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Lymphotoxin Signaling in Immune Homeostasis and the Control of Microorganisms

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Abstract

Lymphotoxin (LT) is a member of the tumour necrosis factor (TNF) superfamily that was originally thought to be functionally redundant to TNF, but these proteins were later found to have independent roles in driving lymphoid organogenesis. More recently, LT mediated signalling has been shown to actively contribute to effector immune responses. LT regulates dendritic cell and $CD4^+$ T cell homeostasis in the steady state and determines the functions of these cells during pathogenic challenges. The LT receptor pathway is essential for controlling pathogens and even contributes to the regulation of the intestinal microbiota, with recent data suggesting that LT induced changes in the microbiota promote metabolic disease. In this Review, we discuss these newly defined roles for LT, with a particular focus on how the LT receptor pathway regulates innate and adaptive immune responses to microorganisms.

Lymphotoxin-α (LTα; also known as TNFSF1) is a member of the tumour necrosis factor (TNF) super-family and was originally identified as a product of lymphocytes that was capable of exerting cytotoxic effects on tumour cells in vitro (hence the name lymphotoxin)[1,2]. LTα forms a homotrimer that binds to herpes virus entry mediator (HVEM; also known as TNFRSF14) with low affinity, and this has unknown biological effects. Homotrimers of LTα also bind to TNF receptor 1 (TNFR1; also known as TNFRSF1A) and TNFR2 (also known as TNFRSF1B) with high affinity, but they have a much less prominent role in driving TNFR signalling than does TNF itself [2–4]. It was later shown that LTα-mediated signalling is essential for the development of secondary lymphoid tissues, whereas TNF-mediated signalling alone has only a minor role in this process[5–7]. Indeed, without LTα, animals lack lymph nodes and Peyer's patches. LTα is also essential for the organization of lymphoid organs, such as the spleen and thymus[4,8–10].

Notably, LTα-mediated induction of TNFR signalling is not the major pathway for promoting lymphoid tissue development; this process is instead dependent on lymphotoxin-β receptor (LTβR; also known as TNFRSF3)-mediated signalling. LTα forms a heterotrimer with LTβ (a product of the nearby LTB gene (also known as TNFSF3)), and this heterotrimer binds to LTβR[4,6,11,12]. $LT\alpha_1\beta_2$ is produced by cells of the lymphocytic lineage, including B cells, T cells and innate lymphoid cells (ILCs) that express the transcription factor retinoic acid receptor-related orphan receptor-γt (RORγt)[2,13,14]. LTα-, LTβ- and LTβR-deficient mice have been shown to have similarly defective lymphoid organogenesis, which indicates that LTβR signalling in response to $LT\alpha_1\beta_2$ binding is crucial for this

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process. LTβR is expressed by fetal stromal cells that are found in the lymph node anlagen during development, as well as by cells of the myeloid lineage, hepatocytes, intestinal epithelial cells and endothelial cells[2,14,15]. Therefore, this cytokine–receptor pair serves as a bridge between lymphoid cells and non-lymphoid cells, including parenchymal and stromal cells.

Although the role of LT was once thought to be restricted to promoting lymphoid tissue development[7], it is now known that LT also maintains the structure of the postnatal spleen[16]. LT regulates endothelial cell expression of mucosal vascular addressin cell adhesion molecule 1 (MADCAM1), an adhesion molecule that is recognized by lymphocytes that express L-selectin. MADCAM1 is detectable 3–5 days after birth in the spleens of $Lta^{-/-}$ and $Ltbr^{-/-}$ mice, but its expression is lost at later time points[16]. Thus, in addition to promoting lymphoid tissue development, LT is also essential for maintaining these structures postnatally.

As LT has such a crucial role in lymphoid tissue organogenesis, it has been more difficult to define roles for LT in effector immune responses that are independent of tissue organogenesis. However, recent studies have shown that LT contributes to the effector responses of both the innate and the adaptive immune systems (discussed below). Several reviews have covered the functions of LT in prenatal lymphoid tissue development[2,14,17]; therefore, in this Review, we focus on the immune functions of the LT pathway in adult mice. In particular, we emphasize the new roles that have been identified for LT in protecting the host against pathogenic insults and in regulating the composition of the host microbiota.

DC regulation depends on the LT receptor

DC derived LT modulates the cellularity of secondary lymphoid tissues

Although endothelial cells are known to be the targets of LT in secondary lymphoid tissues, the cellular source of the LT is less clear. Conventionally, lymphocytes have been considered to be the only producers of $LT\alpha_1\beta_2$, but this has been challenged by recent data that indicate that dendritic cells (DCs) also express LT and can control secondary lymphoid tissue cellularity[18] (FIG. 1). Furthermore, another LTβR ligand, LIGHT (also known as TNFSF14), is expressed by human DCs[19,20].

The classic model for DC function is that DCs patrol tissues in search of invasive pathogens, that they mature in response to ‘danger’ or ‘damage’ signals that they receive in situ and that they subsequently initiate adaptive immune responses by migrating to secondary lymphoid organs where they function as antigen-presenting cells (APCs)[21]; however, the role of DCs in uninfected hosts is less clear[22]. Using the Cd11c–DTR system, one group observed a decrease in overall lymph node cellularity after DC ablation[18]. This finding extended previous work that showed that antigen-pulsed DCs increase lymph node cellularity, but only if the DCs express CC-chemokine receptor 7 (CCR7) and are therefore able to home to the lymph nodes[23]. DC depletion correlated with the reversion of high endothelial venules (HEVs), which regulate lymphocyte entry into lymphoid tissues, from a mature to an immature phenotype[18], in which they lacked expression of high-affinity cell-surface receptors that facilitate lymphocyte recruitment[24–26] (FIG. 1). The phenotype of HEVs that are found in DC-depleted mice is reminiscent of the phenotypes observed in animals that lack the LTβR[18,24,27]. Further analysis showed that classical DCs ($CD11c^{hi}$MHC class II^{int}) that reside in the lymph node can express LT, although migratory DCs ($CD11c^{int}$MHC class II^{hi}) do not express this protein[18]. The same study showed that the elimination of DCs capable of LTα expression also resulted in decreased lymph node cellularity[18].

One potential caveat to the experiments addressing DC expression of LT is that the membrane-bound version of LT was never stained to demonstrate that it was actually expressed by DCs[18,28]. Therefore, more work is needed to confirm the expression of LT by DCs. Classical DCs are a rapidly turning over population of cells[21], and so additional work is needed to determine whether it is DC development or DC function that is directly dependent on LT.

LT is required for DC homeostasis

The relationship between DCs and LT is actually quite complex. Ltbr$^{-/-}$ animals lack specific subsets of splenic DCs[29,30], and mixed chimaeras show that this defect cannot be rescued by the presence of wild-type stromal cells. This indicates that LTβR signalling is a cell intrinsic requirement for DC homeostasis[29].

It was recently shown that Notch-dependent endothelial cell-selective adhesion molecule (ESAM)^{hi} DCs require LTβR for their development, whereas the development of ESAM^{low} DCs is unperturbed in the absence of LTβR[30] (FIG. 1). The newly characterized population of ESAM^{hi} DCs proliferates at a greater rate than their ESAM^{low} counterparts, and ESAM^{hi} DCs are essential for CD4^{+} T cell priming (FIG. 1). OT-II T cells — CD4^{+} T cells with a transgenic T cell receptor (TCR) that is specific for ovalbumin — divide at a much lower rate in animals lacking the LTβR-dependent ESAM^{hi} DC population than in wild-type animals, which indicates that LTβR signalling might promote CD4^{+} T cell priming by regulating the maturation status of APCs[30]. Although DC subsets are reduced in Ltbr$^{-/-}$ mice, the mechanisms behind these selective DC deficiencies are still unclear. Early studies suggested that chemokine production is diminished in the absence of LT signalling, preventing the entry of DCs into tissues[31]. However, mixed bone marrow chimaeras indicate that this explanation is unlikely to be correct[29]. Instead, it seems that the LT pathway is essential for promoting the maturation of DC subsets. In this scenario, one would envision the agonism of LTβR signalling on DCs to alter their cell fate through transcription factors downstream of the LT pathway (BOX 1). This might be a mechanism that enables the maturation of DCs once they have migrated into certain host tissues, such as lymphoid organs or intestinal tissue, where a large proportion of $LT\alpha_1\beta_2$-expressing lymphocyte populations reside[22,32–34].

Box 1

LTβR signalling that enables effector functions

Known ligands for the lymphotoxin-β receptor (LTβR) include $LT\alpha_1\beta_2$ and LIGHT[19]. Agonism of LTβR by either of these ligands initiates both canonical and non canonical nuclear factor κB (NF-κB) signalling[105]. Tumour necrosis factor receptor 1 (TNFR1) signalling preferentially, but not exclusively, results in the translocation of p50–RELA (also known as p50–p65) complexes into the nucleus, whereas LTβR signalling preferentially results in the translocation of p52–RELB complexes into the nucleus, in addition to the aforementioned canonical NF-κB complex[19,105]. The direct cytotoxic effects of LTβR signalling can be initiated by both $LT\alpha_1\beta_2$ and LIGHT in conjunction with interferon γ (IFNγ)[106,107]. Other effector consequences that are initiated by the LT pathway (such as the expression of certain cytokines) are influenced by either canonical or non canonical NF-κB activity. $LT\alpha_3$ is a secreted homotrimer that can initiate cytotoxic functions through TNFR1 (REF. 2).

DC derived LT regulates IgA production

The production of IgA is essential for maintaining symbiotic relationships between the host and its microbiota[1,35]. Recent work has indicated that the effects of LT on DCs might explain why the LT pathway is necessary for IgA production[36]. Animals lacking LTβR or LTα are devoid of both serum IgA and intestinal IgA[37]; remarkably, the defect in IgA production seen in LT-deficient animals is more severe than that observed in $\mu MT^{-/-}$ mice[38]. The inability of $Ltbr^{-/-}$ or $Lta^{-/-}$ mice to produce IgA was originally attributed to defects in stromal cell populations in the small intestine that might have provided signals for B cells to class switch to IgA[37]. Support for this model came from a putative role for $ROR\gamma t^{+}$ ILCs in providing membrane-bound $LT\alpha_1\beta_2$ to stromal cells and in inducing their production of factors that regulate IgA[39,40]. However, it was recently shown that inducible nitric oxide synthase (iNOS)-producing DC subsets, which are absent in the gastrointestinal tract of $Ltb^{-/-}$ and $Ltbr^{-/-}$ mice, contribute to intestinal IgA production and carry out this function independently of LTβR-expressing stromal cells[36] (FIG. 1).

DCs residing in mucosa-associated lymphoid tissue (MALT) express iNOS, and mice lacking iNOS expression have defects in IgA production[41–43]. As mentioned above, it was previously thought that the defect in IgA production seen in $Ltbr^{-/-}$ animals was due to the absence of LTβR on stromal cells in the intestinal lamina propria; this original finding was supported by data which demonstrated that intestinal tissue from recombination-activating gene 1 (RAG1)-deficient mice could rescue IgA production in $Ltbr^{-/-}$ hosts[37]. However, it is possible that $iNOS^{+}$ DCs in the graft could also have been responsible for rescuing IgA production in these earlier experiments. In light of new findings, the main role of ILCs in mediating IgA production might be to promote the organization of structures in which $iNOS^{+}$ DCs can interact with B cells. Thus, the role of LT in IgA production might be multifaceted: LT might recruit B cells and DCs by inducing chemokine expression, it might initiate lymphoid structure organization and it might even function as a signal to induce the development of $iNOS^{+}$ DC subsets. Perturbing iNOS-dependent IgA production, or eliminating class-switch recombination and somatic hypermutation, in mice alters the composition of the host microbiota[36,44]. Clearly, future work that aims to address how LT contributes to DC homeostasis in the intestine and in lymphoid tissues will be crucial for appreciating host–microorganism symbiosis.

Roles for LT in adaptive immunity

LT supports $CD4^{+}$ T cell differentiation

In addition to showing deficiencies in IgA production, mice deficient in LT signalling fail to produce IgE[45]. This inability to produce IgE might reflect a broader imbalance in $CD4^{+}$ T helper (T_H) cell subsets that occurs in $Lta^{-/-}$ and $Ltbr^{-/-}$ mice. Defective $CD4^{+}$ T cell responses have been observed in LT-deficient mice subjected to various immune challenges[28,46–48]. Mice lacking LT express higher levels of T_H1-type cytokines in their spleen and lungs[45]. Although these data indicate that LT might be essential for T_H2 cell development, LT is traditionally thought of as a hallmark cytokine in the T_H1 cell response[49,50]. The generation of T_H2 cells is not completely understood[51,52], and the contribution of LT to T_H1- or T_H2-type immunity reveals how the host creates unique $CD4^{+}$ T cell effector programmes.

Citrobacter rodentium and Leishmania major are pathogens that require T_H1-type immunity to be cleared from the body[46,47]. Blocking LT signalling in wild-type mice results in an increased susceptibility to C. rodentium. Intriguingly, although wild-type splenocytes produce interferon-γ (IFNγ) in response to C. rodentium challenge, splenocytes from $Ltbr^{-/-}$ animals produce interleukin-4 (IL-4) and IL-10 (REF. 46). In addition, $Ltbr^{-/-}$ mice show defective humoral responses during C. rodentium infection and generate higher levels of

pathogen-specific IgG1 and lower levels of pathogen-specific IgG2a compared with wild-type mice. However, Ltbr$^{-/-}$ mice are particularly susceptible to C. rodentium during the first 10 days of infection, which indicates that LT might be important for innate responses; and this might complicate the interpretation of these studies.

Experiments using L. major have also indicated that LT might contribute to T_H cell differentiation[47]. Animals deficient in LT signalling fail to control L. major infection, and, similar to the defect seen during C. rodentium infection, Ltbr$^{-/-}$ mice favour the development of IL-4-producing CD4$^+$ T cells, rather than IFNγ producing T cells, in the spleen. In the case of infection with L. major, blocking peripheral lymph node development by treating animals in utero with soluble LTR-Ig and TNFR-Ig recapitulated the T_H2-type skewing of the immune response observed in the Ltbr$^{-/-}$ and Lta$^{-/-}$ mice, even though animals generated using this technique could still signal through LTβR.

Whether lymph nodes contribute to the immune defence against C. rodentium infection by preventing T_H2-type immune skewing or by another mechanism remains unclear. This is difficult to determine as the LT pathway has dual roles during C. rodentium challenge: hosts require LT for both T_H1-type cytokine expression and innate IL-22 production in response to this pathogen[76,77].

Why does LT deficiency affect T cell polarization?

One might conclude from the data discussed above that LT promotes T_H1-type immune responses by supporting lymphoid tissue development, and that the absence of lymph nodes causes pathogens to evoke an inappropriate T_H2 response. However, other data have demonstrated that Lta$^{-/-}$ mice generate increased levels of T_H1-type cytokines in response to schistosome egg challenge experiments, which normally elicit a TH2-type immune bias[45]. This is a surprising result considering the data discussed above that indicate that LT promotes T_H1-type immunity against various pathogens. The idea that LT influences T_H cell education through lymphoid organogenesis does not explain why one would develop opposing T helper cell biases for both T_H1- and T_H2-type immunity.

It is probably the case that active LT signalling supports both lymph node development and the complex cellular interactions that occur in a fully developed lymph node (BOX 2; FIG. 2). This idea is supported by a recent study that explored LT deficiency in the setting of Heligmosomoides polygyrus infection[53], a pathogen that is known to induce IL-4 and IgE production[28]. The authors found that LT signalling is essential for the generation of IL-4-producing CD4$^+$ T cells in response to this infection. H. polygyrus infection results in considerable DC migration to the perifollicular and interfollicular regions of the lymph node[28]. These DCs express CXC-chemokine receptor 5 (CXCR5), which enables them to migrate in response to CXC-chemokine ligand 13 (CXCL13) that is produced by follicular DCs (FDCs)[28].

Box 2

Lymphoid organogenesis directed by the LT pathway

During embryonic development, retinoic acid is produced by neurons to recruit retinoic acid receptor related orphan receptor-γt (RORγt)$^+$ fetal lymphoid tissue inducer (LTi) cells to the location of future lymph nodes[14]. Once LTi cells have arrived, they express membrane bound lymphotoxin-$\alpha_1\beta_2$ (LT$\alpha_1\beta_2$), which binds to the LT-β receptor (LTβR) on fetal stromal cells; stromal cells undergo transcriptional changes in response to LT signalling. Agonism of the LT pathway results in the local production of vascular cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule 1 (ICAM1), mucosal vascular addressin cell adhesion molecule 1 (MADCAM1), CC chemokine ligand 19

(CCL19), CCL21 and CXC chemokine ligand 13 (CXCL13) to recruit a vast range of cells that ultimately form and populate the lymph node[14]. After birth, the LT pathway has an essential role in the maintenance of lymphoid tissues. For example, active LT signalling, mostly from B cells, is required to maintain splenic architecture in adult mice[108]. In the colon, B cells must express LTα to induce isolated lymphoid follicle expansion[109], and the lack of LTα on B cells results in atrophic follicles[109]. Animals that lack LTi cells as a result of the absence of the transcription factor RORγt lack secondary lymphoid tissues, which includes isolated lymphoid follicles, but can develop tertiary lymphoid follicles from chronic inflammatory stimuli in similar locations[110].

In addition, follicular helper T (T_{FH}) cells are also recruited to the perifollicular regions during helminth challenge[28]. The authors speculated that LT might contribute to this multicellular organization process in the lymph node, as it does in the spleen[16,31]. Blockade of LT signalling results in diminished CXCL13 production, and it was also shown that B cells need to express LT to coordinate interactions between T cells, DCs and themselves in the lymph node in order to promote the development of a T_H2-type immune response following helminth infection[28] (FIG. 2). The role of B cells in this coordination may be antigen nonspecific as both pathogen-specific and pathogen-nonspecific B cells upregulate LT in response to helminth infection[28]. Understanding the exact role of LT during helminth infection might be essential for appreciating the general mechanisms that are involved in the generation of T_H2 cells. Furthermore, it is unclear why LT has seemingly opposing effects in different infections.

Accounting for B cell defects in LT deficient mice

Reconsidering the humoral defects in LT-deficient animals, it is possible that LT might contribute to B cell class-switching responses through its effects on lymph node architecture. One explanation for why animals that lack the LT signalling pathway have defective humoral immunity is that LT is essential for the formation of FDCs and germinal centres[54]. The microRNA miR-155 directs the expression of TNF and LTα in mature B cells, and deficiencies in miR-155 or LTα result in the abrogation of the germinal centre response[55] (FIG. 2). Inducible T cell co-stimulator (ICOS)-deficient animals also have abnormal germinal centres and experience defects in humoral immunity similar to those seen in LT-deficient animals[56]. Interestingly, ICOS crosslinking results in T cell expression of $LT\alpha_1\beta_2$, which is essential for the chemokine expression that is necessary to organize the germinal centre[56]. Induction of LT expression via the ICOS pathway is also dependent on CD40, but the overall contribution of CD40 to this pathway is unclear. However, it is clear that both B cells and T cells have unique mechanisms to induce LT expression and that this is essential for normal germinal centre responses[55,56].

Roles for LT in immunity to viruses

LT supports SCS macrophages

The ability of B cells to produce LT is essential to control viral infections. During vesicular stomatitis virus (VSV) infection, virions are normally captured by subcapsular sinus (SCS) macrophages, which produce type I IFNs and which prevent the systemic spread of the pathogen[57] (FIG. 3). The lymph nodes of B cell-deficient mice have significantly lower numbers of SCS macrophages, and these mice are more susceptible to infection with VSV. B cell-derived LT has now been linked to SCS macrophage development and the generation of B cell-specific immunity[58,59]. Mice lacking conventional B cells fail to survive following VSV challenge; however, D_HLMP2A animals, in which B cells contribute to normal lymphoid tissue development but do not generate antigen-specific B cell responses, are

resistant to VSV infection[60]. The role of B cells in VSV immunity was therefore attributed to their ability to express LT. Mice with a B cell-restricted deficiency in LT expression are susceptible to VSV infection owing to defects in SCS macrophage development[60]. Therefore, LT produced by B cells affects SCS macrophages and contributes to their ability to prevent the systemic spread of pathogens[60] (FIG. 3).

LT supports type I IFN responses

The LT signalling pathway has different protective functions in other viral infection models. The importance of type I IFNs in viral immunity has been known for some time[61], and a recent study has demonstrated that LT is part of a dual mechanism which results in IFNβ production in response to murine cytomegalovirus (MCMV)[62]. $Lta^{-/-}$, $Ltb^{-/-}$ and $Ltbr^{-/-}$ mice fail to control MCMV infection and, compared with wild-type mice, their viral loads increase by nearly 100-fold[63]. It is thought that plasmacytoid DCs (pDCs), which are activated by virally derived Toll-like receptor 9 (TLR9) ligands, are the main source of IFNβ during viral infection. During MCMV infection, serum levels of IFNβ exhibit a biphasic expression pattern, with peak levels occurring at 8 hours and 36–72 hours after MCMV infection. Mice lacking LTβ and LIGHT do not produce IFNβ 8 hours after MCMV infection, but animals lacking TLR9 signalling are still able to produce IFNβ at this early time point. B cells must express $LT\alpha_1\beta_2$ for the early IFNβ peak in response to MCMV infection[62], and radio-resistant cells in the spleen are the source of the type I IFN peak at 36–72 hours during infection with MCMV[62] (FIG. 3).

Considering a different viral infection model, it was shown that, in mice infected with lymphocytic choriomeningitis (LCMV), B cell-derived LT is important not only for type I IFN production, but also for the reorganization of the lymphoid architecture[64,65]. In the lymph node, LTβR is required for LCMV-induced B cell follicle formation, and the absence of B cell-derived LT results in unorganized follicles[64]. In response to LCMV challenge, $Lta^{-/-}$ mice produce only 3% of wild-type levels of type I IFN[65]. It is thought that the effects of LT on splenic macrophages is necessary for the production of type I IFNs, in a similar manner to the way in which LT induces type I IFN production by SCS macrophages in the lymph node[65] (FIG. 3).

LT promotes antiviral T cell responses

The induction of IFNβ is crucial for protective antiviral $CD8^+$ T cell responses[66]. LT-mediated regulation of DCs might provide an important source of type I IFN for $CD8^+$ T cell viability during viral infection. Both LTα and LTβR are required to prevent $CD8^+$ T cell apoptosis in response to MCMV challenge[67], and it was recently demonstrated that LT might contribute to $CD8^+$ T cell homeostasis by inducing the expression of type I IFNs[68]. In this study, blockade of LT signalling with LTβR-Ig resulted in a decreased capacity of the OVA-specific $CD8^+$ T (OT-I) cell population to expand in response to cognate antigen delivery[68]. Using the CD11c–DTR system, investigators demonstrated that DCs were required for $CD8^+$ T cell proliferation[68]. They speculated that DCs might produce type I IFNs in response to LT signalling to maintain adequate $CD8^+$ T cell homeostasis. In vitro experiments showed that, in conjunction with lipopolysaccharide (LPS; a TLR4 ligand), agonist antibodies against LTβR induce IFNβ production from bone marrow-derived DCs[68]. $Ltbr^{-/-}$ DCs are less efficient at inducing the expansion of the $CD8^+$ T cell population in vivo, and this defect can be rescued by the exogenous delivery of type I IFNs[68].

Why the LT pathway regulates type I IFNs during viral infection is not immediately obvious, but it might be linked to the role of LT in host defence mechanisms against viruses. It is important to note that the LT pathway has a non-redundant role in promoting protective immunity in the fully developed lymph node, where LT prevents the spread of viruses into

the blood[57,69,70]. The ability of the host to induce type I IFN production by this pathway is of dual importance in that it helps to initiate adaptive immune responses, and it can also prevent the sudden death of T cells in response to viral challenge[67].

The role of LT in immunity is specific

One might erroneously conclude from the work on the role of LT in lymphoid tissue development (BOX 2) and the extensive work detailing the multicellular interactions coordinated by the LT pathway (FIGS 2,3) that LT only contributes to the adaptive immune response by supporting lymphoid tissue development and organization. In fact, the importance of the studies highlighted above is that they demonstrate the unique requirement of LT in active immune processes, and show the distinct and specific roles for the LT receptor. LT is not required for the development of all lymphoid tissues; for example, bronchus-associated lymphoid tissue (BALT) develops in the absence of LT and is able to support immunity to influenza[71]. However, even in the lung, the importance of LT is still apparent, though poorly understood, because host survival relies on the LT receptor pathway during challenge with other airborne pathogens, such as Mycobacterium tuberculosis[72]. The above studies emphasize the important and unique roles of the LT receptor pathway in modulating adaptive immunity.

LT regulates mucosal immune responses

Regulation of immunity to mucosal pathogens

The LT pathway is essential for the control of enteric pathogens[73–78]. Investigators studying MALT found that Ltbr$^{-/-}$ mice have a greater systemic spread of Salmonella typhimurium[73,74], but the mechanisms responsible for this have not been well defined. It was found that B cell localization in response to S. typhimurium is dependent on the LT pathway[73], but how B cell localization might contribute to host defence is unclear because wild-type and Ltbr$^{-/-}$ animals seem to be equally susceptible to initial infection with S. typhimurium. A major caveat to these studies on S. typhimurium is that they rely on the pretreatment of the mice with streptomycin, which may alter the composition of the commensal microbiota and change undetermined variables in both wild-type and Ltbr$^{-/-}$ mice.

Toxoplasma gondii, a neuroinvasive pathogen that infects the host via the terminal ileum, induces fatal infection in hosts lacking the LT pathway[78]. However, as in the case of S. typhimurium infection, it is not clear how the LT receptor pathway protects the host from T. gondii. By contrast, we have a better understanding of the role of the LT pathway in response to C. rodentium infection, a mucosal pathogen that is controlled by ILCs and elements of the adaptive immune response (discussed below).

The role of LT in immunity to C. rodentium

In the past few years, there has been much interest in understanding the development and the function of ILCs that express the transcription factor RORγt[79–83] and that contribute to tissue immunity in response to IL-23 (REF. 84). Although there has been great debate about the origin of these cells, a consensus is beginning to be reached, that they might be an adult version of lymphoid tissue-inducer cells; however, whether these cells differentiate from their fetal counterparts is still a contentious topic[85–87]. Although LT does not contribute to the development of ILCs[88], RORγt^{+} ILCs have crucial effector functions, including IL-22 production, via the LT pathway[76,77,89].

During C. rodentium infection, IL-22 from RORγt^{+} ILCs is required for the clearance of the bacteria. The role of the LT pathway in the response to C. rodentium challenge was

originally attributed to the capacity of LT to regulate the T_H1 and T_H2 cell balance. However, $Ltbr^{-/-}$ mice are ill as early as 7 days following initial challenge with C. rodentium and some succumb to C. rodentium infection 10 days after challenge[75]. This early onset of disease indicated a possible innate role for LT in this infection model. Indeed, it was found that LTβR expression was required by both myeloid cells and intestinal epithelial cells for mice to control C. rodentium[75]. Blockade of LT renders $Rag1^{-/-}$ hosts more susceptible to C. rodentium infection, and $ROR\gamma t^+$ ILCs are the innate source of the $LT\alpha_1\beta_2$ that promotes host resistance to C. rodentium[75].

It was initially reported that $Ltbr^{-/-}$ mice have normal levels of IL-22 but fail to clear C. rodentium[88], which caused confusion as to what role LT has in promoting innate immunity to this pathogen. As indicated above, numbers of $ROR\gamma t^+$ ILCs are not decreased in $Ltbr^{-/-}$ mice. It was later suggested that the susceptibility of $Ltbr^{-/-}$ mice to C. rodentium might, after all, occur because these animals fail to express IL-22 (REFS 76,77). Treatment of animals with an agonist antibody against LTβR can rescue IL-22 production in $Ltb^{-/-}$ mice infected with C. rodentium[77]. Furthermore, addition of exogenous IL-23 induces IL-22 production in mice infected with C. rodentium when LT signalling is blocked and rescues these animals from death[76]. The ability of IL-23 to rescue this defect indicates that agonism of the LT signalling pathway might control IL-22 by regulating the production of IL-23.

Microscopy revealed that providing exogenous IL-22 to mice after blockade of LTβR signalling restored normal lymphoid structures in these animals during C. rodentium infection[76]. Furthermore, isolated lymphoid follicles (ILFs) were identified as the structures containing protective ILCs, and $ROR\gamma t^+IL\text{-}22^+$ ILCs were found in immediate proximity to DCs in ILFs[77] (FIG. 4). Mice in which LTβ deficiency was restricted to ILCs had lower expression of IL-22, but treatment with exogenous IL-22 rescued the ability of these mice to resist C. rodentium. This was an interesting result because $ROR\gamma t^+$ ILCs were required to express membrane-bound LT to promote IL-22 production, but these were the same cells that produced the IL-22 that was necessary to control C. rodentium infection[77]. Furthermore, LTβR is not expressed by ILCs. This confusion was resolved when it was discovered that, in the absence of LTβR, DCs could not express wild-type levels of IL-23 in response to C. rodentium[77]. Conditional deficiency of LTβR on DCs renders animals more susceptible to C. rodentium and reduces IL-22 production by ILCs[77].

These studies have resulted in the development of a model to describe the roles of the LT pathway in mucosal defences. It seems that there is an ILC–DC feedback loop in ILFs, in which LT is produced by ILCs and induces IL-23 production by local DCs, which in turn induces IL-22 production by the ILCs (FIG. 4). As the LT pathway also regulates T_H cells, it is important to determine whether ILCs regulate T_H cells that contribute to similar infection models.

Regulation of the microbiota by LT

There is growing evidence that in addition to promoting immunity to intestinal pathogens, such as C. rodentium, LT signalling also contributes to the regulation of the intestinal microbiota. The role of the microbiota in human disease is complex and multifold. This is perhaps best demonstrated by the landmark discovery of an ‘obesity-associated microbiome’ in 2006, which demonstrated that specific changes in the host microbiota could contribute to the development of obesity[111].

Obesity is also significantly linked to natural polymorphisms that occur in LTA itself[90]. The idea that LT contributes to metabolic disease is not without precedent[91–93]. LTβR has previously been reported to regulate hepatic lipid production and atherosclerosis[91,92]; this occurs in the setting of the pathological overexpression of LIGHT by T cells[91] or in mice

that have abnormalities in lipid homeostasis through other genetic mechanisms[92]. However, despite epidemiological studies that indciate that there might be a link between obesity and LT signalling, the exact mechanisms by which LT contributes to weight gain are unclear.

A link between LT and metabolic disease

One group previously identified single nucleotide polymorphisms in the TNF–LTA gene locus in the Pima Indians[94]. Their study reports the significant association between a single nucleotide polymorphism in the dinucletotide repeat marker TNFir24, which is located 10 kilobases away from the TNF gene, and changes in body fat percentage and body mass index (BMI)[94]. However, sequencing of the TNF gene in obese and lean subjects of Pima Indian origin did not reveal any differences in TNF alleles between the obese and lean subjects. At the time, it was suggested that this polymorphism could indicate a link between the nearby LTA gene and obesity.

Nearly a decade later, these findings were revisited by another group, who hypothesized that the LT signalling pathway might contribute to obesity[95]. It was discovered that the Thr60Asn mutation in LTα significantly correlated with an increased incidence of type 2 diabetes in a Danish cohort of patients. The incidence of type 2 diabetes in Thr60Asn/Thr60Asn homozygous individuals was 1.24-fold higher than in control subjects who had a Thr60Thr/Thr60Thr genotype. Closer examination revealed that this allele did not influence BMI, but other indicators of increased adiposity, including waist-to-hip ratio, were significantly increased in individuals who were homozygous for the mutation compared with those who were either heterozygous for the mutation or had two normal alleles[95].

Three important considerations caused investigators to later consider the possibility that LT might regulate changes to the microbiota that are causative of obesity: the link between the LT pathway and obesity, the role of the microbiota in metabolic disease, and the regulation of gut immunity by LT[96]. Indeed, two groups independently demonstrated that LT-deficient animals are resistant to diet-induced obesity[96,97]. One group found that $Lta^{-/-}$ animals resisted excess weight gain that is induced by a high-fat diet, and that deficiency in the LT pathway resulted in increased lymphocyte infiltration into the perigonadal fat pads of animals, regardless of the diet they received[97]. In this study, the role of LTα in weight gain was considered to be redundant to that of TNF[97]. The second study showed that animals deficient in LTα, LTβ or LTβR resisted high-fat diet-induced weight gain[96]. However, which cells usually express LT in this setting remain to be determined. It is also possible that more than one type of cell is involved.

LT regulates the commensal microbiota

Although animals lacking elements of the LT pathway resist excessive weight gain induced by a high-fat diet, they still consume similar amounts of food[96,97]. This is a unique finding because excess weight gain is driven by a positive energy imbalance induced by a high-fat diet[98]. Given the role of the LT pathway in regulating C. rodentium and other mucosal pathogens[73–78], it was speculated that the LT pathway might influence host weight gain by regulating the commensal microbiota of the host.

16S rRNA sequencing revealed that the microbial communities of $Ltbr^{-/-}$ mice differ from those of their $Ltbr^{+/-}$ siblings, both on a normal chow diet or on a high-fat diet[96]. Notable differences include the sustained overgrowth of segmented filamentous bacteria (SFB) in $Ltbr^{-/-}$ mice on a high-fat diet, whereas SFB are cleared by $Ltbr^{+/-}$ mice fed on the same diet. Furthermore, members of the Erysipelotrichi class were found to be enriched in $Ltbr^{+/-}$ animals fed a high-fat diet, but are not enriched in $Ltbr^{-/-}$ mice on the same diet[96]. Importantly, outgrowth of Erysipelotrichi class members was previously reported to occur in

response to a high-fat diet, and this bacterial class might contribute to obesity[99]. Which species have essential roles for LT-controlled diet-induced obesity remain to be determined. Co-housing Ltbr$^{-/-}$ mice with their obesity-prone littermates actually rescued their capacity to gain weight and restored control of the SFB population[96]. This confirms that the LT pathway influences weight gain by regulating the composition of the microbiota. LT is essential for the production of IL-23 and IL-22 in response to a high-fat diet, and restoring IL-22 levels in Ltbr$^{-/-}$ mice rescued the defective SFB homeostasis and the metabolic abnormalities, including perigonadal fat depot expansion, that occur in Ltbr$^{-/-}$ mice[96].

Although the role of the LT pathway in promoting protection against invasive pathogens such as C. rodentium, S. typhimurium and T. gondii might originally have been thought to be its primary immune function at mucosal sites, these recent data show that the LT pathway is also involved in regulating the symbiotic relationship between the host and their commensal microbiota. Notably, some of the mechanisms by which the LT pathway contributes to protective immune defences could also contribute to weight gain through regulation of the microbiota (FIG. 4).

Breaking (mucosal) barriers with the LT receptor

The LT pathway has an important role at mucosal surfaces, where it facilitates the formation of unique, dynamic lymphoid structures, such as ILFs, in the gut[76]. ILFs help to coordinate the symbiotic relationship between the host and their commensal microbiota[1,100]. Although the role of the LT receptor pathway in ILF formation is well understood in healthy host physiology, this process might also highlight the ability of the LT pathway to coordinate the dynamic lymphoid tissue neogenesis that can occur at all epithelial surfaces. This would be an unappreciated property of epithelial surfaces, but would explain why many autoimmune disorders are characterized by lymphoid aggregation near epithelia, and would also highlight modulation of the LT receptor pathway as a new avenue of therapeutics for people with autoimmune diseases. For example, in a mouse model of Sjögren's syndrome, lymphoid tissue aggregates form near the ductal epithelium as a result of CXCL13 overexpression, and the use of LTβR-Ig decreases lymphocyte recruitment to these sites[101].

Furthermore, recent work in mouse models has shown that autoimmunity might be partly driven by alterations in the commensal bacteria present at mucosal surfaces, and therefore breaks in immune tolerance could be induced by previously unappreciated factors, such as milk fat-containing diets[102–104]. Given the unique position of the LT receptor pathway in regulating both the lymphoid follicle formation and the commensal microbiota, it is intriguing to consider possibilities such as a combination of biological and probiotic therapies. For example, beneficial probiotic bacteria could be reseeded into hosts when disease is driven by ectopic lymphoid follicles and dysbiosis. The combination of LTβR-Ig in conjunction with this probiotic agent might be more effective than either agent in isolation at tackling complex human diseases. The ability to block ectopic lymphoid follicle formation and to control microbial communities by modulating the LT receptor pathway might, therefore, be a future avenue to explore to treat autoimmune diseases, or other inflammatory host states, such as obesity.

Concluding remarks

The history of our understanding of the role of LT has many chapters. It was initially considered a redundant cytokine, and was later thought to be the focus of lymphoid organogenesis. In recent years, it has become clear that LT is an important component of effector immune responses. LT is essential for host defences against specific pathogens and even contributes to the regulation of the gut microbiota. It is now widely appreciated that the LT receptor pathway is essential for protective innate and adaptive immune responses, but

LT signalling may also contribute to the development of metabolic disease through the regulation of commensal microbiota. It will be interesting to explore whether manipulation of the LT receptor pathway could be useful in the clinic.

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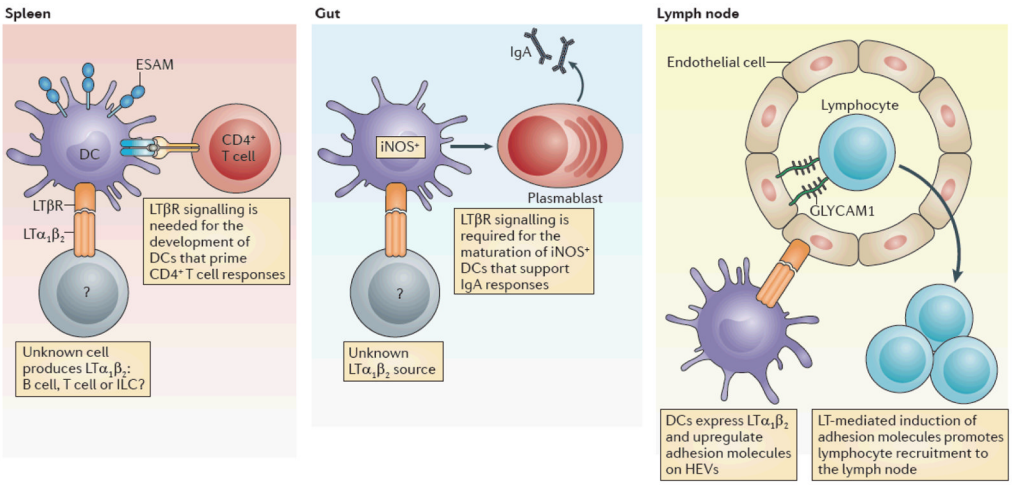

Figure 1. The LT pathway regulates DC fates

Dendritic cells (DCs) require lymphotoxin-$\alpha_1\beta_2$ ($LT\alpha_1\beta_2$) that is provided by a currently undefined cell type. Endothelial cell selective adhesion molecule $(ESAM)^{hi}$ splenic DCs, which are essential for priming $CD4^+$Tcells, require T signaling for their development[29,30]. In the gut, LTβR induces the maturation of inducible nitric oxide synthase $(iNOS)^+$ DCs, which then induce the development of IgA producing plasmablasts[36]. In both of these cases, further studies are required to define the cellular source of the LT. Additionally, in the lymph nodes, DCs have recently been shown to express $LT\alpha_1\beta_2$ (REF. 18), which is recognized by high endothelial venules (HEVs) and leads to their upregulation of adhesion molecules, such as glycosylation dependent cell adhesion molecule 1 (GLYCAM1), and is important for maintaining lymph node cellularity in the steady state. ILC, innate lymphoid cell.

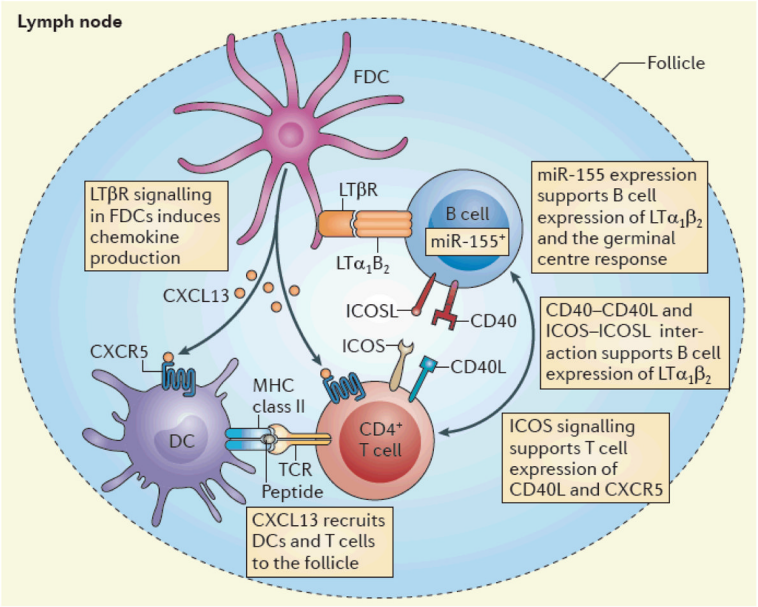

Figure 2. LT coordinates adaptive immunity

The lymphotoxin (LT) pathway organizes multicellular interactions in the lymph node. B cell-derived $LT\alpha_1\beta_2$ is recognized by the LTβ receptor (LTβR), which is expressed on follicular dendritic cells (FDCs), and this interaction promotes the secretion of chemokines that recruit antigen presenting dendritic cells (DCs) and $CD4^+$ T cells to facilitate the development of T helper cell responses[28,47]. In the germinal centre, miR-155 expression in B cells maintains LT expression. Ligation of inducible T cell co-stimulator (ICOS) on T cells to ICOS ligand (ICOSL) on B cells and the CD40–CD40 ligand (CD40L) interaction are also essential for the upregulation of LT on B cells that enables a germinal centre response [55,56]. CXCL13, CXC chemokine ligand 13; CXCR5, CXC chemokine receptor 5; TCR, T cell receptor.

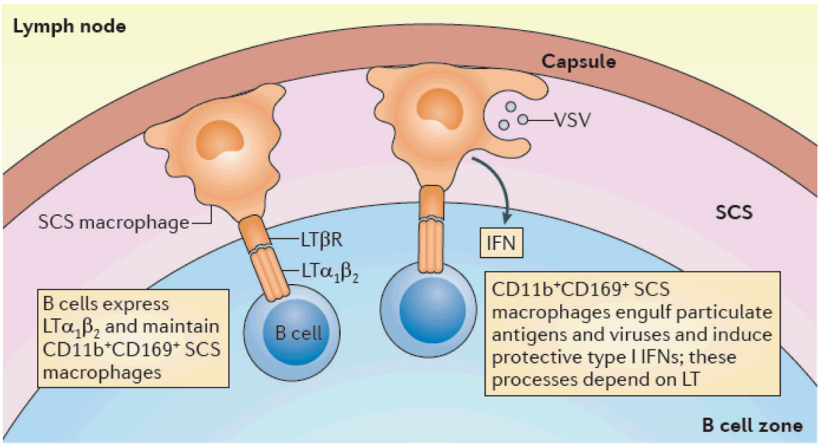

Figure 3. Regulation of viral infections with LT

In the fully developed host, B cells provide lymphotoxin (LT), which is recognized by the macrophages that populate the subcapsular sinus (SCS) of the lymph node and causes them to adopt an SCS macrophage phenotype ($CD11b^+CD169^+$)[60]. These macrophages engulf particulate antigens, including vesicular stomatitis virus (VSV), and prevent their spread to the periphery[57,70]. The macrophages also produce protective type I interferons (IFNs). During lymphocytic choriomeningitis (LCMV) infection, active LT signalling is required to organize B cell follicles; the cell type sensing this LT signalling is currently uncharacterized, but might be the high endothelial venule (HEV)[64] (not shown). The LT pathway is also essential for the production of type I IFNs from radio-resistant cells in the spleen that protect the host during viral infection[63,65] (not shown). In addition, dendritic cells (DCs) that produce type I IFN in response to LTβ receptor (LTβR) agonism and Toll like receptor (TLR) signalling promote $CD8^+$ T cell homeostasis in the spleen[63,68] (not shown).

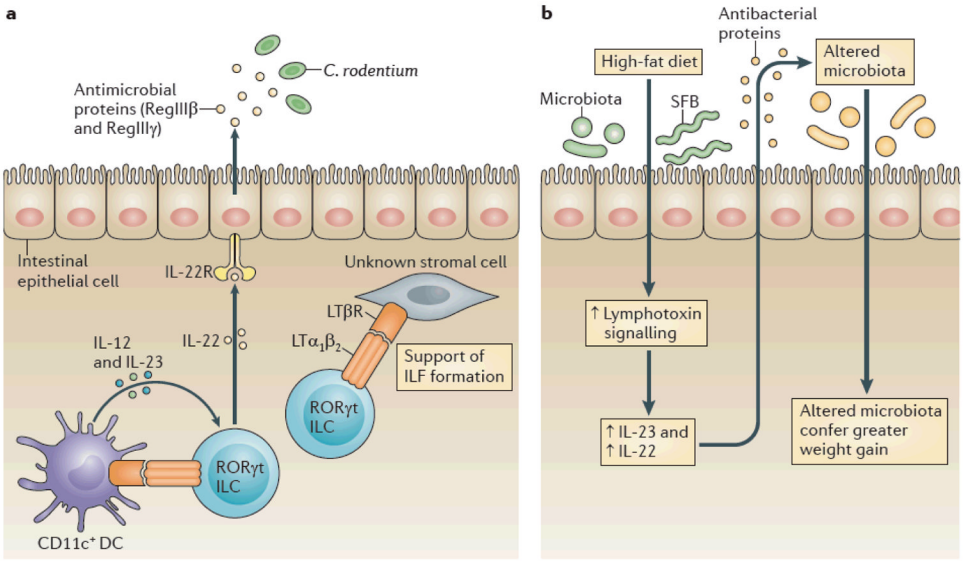

Figure 4. LT regulates responses to microorganisms at mucosal surfaces

a|During Citrobacter rodentium infection, the lymphotoxin (LT) pathway is part of an innate feedback loop in which retinoic acid receptor related orphan receptor γt(RORγt) innate lymphoid cells (ILCs)induce interleukin 12(IL 12) and IL 23production by local dendritic cells (DCs) via the LT pathway[76,77]. In response to IL 23, these ILCs produce IL 22, which promotes gut epithelial cells to produce antimicrobial peptides, such as RegIIIβ and RegIIIγ[77]. These antimicrobial peptides have direct killing functions on C. rodentium and members of the commensal microbiota. LT dependent interactions between ILCs and stromal cells can also support the formation of isolated lymphoid follicles (ILFs) during infections. **b** | In response to a high fat diet, LT signalling is increased within the colon (V.U. and Y. X.F., unpublished observations) and this increases IL 23 and IL 22 production[96]. This then induces the production of antibacterial proteins, which are essential for the clearance of some microorganisms (such as segmented filamentous bacteria (SFB)) in response to high fat diet administration and which contribute to the alteration of the microbiota, which in turn confers greater weight gain[96]. IL 22R, interleukin 22 receptor; LTβR, LTβ receptor.