-specific CD8+ T cells following LTβR IgG treatment. Overall antiviral cytotoxic-T-cell immune responses were more or less impaired, and the clearance of the virus was slowed down or inhibited, leading to a lethal course in influenza A virus (40), murine cytomegalovirus (MCMV) (5), and Theiler’s virus (37) infections. In the extensively studied lymphocytic choriomeningitis virus infection model, the defective antiviral immune response was secondary to the loss of the marginal zone in the spleen (6, 45) but not due to the absence of LTβ itself. Similarly, treatment of adult wt mice with LTβR IgG did not affect immunity against Theiler’s virus infection, while LTα−/− and LTβR−/− mice failed to mount appropriate antiviral cytotoxic-T-cell responses (37), suggesting that changes to splenic and lymph node architecture, but not the presence of LTβ, were critical for clearing of the infection.

**Parasite infections.** Studies investigating the role of LT in parasite infections are summarized in Table 5.

(i) *Toxoplasma gondii.* Schlüter et al. compared the course of experimental toxoplasmosis in wt, TNF-α−/−, LTα−/−, and TNF/LTα−/− mice in order to dissect the roles of both ligands of the TNF receptors in this infection (62). TNFR-I plays a predominant role in experimental toxoplasmosis. TNF-α induces toxoplasmastmatic gamma interferon secretion in macrophages and microglial cells in the central nervous system (9, 34).

All gene-deficient mice tested in this study failed to control
intracerebral *T. gondii* and succumbed to acute necrotizing *Toxoplasma* encephalitis. The lethal course of disease was associated with reduced intracerebral expression of iNOS and lower splenic NO levels. Experiments with bone marrow reconstitution chimeras demonstrated an exclusive role of TNF-α and LTα-producing hematopoietic cells for surviving toxoplasmosis.

(ii) *Leishmania*. Infection of LTβ−/− mice with *Leishmania major* was associated with a fatal course of disease with visceral spread of parasites despite the resistant genetic background of the C57BL/6 mice used in this study (70). The impaired and delayed cellular and humoral anti-L. major immune response in LTβ−/− mice was secondary to changes in the lymphoid architecture. Reconstitution of LTβ−/− mice with wt bone marrow failed to restore effective antiparasite immunity, whereas wt mice receiving LTβ−/− bone marrow were not immunocompromised.

Murine *Leishmania donovani* infection induces visceral leishmaniasis and is more severe in both TNF-α−/− and LTα−/− mice (15). Experiments with bone marrow radiation chimeras indicated a critical role for liver-generated LTα in the migration of leukocytes from peripoortal to sinusoidal areas, while T-cell-generated TNF-α and LTα were required for the control of parasite growth.

(iii) *Trypanosoma brucei*. Infection of LTα−/− and TNF/LTα−/− double-gene-deficient mice with the extracellular parasite *Trypanosoma brucei* was associated with control of disease and slightly prolonged survival of LTα−/− mice following infection (43). *Trypanosoma*-specific IgM and IgG2a serum antibody titers were increased in LTα−/− mice, indicating that germinal centers and FDC networks were not required for this antiparasite humoral immune response.

(iv) *Cerebral malaria*. Infection of mice with *Plasmodium berghei* serves as an animal model for human cerebral malaria. LTα−/− mice were protected against cerebral malaria, as they did not develop perivascular cerebral hemorrhage. Bone marrow chimeras experiments indicated that a radioresistant cerebral cell population is the source of the LTα required for extravasation of malaria-infected erythrocytes (16).

**Prion disease/scrapie.** Transmissible spongiform encephalopathies (TSEs), or “prion diseases,” are chronic neurodegenerative diseases that affect humans and animals. Most TSEs, including human variant Creutzfeldt-Jakob disease and experimental prion disease in mice, are transmitted by peripheral exposure. TSE infection results in conversion of normal prion protein (PrP) to the disease-associated form, PrPsc. Intracerebral or peripheral administration of prions to mice induces a rise of infectivity in the spleen and in other lymphoid organs long before the development of neuropathological changes. PrPsc migrates from the lymphoid compartments to the central nervous system by neuronal transport. FDCs in the germinal centers of lymphoid organs have been implicated as initial sites of accumulation of PrPsc. FDCs trap antigen-antibody complexes. Studies using intraperitoneal (i.p.) (41, 54) and oral (50) routes of scrapie infection provided different results regarding the role of FDCs and LT ligands/receptors in this infection. LTα−/−, LTβ−/−, TNF/LTα−/−, and LTBR−/− mice with disrupted LTαβLTβR signaling undergoing i.p. inoculation resisted infection and contained no infectivity in spleens and lymph nodes (54). Similarly, pretreatment of wt mice with
LTβR IgG prior to i.p. scrapie infection blocked early PrP\(^{\text{sc}}\) accumulation in the spleen and reduced disease susceptibility. In contrast, LT\(\alpha\)/−/− mice orally infected with scrapie were susceptible to disease while LTβ/−/− mice were resistant (50). However, pretreatment of wt mice with LTβR IgG prior to oral infection with scrapie blocked PrP\(^{\text{sc}}\) in PP and MLN and prevented neuroinvasion (41).

As FDCs were similarly absent in TNF-\(\alpha\)/−/−, TNFR-1/−/−, and LT\(\alpha\), LTβ/−/− mice but only LT gene-deficient mice were protected against experimental scrapie, FDCs are not required for the replication of scrapie in lymphoid tissue following i.p. infection. More likely, some other yet-undefined effect of impaired LTβR-mediated signaling is critical for control of the expansion of scrapie protein in lymphoid tissues. The susceptibility of LT\(\alpha\)/−/− mice to oral scrapie and the resistance of LTβ/−/− mice and LTβR IgG-pretreated mice to oral infection are two controversial observations which require further investigation.

**SUMMARY AND CONCLUSIONS**

The studies of experimental infectious diseases summarized in this review reveal the complex biological functions of the LT\(\alpha\)/β/LIGHT-LTβR pathway in immunity to infectious agents. As the courses of the respective infections were attenuated, unchanged, or even more severe, the role of LTβR signaling in host defense depends on the respective pathogens. Animal models predominantly requiring cellular immunity or T helper cell-mediated humoral immunity for clearance of the respective infectious agent have been investigated. Overall, the contribution of the LTβR pathway to host defense against the respective pathogen depended on the antigenic properties of the pathogen, but not on the type of immune response induced by it.

Host defense against bacterial intracellular pathogens such as mycobacteria and *Listeria* mediated by cellular immune mechanisms in mice with disrupted LTβR-mediated signaling was severely impaired in most studies. Similarly, in most models of viral infections, cytotoxic-T-cell responses were diminished, although the defect in host defense observed was secondary to changes in lymphoid microarchitecture and not caused by the absence of LT. The elimination of the obligate intracellular parasite *Toxoplasma gondii* depends on T-cell responses and on the presence of LT\(\alpha\). Conversely, the clearance of the intracellular parasite *Leishmania major* depends on T helper 1-mediated cellular immunity and is independent of LTβR-mediated signaling, while the extracellular parasite *Trypanosoma brucei* is similarly cleared in LT\(\alpha\)/−/− mice and in wild-type mice (43), indicating that there is no common pattern for LTβR signaling in the host defense against intracellular or extracellular parasites. The immune responses against bacterial pathogens inducing combined T- and B-cell immunity varied in mice with blocked LTβR activation with the different models tested, suggesting that certain bacterial antigens (*Citrobacter rodentium*) required LTβR mediation while others (*Salmonella enterica*) were cleared in an LTβR-independent fashion.

The beneficial effects of anti-LTβR therapy observed in experimental virus- and LPS-induced shock, cerebral malaria, and prion disease call for further studies of the role of LTβR signaling in the pathogenesis of similar human disease conditions and suggest that anti-LTβR therapy might also be a future treatment for these diseases.

Few studies using bone marrow chimeras and soluble antagonist LTβR IgG fusion protein in wt mice have demonstrated differential roles of secondary lymphoid organs and the cytokines LT\(\alpha\)3 and its membrane-bound heterodimers. Thus, soluble LT\(\alpha\)3 and the LTβR play pivotal roles in immunity against mycobacterial infections and *C. rodentium*-induced colitis. The presence of the LTβR on bone marrow-derived cells is required to clear these infections in mice. In contrast, LT\(\alpha\)3/−/− LIGHT-LTβR interactions are not required to clear experimental *L. major* infection, while a normal splenic, PP, and LN microenvironment is required to overcome experimental leishmaniasis (70). Similarly, an intact splenic microenvironment is required for the induction of appropriate antiviral immune responses in the lymphochoriomeningitis virus model (6, 45).

Inhibition of the LTβR is a future therapeutic concept in treatment of autoimmune diseases (22). The effects of such treatment are secondary to changes to the lymphoid microenvironment and have also been demonstrated in the spleens of nonhuman primates (23). Compared to the effects observed in LT gene-deficient mice, changes following short-term LTβR IgG treatment are moderate (Table 1). However, long-term treatment with LTβR IgG in mice also deletes PPs and colonic patches and reduces the number of intestinal DCs (13).

The treatment of adult mice with anti-LTβR agents is, considering the substantial differences between human and murine immune systems, a situation comparable to the treatment of humans with anti-LTβR therapy. Impaired host defense following LTβR IgG treatment has been observed in the BCG and *C. rodentium* models, while Thiefer’s virus infection was not affected by LTβR IgG treatment (37, 39, 65). Thus, bearing in mind the different modes of action in experimental murine infections and spontaneous infections of humans, immunity against mycobacterial infections and infectious colitis induced by enteropathogenic and enterohemorrhagic *E. coli* might be impaired in humans undergoing anti-LTβR treatment. The immunosuppressive and thus host defense-suppressive effect of anti-LTβR therapy will probably depend on the dose and duration of such treatments.

Gestational treatment of mice with LTβR IgG results in permanent changes to the development of lymphoid organs (56). Similar to other potent immune-modulating therapies, the treatment of pregnant women should be strictly prohibited, and preventive measures, such as the use as of oral contraceptives, should be mandatory in sexually active women undergoing such treatment.

Considering the need for new and effective treatment modalities of human inflammatory and autoimmune diseases, LTβR blockade might be a potent biological tool which has to be carefully tested in clinical trials, considering the delicate balance between sufficient host defense and the suppression of autoimmunity.

**REFERENCES**


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