Linking the microbiota and metabolic disease with lymphotoxin

Vaibhav Upadhyay¹² and Yang-Xin Fu¹²

¹Committee on Immunology, University of Chicago, Chicago, IL 60637, USA
²Department of Pathology, University of Chicago, Chicago, IL 60637, USA

Correspondence to: Vaibhav Upadhyay, Committee on Immunology, University of Chicago, 924 E 57th Street, Chicago, IL 60637, USA; E-mail: vupadhyay@uchicago.edu
Received 18 March 2013, accepted 3 April 2013

Abstract

The field of lymphotoxin biology has seen many advances in the past decade. Notably, a role for lymphotoxin as a key effector cytokine has emerged to add to its foundational contribution to lymphoid organogenesis. It is now clear that lymphotoxin contributes to host defense for a wide variety of pathogens, and the lymphotoxin receptor is a defining feature of and regulatory mechanism in both innate and adaptive immunities. Specifically, lymphotoxin contributes to Th education, licensing of IL-22 production from type 3 innate lymphoid cells, and even maintains innate myeloid populations within the fully developed lymph node. Most recently, lymphotoxin has been implicated in regulation of the microbiota and metabolic disease. Early studies revealed that lymphotoxin might influence composition of the commensal microbiota through its regulation of immunological compartmentalization in the gut. Additionally, several epidemiological studies have linked polymorphisms in lymphotoxin to metabolic disease. Studies exploring the role of lymphotoxin in metabolic disease have demonstrated that lymphotoxin may influence metabolism both directly in the liver and indirectly through regulation of gut immune responses. It now appears that lymphotoxin may bridge the gap between altered composition of the commensal microbiota and metabolism.

Keywords: diabetes, lymphotoxin, metabolism, microbiota, obesity, SFB

Introduction

The terms ‘lymphotoxin’ and ‘tumor necrosis factor’ were used initially to describe, respectively, lymphocyte-derived and monocyte-derived molecules that killed fibrosarcoma cells (1). Both molecules were found to have similar amino-acid sequences and to bind the same receptor, so they were renamed tumor necrosis factor β (TNFβ) and TNFα, respectively. TNFα is now termed TNF or TNFSF2 (TNF superfamily, member 2); lymphotoxin is now used to describe combinations of lymphotoxin α (LTα; TNFSF1) and LTβ (TNFSF3). TNF signals through two receptors: TNFR1 (TNFRSF1A) or TNFR2 (TNFRSF1B), and LTα was believed to function primarily by signaling through these TNFRs (2).

It is now widely appreciated that there is a distinct pathway triggered by lymphotoxin binding to the so-called lymphotoxin receptor, which is also called the LTβ receptor (LTβR; TNFRSF3), that plays a critical role in the development and maturation of secondary lymphoid tissues, maintenance of the immunological steady state, cell-mediated immunity and control of both pathogenic and commensal micro-organisms (3). Recent reports demonstrate that lymphotoxin plays a key role in regulation of the microbiota and contributes to metabolic disease in the host (4–6). Lymphotoxin’s role in immunity, including its regulation of the commensal microbiota and contribution to metabolic disease, will be visited in this review.

The lymphotoxin receptor: discoveries in lymphoid organogenesis

One molecule of LTα and two molecules of LTβ form a membrane-bound heterotrimer known as LTα1β2, which binds LTβR. The ligand, LTα1β2, is expressed on lymphocytes, whereas LTβR is expressed on myeloid, parenchymal and stromal cell populations, and therefore LTα1β2 and LTβR form a unique bridge among multiple cell types. Although LTα can signal through both TNFR1 and TNFR2 as a secreted homotrimer (LTα1α), unlike TNF-deficient mice, LTα-deficient mice lack lymph nodes, Peyer’s patches and isolated lymphoid follicles (ILFs), which originally suggested that
there was a lymphotoxin-specific receptor (7). It was also appreciated mice lacking LTβ have similar defects in lymphoid organogenesis to those in LTα-deficient mice. Later on, LTβR was discovered and its deficiency recapitulated identical deficits in lymphoid structure to those in LTα-deficient mice and identified it as the enigmatic receptor of the lymphotoxin pathway (8, 9). During fetal development, lymphoid-tissue inducer (LTI) cells migrate to the site of the lymph node anlagen and express LTαβ, which is recognized by LTβR. Agonism of LTβR results in signaling through either p50–RelA or p52–RelB heterodimers into the nucleus; the ability of the lymphotoxin receptor to initiate both canonical and non-canonical NF-kB signaling in this way is essential for its ability to initiate transcriptional changes in stromal cells that enable it to form a lymph node (10).

An immunological effector

In addition to influencing lymphoid development, another role of the lymphotoxin-receptor pathway is as a key effector cytokine. This was initially hinted at in mucosal surfaces where it was discovered that lymphotoxin is essential for the formation of IgA and IgE (11, 12). In the absence of the lymphotoxin-receptor pathway, animals develop aberrant immune responses to a wide variety of mucosal pathogens including Mycobacterium tuberculosis, Leishmania major, Toxoplasma gondii, Listeria monocytogenes, Citrobacter rodentium, and Heligmosomoides polygyrus (13–19). Lymphotoxin is believed to play two crucial roles in defense against the abovementioned pathogens: it serves as a cytokine to balance Th1-cell immunity and plays a key function in licensing IL-22 production from retinoic acid receptor γt (RORγt+) innate lymphoid cells (ILCs).

In the absence of the lymphotoxin pathway, the Th1-cell bias is abnormal and Th2 responses are generated where Th1 responses are protective (i.e. in the case of Leishmania major and C. rodentium) (2, 14, 16) and in cases where Th2 immunity is physiologically appropriate, it is not generated (i.e. in the case of H. polygyrus and schistosome eggs) (3, 12, 20). In addition to its role in the formation of lymph nodes, it is now appreciated that lymphotoxin organizes complex multicellular interactions within fully developed lymphoid tissues that are essential for interactions between B cells, T cells, and dendritic cells (DCs) for appropriate adaptive immune responses, including Th1-cell education (4–6, 20).

The role of the lymphotoxin receptor is also important in licensing IL-22 production from type 3 ILCs (i.e. the ILC subset that produces IL-22 and expresses RORγt), which protects the host from mucosal pathogens (7, 17, 19). An emerging model in the field is that in response to mucosal pathogens, type 3 ILCs express LTαβ, which is recognized by LTβR on DCs that localize with type 3 ILCs in ILFs. Agonism of LTβR on DCs induces IL-23 production from local DCs, which provokes IL-22 expression in type 3 ILCs (8, 9, 17, 19). IL-22 binds to IL-22R expressed by intestinal epithelial cells and induces expression of anti-microbial peptides, which have a direct role in pathogen defense (10, 21). Importantly, the lymphotoxin receptor has additional attributes as an effector cytokine on both macrophage and intestinal epithelial cell populations (11, 12, 18), but its function on these cell types requires further definition.

The lymphotoxin receptor is also essential in antiviral immunity. This has been illustrated with multiple virus types and centers on the lymphotoxin receptor’s role in stabilizing the subcapsular macrophage phenotype and inducing type I interferon production (13–19, 22–26). For example, vesicular stomatitis virus (VSV) is taken up by subcapsular sinus (SCS) macrophages that line the lymph node and process lymph flowing from the periphery toward the circulation system; B cells express lymphotoxin to maintain the SCS-macrophage phenotype, and without B-cell-derived lymphotoxin, SCS macrophages do not populate the lymph node. Their absence leaves animals susceptible to VSV and potentially other pathogens present in the lymph (22–24). In the case of murine cytomegalovirus and lymphocytic choriomeningitis virus, it is appreciated that the lymphotoxin-receptor axis is part of a biphasic production of type I interferon that is essential to control viral load and key to prevent virally induced death of cytotoxic T-cell populations that have the potential to contribute to host defense (27).

Lymphotoxin-receptor-mediated regulation of commensal bacteria

The initial possibility that lymphotoxin contributed to regulation of the commensal microbiota came from early studies demonstrating IgA deficiencies in lymphotoxin-deficient animals that are even more severe than the IgA defects observed in Ighm−/− mice (which lack nearly all mature B cells) or mice that lack T cells (28, 29). IgA is a key component of the host’s ability to prevent colonization of harmful bacteria at mucosal surfaces and has even been suggested to serve as an anchor for certain commensals (30).

The notion that the lymphotoxin receptor would serve a key function in regulating the composition of the commensal microbes occupying the distal gut was definitively demonstrated by the study by Bouskra et al., in which treatment of animals with LTβR–Ig, a decoy receptor that prevents lymphotoxin signaling and subsequent lymphoid-structure formation, resulted in aberrant overgrowth of certain microbial community members, including segmented filamentous bacteria (SFB) (31). SFB is a community member that lives in proximity to Peyer’s patches and has been linked to T17-mediated immunity (32). Here, the role of the lymphotoxin receptor in commensal homeostasis was attributed to its regulation of IgA. Further support for the notion that specific commensal bacteria rely on lymphotoxin for regulation came from the demonstration that Alcaligenes species also live in Peyer’s patches and rely on the formation of these structures to induce Alcaligenes-specific IgA responses (33).

A key dichotomy began to emerge from these early studies, which was that compartmentalization was particularly important for commensal homeostasis. It is natural to consider the importance of the lymphotoxin receptor in compartment-mediated commensal homeostasis because the formation of lymphoid structure is essential for immunological compartmentalization. Recent work revealed the importance of compartmentalization in commensal tolerance, which is a function of both adaptive immunity and unique
antigen-presenting-cell subsets (34). Although IgA was a focus of this early work, the multiple roles of the lymphotoxin pathway in lymphoid-structure development, cell trafficking and as an effector cytokine suggested that IgA was just an initial window for the many roles lymphotoxin could play in regulating commensal bacteria.

**Metabolic disease and commensal bacteria**

In 2006, a landmark study demonstrated the existence of an altered microbial community that accompanies the obese state and that may actually contribute to metabolic disease (35). Specifically, a reduction of Bacteroidetes phyla with a corresponding increase in Firmicutes phyla and loss of overall commensal diversity occur in obese mice and were also demonstrated to occur in humans (36). More recently, a first of its kind metagenome-wide association study (MGWAS) demonstrated that type 2 diabetes has its own metagenomic characteristics; quite impressively, some of the features of the type 2 diabetes metagenome provide odds ratios for type 2 diabetes of 23.1, suggesting a significant role for commensal bacteria in this metabolic disease (37). However, as with many studies regarding the microbiota, it was still not known whether microbial changes associated with metabolic disease are causative of illness or simply an epiphenomenon.

Complementary to this recent MGWAS data, fecal transplantation from healthy donors to glucose-intolerant recipients was shown to improve parameters of glucose tolerance, which suggests that correlative findings of microbial dysbiosis and human disease may be causal in nature with regard to metabolic disease (38). However, the role of the microbiota in metabolic disease is not only restricted to obesity or type 2 diabetes. Along the same lines of thought, a recent study in humans and mice demonstrated that the microbiota metabolizes phosphatidyl choline into products that mediate cardiovascular disease (39); these metabolites are modified by the host and ultimately activate macrophages to mediate atherosclerotic plaque formation (39). This study on atherosclerosis is particularly powerful because investigators were able to establish key microbial correlations in humans and then definitively demonstrate causation in mice.

**Metabolic diseases epidemiologically linked to lymphotoxin**

The genes encoding LTα (LTA) although previously annotated as TNFB and LTβ (LTB) are located proximal to TNF (~1.3 kB away from TNF in the case of LTA) within the MHC (40). The first suggestion that lymphotoxin may be connected to metabolic disease was a study that revealed a polymorphism within the TNF–LTA gene locus that positively correlated with obesity in Pima Indians (Table 1); in this early study, ‘obese’ and ‘lean’ TNF alleles had identical sequences, and the authors suggested that polymorphism linking this locus to increased body size may be suggestive of a connection between obesity and the nearby lymphotoxin gene that may have actually been mutated (41).

Nearly a decade later, this earlier work was revisited in a Danish cohort where it was demonstrated that the T60N polymorphism of the LTA gene positively correlated with type 2 diabetes (Table 1) (42). Although the original report by Norman et al. (41) suggested that lymphotoxin may contribute to body size, closer examination of the study by Hamid et al. (42) revealed that the T60N polymorphism did not influence the body mass index; however, other surrogates of excess adiposity, including waist-to-hip ratio, were significantly increased in individuals with the N/N genotype compared with the control N/T or T/T genotypes (42).

Building upon these epidemiological studies, multiple groups chose to examine whether there was a direct role for the lymphotoxin pathway in metabolic disease. Among the first of such studies, it was demonstrated that animals over-expressing lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells (LIGHT; this binds LTβR) experienced exaggerated hyperlipidemia; mechanistically, it was demonstrated that over-expressing LIGHT in this manner antagonized hepatic-lipase expression within the liver through LTβR and resulted in dyslipidemia (4). However, there was not a significant impact on

<table>
<thead>
<tr>
<th>Polymorphism description</th>
<th>Disease observation</th>
<th>Patient population</th>
<th>Explanation and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFB.IR2/TNFB.IR4</td>
<td>Obesity</td>
<td>United States (Pima Indians)</td>
<td>Polymorphism associated with obesity. Sequencing revealed no difference in TNF alleles suggesting a closely linked gene (i.e. LTA) maybe different (41).</td>
</tr>
<tr>
<td>rs1041981</td>
<td>Type 2 diabetes</td>
<td>Denmark (no further specification)</td>
<td>Mutation results in a T60N mutation in LTα and increased incidence of type 2 diabetes.</td>
</tr>
<tr>
<td>rs2229094</td>
<td>Type 2 diabetes</td>
<td>India (North Indians)</td>
<td>Potential gain of function mutation (42). Mutation results in C13R variation in LTα and associates with decreased type 2 diabetes (40). Originally reported as linked to myocardial infarction, but later not confirmed. Later confirmation associated allele with increased incidence of type 2 diabetes (51, 52).</td>
</tr>
<tr>
<td>LTA intron1 252A→G</td>
<td>Myocardial infarction and type 2 diabetes</td>
<td>Japan (no further specification)</td>
<td></td>
</tr>
</tbody>
</table>

This table summarizes epidemiological studies linking single-nucleotide polymorphisms in the TNF–LTA gene locus and their relationship to metabolic disease. Similar studies have been carried out for other diseases, but only metabolic diseases are shown here. TNFB refers to an older annotation scheme and is the LTA gene (40–42, 51, 52).
hepatic-lipase expression or lipidemia between wild-type animals and animals lacking Ltbr (Libr−/−) that were not simultaneously over-expressing LIGHT on T cells, suggesting that this phenomenon occurred in the inflamed state, when T cells over-expressed LIGHT (4). A compound dependence of the lymphotoxin pathway in atherosclerotic disease was revealed when it was demonstrated that the lymphotoxin receptor is essential for the formation of tertiary lymphoid structures within atherosclerotic plaques (43).

In addition to hyperlipidemia and atherosclerosis, it has been more recently revealed that animals lacking the lymphotoxin pathway resist diet-induced obesity despite consuming similar amounts of feed (5, 6). Although T-cell infiltration into perigonadal adipose tissue appears altered in lymphotoxin-deficient animals, it does not correlate with weight gain in this model system (5).

**Lymphotoxin regulates weight gain through the commensal bacteria**

Given the important role the lymphotoxin pathway plays in mucosal defense and the lack of an ability to explain changes in weight gain apparent in lymphotoxin-deficient mice by hepatic-lipase expression or T-cell infiltration of adipose tissue, investigators considered the possibility that lymphotoxin regulated weight gain through a mechanism dependent on commensal bacteria (6). Analysis of fecal communities from Libr−/− and Libr−/− littermates revealed that feeding with a high-fat diet (HFD) resulted in a reduction of SFB and expansion of Erysipelotrichi class members in Libr−/− mice, which did not occur in Libr+/− mice. Of note, SFB has been linked to a Tγ17-mediated immune response, and Erysipelotrichi class members have been demonstrated to overgrow in response to HFD feeding in other systems (32, 44). Closer examination of Tγ17 cytokines revealed that IL-23 and IL-22 were influenced by HFD feeding through a mechanism dependent on lymphotoxin; restoration of these cytokines into Libr−/− mice restored the ability of these mice to regulate SFB and in the case of IL-22 correlated with increased body size (6).

These data complemented earlier work regarding lymphotoxin-dependent control of SFB to demonstrate that Tγ17 cytokines, which are inducible by SFB, actually have a function in controlling this organism in some settings. This may be through their regulation of anti-microbial-peptide production from intestinal epithelial cells. This is supported by the demonstration that animals lacking the anti-microbial peptide RegIIIγ (regenerating islet-derived protein IIIγ), which is also diminished in Libr−/− mice (6), actually experience a modest overgrowth of SFB (45). The contribution of anti-microbial peptides to weight gain revealed by lymphotoxin-deficient mice is not without precedent, as another report has revealed that mice deficient in the anti-microbial peptide RELMβ (resistin-like molecule β) resist weight gain and have altered microbial communities (46).

An important issue raised by these studies is whether active lymphotoxin signaling can contribute to weight gain through anti-microbial-peptide production or instead whether lymphotoxin contributes to the appropriate microenvironment to enable the function of other elements of immunity. Active lymphotoxin signaling may function to alter anti-microbial-peptide expression or even other elements of immunity because LTβR is expressed on intestinal epithelial cells where it plays an important role in host defense (18). However, given the recent work suggesting that lymphotoxin enables IL-22 production from type 3 ILCs (17, 19), it seems likely that lymphotoxin is serving the function of creating the appropriate microenvironment in which to enable the function of cells that agonize anti-microbial peptides through IL-22 production such as type 3 ILCs or even Tγ17 cells. This is still an open issue for exploration.

**The host–commensal paradigm integrates immunity, the microbiota and metabolism**

The multiple roles of the lymphotoxin receptor in metabolic disease and mucosal immunity have been part of a spectrum of revelations regarding the intersection of immunity and the microbiome with regard to health. Lymphotoxin is an example of an immune pathway that coordinates changes in the distal gut microbiota, which in turn contributes to host growth in response to HFD. The lymphotoxin pathway may help provide definition for the host–commensal paradigm as it relates to metabolic disease, at least in the case of weight gain. For example, altering the microbiota in response to HFD relies on lymphotoxin; expansion of some community members (i.e. Erysipelotrichi class members) occurs, whereas others diminish (i.e. SFB) through a mechanism dependent on lymphotoxin. However, some changes in microbial species composition occur independently of the lymphotoxin pathway, namely expansion of Firmicute family members (6). A model for these relationships is depicted in Fig. 1A.

However, we are beginning to appreciate that the intersection between immunity, microbiota and metabolism has many additional components, both immunological and microbiological, that are essential for health. Figure 1B demonstrates the great deal of work required to complete our current understanding with respect to the model presented in Fig. 1A.

Much work has already been initiated that may fill in these gaps. Pattern recognition receptors (PRRs) may be the link between HFD and induced immunity within the colon. Work implicating PRRs in metabolic disease has recently shed light on possible connections. Tlr5−/− mice, for example, experience a metabolic syndrome that is driven by a dysbiosis (47). Analogously, additional players that may provide key definition at this intersection have been identified, for example, by the demonstration that the inflammasome prevents the dysbiosis that exaggerates the effects of non-alcoholic steatohepatitis (a type of hepatitis that features fat accumulation) when animals are challenged with diets deficient in methionine or choline (48). Both of these phenotypes implicate a PRR or PRR-associated pathway that represses metabolic disease in mice, and neither PRR was implicated in HFD feeding. However, proteins in these families may serve analogous functions to promote metabolic disease in response to HFD feeding. Additional cytokines will likely fit into this model as well. Recently, II10−/− mice were demonstrated to experience exaggerated colitis when placed on HFD due to an overgrowth of Bilophila wadsworthia; this is a particularly intriguing intersection of immunity, metabolism and the microbiota because bile acids utilized to absorb milk fat in the diet support bacterial overgrowth and a Tγ17-driven colitis (49). The balance between colitis and nutritional uptake may unearth
additional components to show how the immune system regulates the microbiota to promote weight gain.

**Conclusion**

The role of the lymphotoxin receptor as a key host component for microbial regulation and metabolic disease is just a small part of an incredible paradigm shift that is ongoing in biology. It appears that health is the sum of manifold interspecies interactions in the composite host–commensal organism. The major impetus for this revolution is the increasing availability of gnotobiotic technology and the expedient interrogative efforts that can be undertaken for a complex microbial community with next-generation sequencing technology (50). Not surprisingly,
immunity is now actively being uncovered as a key component of the host–commensal paradigm, and the lymphotoxin receptor is just one among many additional pathways that will likely be revealed to be important for overall health as defined by both host and commensal micro-organism.

References


