Published in final edited form as:

Cytokine Growth Factor Rev. 2014 April ; 25(2): 227–233. doi:10.1016/j.cytogfr.2013.12.007.

Lymphotoxin organizes contributions to host defense and metabolic illness from innate lymphoid cells

Vaibhav Upadhyay and Yang-Xin Fu^{1,2}

¹Committee on Immunology, University of Chicago

²Department of Pathology, University of Chicago

Abstract

The lymphotoxin (LT)-pathway is a unique constituent branch of the Tumor Necrosis Superfamily (TNFSF). Use of LT is a critical mechanism by which fetal innate lymphoid cells regulate lymphoid organogenesis. Within recent years, adult innate lymphoid cells have been discovered to utilize this same pathway to regulate IL-22 and IL-23 production for host defense. Notably, genetic studies have linked polymorphisms in the genes encoding LT α to several phenotypes contributing to metabolic syndrome. The role of the LT-pathway may lay the foundation for a bridge between host immune response, microbiota, and metabolic syndrome. The contribution of the LT-pathway to innate lymphoid cell function and metabolic syndrome will be visited in this review.

Keywords

Lymphotoxin; Innate Lymphoid Cells; Host defense; IL-22; IL-23; NK development; Metabolic Syndrome; Microbiota

Introduction

Lymphotoxin α (LT α) and Lymphotoxin β (LT β) are Tumor Necrosis Factor Superfamily (TNFSF) members TNFSF1 and TNFSF3, and these two species form a heterotrimer with distinct molecular function to that of TNF. LT α and TNF are archetypical members of the TNFSF and obtained their distinct monikers by the observation that these molecules could induce death in fibrosarcoma cells and were secreted by lymphocytes and monocytes respectively [1,2]. Due to the structural homology between LT α and TNF [3,4], it was

© 2013 Elsevier Ltd. All rights reserved

Correspondence should be addressed to Yang-Xin Fu, Department of Pathology, University of Chicago, 924 E, 57th street, BSLC building R112, Chicago, IL 60637. Phone: 773-702-0929. Fax: 773-834-8940. yfu@uchicago.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author contributions

VU and YXF wrote the manuscript.

Competing financial interests and conflicts of interest.

The authors declare no competing financial interests or conflicts of interest.

originally unappreciated that there was a distinct signaling pathway directed completely by LT α [5-7]. Sequencing and discovery of the gene encoding LT β immediately next to LT α sparked speculation that there may be a unique biological pathway from TNF regulated by these cytokines [8]. This speculation came to fruition with the observation that *Lta*^{-/-} mice lacked lymphoid structure while *Tnf*^{-/-} mice did not share this defect; this observation opened the field up for exploration of the LT-specific pathway [9].

Shortly after this discovery, an LT β -receptor (LT β R, TNFRSF3) was identified and the absence of this receptor rendered animals devoid of secondary lymphoid structure, similar to LT α deficient mice [6,7]. In this way, diligent efforts spanning over years extended the field of LT-biology from the study of a redundant cytokine to the appreciation of a new molecular pathway. Recently it has become appreciated that the LT-pathway contributes to host defense by the regulation of the function of innate effector lymphocyte populations and that this may be a link between the LT pathway and metabolic syndrome. These recent developments as well as a brief history of other key moments in LT-biology will be visited in this review.

The LT ligand-receptor: A bridge between lymphocytes and non-lymphocytes

LT α and LT β form a membrane bound heterotrimer with a 1:2 stoichiometric ratio, LT $\alpha_1\beta_2$, which binds to LT β R [10,11]. LT β R signaling results in translocation of the non-canonical p52-RelB complex into the nucleus; notably, canonical NF κ B activation via the p50-RelA complex can also be initiated by LT β R [12,13]. LT $\alpha_1\beta_2$ is expressed by cells of the lymphocyte lineage including B cells, T cells, lymphoid tissue inducer cells (LTi cells), and closely related adult innate lymphoid cells that express the transcription factor ROR γ t [14-18]. LT β R, on the other hand, is expressed by cells of the myeloid lineage, including macrophages and dendritic cells (DCs); LT β R is also expressed by non-hematopoietic cells including hepatocytes, intestinal epithelial cells, follicular dendritic cells, high endothelial venules, and many others [19-23]. In this way, the LT-signaling axis forms a unique bridge between lymphoid and myeloid populations and also lymphoid and stromal cell populations. Recently, it has come to light that CD11c+ cells, presumably DCs, may exploit LT $\alpha_1\beta_2$ expression in order to remodel the lymph node and maintain adequate cellularity in the immunological steady state [24]. This recent observation may engender future work establishing LT-ligand expression on non-lymphoid cell types (for further discussion see [25]).

Although LT $\alpha_1\beta_2$ is only known to bind LT β R, LT β R has another ligand called lymphotoxin-like inducible protein that competes with glycoprotein D for herpesvirus entry on T cells (LIGHT, TNFSF14) [13]. LIGHT is expressed on activated T cells, DCs, and has even been reported to be expressed on some granulocytes and monocytes (see Figure 1) [5,26-28]. The relationship between LT $\alpha_1\beta_2$ and LT β R is relatively straightforward because of their discrete expression patterns and relative fidelity in binding, however LIGHT adds additional nuances to this biological pathway by interacting with both LT β R and another binding partner known as Herpes Virus Entry Mediator (HVEM, TNFRSF14). HVEM is particularly enigmatic because similar to LIGHT, HVEM can be expressed on T cells, and similar to LT β R, HVEM has been described on a variety of non-hematopoietic cells

including intestinal epithelium and hepatocytes [29-31]. There are additional signaling partners for both LIGHT and HVEM, but for simplicity's sake, those partners and pathways will be left out of this discussion where we will focus primarily on the LT-pathway and its immediate signaling partners.

Lymph nodes and other lymphoid structures

The original observation that LT α contributed to lymph node development not only suggested the existence of biology directed by LT, but it also enabled the study of the secondary lymphoid structure development [9,32]. In a landmark study, Cupedo et al. demonstrated that CD45⁺ lymphocytes which expressed LT α induced signaling via LT β R on CD45⁻ stromal organizer cells to initiate lymphoid structure formation [33]. Further work revealed such CD45⁺ lymphocytes to express the CD4 coreceptor and the transcription factor ROR γ t; in the fetal stage these lymphocytes are known as LTi cells [33-35]. During embryonic development, retinoic acid is produced by neurons to recruit LTi cells to the location of future lymph nodes [36]. Once LTi cells have arrived, they express membrane bound LT α ₁ β ₂, which binds LT β R on stromal cells initiating transcriptional changes and chemokine recruitment to ultimately form a lymph node [33]. A model for this function is depicted in Figure 1A.

The LT β R pathway is also essential for the formation of Peyer's patches and colonic patches within the gastrointestinal tract [35]. These are developmentally fixed structures and share similar features to lymph nodes with the exception of their location and slightly altered cellular makeup [35]. In this same body site, LT β R is also essential for the formation of isolated lymphoid follicles (ILFs), which have been dubbed a dynamic lymphoid structure whose development occurs after birth and is unique from that of Peyer's patches or colonic patches [37]. Unlike lymph nodes, B cells must also express LT α to induce ILF expansion and size [38]. B-cells are essential in functionally transforming primitive cryptopatches into ILFs [39]. The lack of LT α on B cells results in atrophic follicles [38].

Interestingly, the major stimulus for ILF formation appears to be the microbiota which is distinct from the requirement of retinoic acid for lymph node formation (see Figure 1A) [35,40]; this notion is supported by the observation that germ free mice lack ILFs which can be rescued by microbial colonization [38,41]. LTi cells appear to play a key role in chemokine recruitment of B cells for ILF formation and are not completely dispensable for the formation of this structure, suggesting a dual role for the LT-pathway in ILF formation [42]. However, it also appears that lymphoid structures closely resembling ILFs can form within the colon of mice lacking LTi cells when the colon is exposed to a noxious stimulus; in this case, B cells can overcome the lack of LTi cells by stimulating the LT β R pathway [43]. A recent study revealed that expression of TNF may be sufficient to induce lymph node in the absence of LTi cells, but the contribution of TNF to this process in wild-type hosts appears to be less influential than that of the LT-pathway [44]. As the cellular players in ILF formation or tertiary lymphoid tissue neogenesis continue to be explored, the LT β R pathway continues to be an important experimental tool and cornerstone in the development of these and other lymphoid structures.

From the fort to the battlefield: ILC3s bridge the gap between lymphotoxin and host defense

In the past several years, there has been a great effort in understanding the developmental control and functional fate of innate lymphoid cells that express the transcription factor ROR γ t. One class of such cell types, dubbed type 3 innate lymphoid cells (ILC3s), appears to uniquely utilize the LT-pathway to initiate host defense and even contribute to abnormal pathology. Fetal LTi cells and ILC3s are phenotypically similar and their precise developmental relationship is still unfolding. It is clear that LTi cells utilize the LT-pathway during development to initiate the site of organized immune defense, and it appears that ILC3s utilize the LT-pathway in the adult animal as a mechanism of active host defense (Figure 1) [33,45,46].

As discussed above, the contribution of ROR γ t+ cells to lymphoid development was determined by generating animals that lacked this transcription factor and the demonstration that such animals phenocopied many defects in lymphoid structure present in LT-deficient mice [34,37,47]. Quite separately, ROR γ t was later found to play a key role in autoimmunity by the regulation of T_H17 T cell development. This observation occurred in part by resolving an inappropriate assumption that IL-12 signaling contributed to autoimmunity, when in fact IL-23, which shares a heterodimeric subunit with IL-12, contributes to autoimmunity by regulating the function of T_H17 cells [48,49]. As study of T_H17 cells and associated cytokines continued, it was enticing to discover that CD4⁺CD3⁻ innate lymphoid cells that express the transcription factor ROR γ t also contribute to tissue immunity in response to IL-23 in fully developed hosts [50]. Such cells are currently known as ILC3s [45]. Although there has been great debate as to the origin of these lymphocytes, consensus is beginning to be reached that this cell population may be functional analogues to LTi cells, but whether these cells differentiate from their fetal counterparts (currently thought to be LTi cells) or arise as ILC3s from a distinct progenitor is currently unknown [51-53].

Although LT does not contribute to the development of such lymphocytes [54], ILC3s execute critical effector functions, including IL-22 production, by exploiting the LT-pathway [17,55,56]. This is not completely surprising given the phenotypic similarities between LTi cells and ILC3s, and the additional consideration that LTi cells also exploit the LT β R pathway to stimulate lymphoid organogenesis [42]. An interesting biological redundancy can be noted here: LTi cells utilize the LT β R pathway to regulate the development of lymphoid structures within the fetus, while ILC3s, which closely resemble LTi cells, utilize the same pathway for host defense in the adult host.

C. rodentium is a particularly illustrative mouse pathogen because it relies on both innate and adaptive immunity for clearance, including IL-22 production from ILC3s. IL-22 was originally identified as a member of the IL-10 family whose receptor was expressed by epithelial cells; IL-22 was originally considered to be uniquely expressed by T_H17 cells [57,58]. An early study by Zheng et al. revealed that the protective effect of IL-22 in response to *C. rodentium* came from an innate cell-type and resulted in the production of antimicrobial peptides [59]. This study coincided with the observation by Cella et al. that a

group of innate lymphocytes that expressed NKp46 could produce IL-22 in response to IL-23 stimulation [46]. These cells were found express the transcription factor ROR γ t and were essential in host defense against *C. rodentium* (see Figure 2) [60].

The LT-pathway was also appreciated to play a critical host role in host defense in *C. rodentium*. The role of the lymphotoxin pathway in *C. rodentium* infection was originally attributed to the capacity of lymphotoxin to regulate the T_H1/T_H2 helper T cell balance [61]. Revisiting this earlier data, Wang et al. speculated that the deaths occurring in *Ltbr*^{-/-} and *Lta*^{-/-} mice in response to *C. rodentium* were unlikely to be related to helper T cell balance because *Ltbr*^{-/-} animals start to get sick on as early as 7 days and some succumb to *C. rodentium* infection 10 days after *C. rodentium* challenge; this is a very early time point for the host to mount a classical, adaptive immune response to the pathogen [21]. Such early onset of disease suggested an innate role for the LT-pathway in this mucosal infection model.

Further work demonstrated that LT β R signaling was essential in innate immune defense by the demonstration that blockade via administration of the decoy receptor LT β R-Ig rendered *Rag1*^{-/-} animals more susceptible to *C. rodentium* challenge [21]. A requirement for LT β R was observed on macrophages, dendritic cells, and intestinal epithelial cells for defense against this intestinal pathogen [21]. However, the role which LT β R played in host defense against *C. rodentium* was thought to be via recruitment of neutrophils by inducing intestinal epithelial cells to express CXCL1 and CXCL2 thereby reducing the *C. rodentium* burden (Figure 1B) [21]. Although neutrophils play some role in this process, the depletion of neutrophils does not recapitulate the profound susceptibility to *C. rodentium* seen in LT-deficient mice.

Building upon this earlier work, two separate studies demonstrated that LT β R was a mechanism by which ILC3s initiated IL-22 dependent host defense in response to *C. rodentium*. *Ltbr*^{-/-} animals do not produce IL-22 in response to challenge with *C. rodentium* [17,56]. Furthermore, the IL-22 pathway can be rescued in LT-deficient animals by treatment with an agonist antibody and be blocked by treatment of wild-type animals with the LT β R-Ig decoy receptor demonstrating an exquisite regulation of IL-22 via the LT β R [17,56]. The role of LT β R in this IL-22 regulation is via control of IL-23 production by DCs that are immediately proximal to ILC3s within ILFs [17,56].

A current model for the regulation of this protection pathway (see Figure 1) is that DCs sense MyD88 dependent signals during colonic infection, which results in their secretion of IL-23 (Figure 1B) [17,62]. It also appears that MyD88 dependent signals on ROR γ t+ cells themselves can shape their ability to produce IL-22, but this is still not well understood [63]. It is clear that IL-23 produced by DCs is recognized by IL-23R on adult LT_i cells, which then produce IL-22 [46]. IL-22 acts on intestinal epithelial cells, which respond to IL-22R signaling by up regulating proteins that prevent apoptosis and antimicrobial peptides that have a direct killing function on members of the microbiota (Figure 1B) [64]. Clearly, the lymphotoxin pathway is essential for key events along the IL-22 activation pathway. LT β R is key for IL-23 production from DCs in response to pathogenic challenge [17,56]. Furthermore, it has been suggested that LT β R is essential for the formation of innate

immunological synapses between LT α cells and DCs. The close proximity of IL-23 producing DCs directly in contact with IL-23R expressing adult LT α cells within ILFs supports the notion that such synapses may exist; the importance of this colocalization is also apparent in other body sites including joints as described by Sherlock et al. [17,65]. Within the colon, localization occurs in ILFs, which are also dependent on the LT β R pathway for development. Therefore the LT-pathway plays an additional role in architecture formation because the localization of ILC3s near DCs is thought to be essential for the activation of the IL-22 protection pathway in response to mucosal pathogen.

Several recent studies have suggested new avenues for exploration along this theme as it is now becoming appreciated that ILC3s also express the Aryl Hydrocarbon Receptor (AHR), a ligand inducible transcription factor that contributes both to ILC3 function and lymphoid organogenesis (Figure 1B) [66,67]. Of note *Ahr*^{-/-} mice are susceptible to *C. rodentium* infection due to their diminished production of IL-22 and also lack ILFs within the colon. The lack of ILFs is apparently due to the inability of ILC3s to expand in the absence of this transcription factor. Notably, *Ahr*^{-/-} mice also fail to control segmented filamentous bacteria, which also overgrows in *Ltbr*^{-/-} mice [67,68]. Although it is unlikely that AHR is directly controlling the LT-pathway, the intersection of this newly appreciated transcription factor and a key effector cytokine for ILC3s will help tease apart different roles of ILC3s in immunological development and effector function (Figure 1).

The LT-pathway is linked to metabolic syndrome

Current approximations are that 37.4% of US adults were obese in 2010, with estimates as high as 39.2% [69]. Overweight and obese states independently increase the risk of cardiovascular sequelae and death from cardiovascular disease (CVD) in both men and women, and the increased risk of death from CVD is independent of other known obesity associated pathologies, such as diabetes [70-72]. The clinical constellation of central obesity, glucose intolerance, hyperlipidemia either from elevated LDL or elevated triglycerides, and hypertension formulate metabolic syndrome, whose prevalence estimates are not far off those of the obesity epidemic itself and constitute a major disease burden in the US [73]. Somewhat unexpectedly, the LT-pathway has been linked to three constituents of metabolic syndrome – body size, glucose intolerance, and hyperlipidemia – and may have a multi-modal effect in the initiation of this disease (Figure 2).

Multiple genetic studies have demonstrated links between polymorphisms in the *LTA* gene and either body size, glucose tolerance, and atherosclerosis. Some of this work was initiated by early studies which revealed that adipose tissue was not an inert storage depot of excess nutrition, but that adipocytes secreted protein products which could influence the host; TNF was one such secreted protein product of adipocytes and of great interest due to its inflammatory properties [74,75]. Because obesity is characterized by excess adipose tissue and TNF is secreted from adipocytes, Norman and colleagues performed an association study between this gene locus and obesity with the hypothesis that TNF was an obesity-inducing gene. Norman et al. looked for changes in single nucleotide polymorphisms within the *Tnf-Lta* gene locus in a genetically homogenous population characterized by high rates of obesity – the Pima Indians [76]. However, sequencing of the TNF gene in Pima obese and

lean subjects did not reveal any differences between the obese and lean TNF alleles linked to TNF α 24.

As a result, Norman and colleagues concluded that while a polymorphism was found to link this genetic region to obesity, this polymorphism could actually be informative of the link between obesity and another gene product from this locus. Hamid and colleagues revisited the associations found in the TNF gene locus with the hypothesis that there may be alterations in the nearby *Lta* gene that correlated with the incidence of type II diabetes [77]. In this study, it was discovered that the T60N mutation in LT α , which results in a threonine to arginine substitution, significantly correlated with increased incidence of type II diabetes in a Danish cohort of patients. Hamid et al. reported that the odds ratio for type II diabetes incidence in N/N homozygous individuals was 1.24 compared to control subjects [77]. Equally important, Mahajan et al. reported that a different variant of the *LTA* gene which results in a C13R substitution, a potentially destabilizing mutation, actually correlated with decreased incidence of diabetes in a population of North Indian individuals [78]. This study is particularly powerful in light of the study by Hamid et al. as it illustrates that there are both risk and protective variants of the *LTA* gene that contribute to the same human illness.

With regard to metabolic syndrome, polymorphisms in the *LTA* gene have also been linked to increased incidence of atherosclerosis induced myocardial infarction but follow up studies reveal that this association may actually be between *LTA* and type II diabetes as opposed to atherosclerosis [79,80]. Integrating all of the data, it appears that there are multiple aspects of metabolic syndrome to which the LT-pathway may contribute. For a more detailed discussion see ref [81].

Integrating LIGHT and ILC3s may reveal multiple contributions for LT β R in metabolic syndrome

The above-discussed epidemiological evidence regarding an association between the LT-pathway and metabolic illness has actually been built upon by several mechanistic studies in rodents and results in an integrative model that may connect immunity to metabolic syndrome [22,68,82-84]. One such study stimulated by the potential link between the LT-pathway and atherosclerosis revealed that transgenic overexpression of LIGHT on T cells results in hyperlipidemia in mice [22]. Mice lacking HVEM continued to be susceptible to LIGHT induced dyslipidemia, while mice lacking LT β R were no longer sensitive to transgene overexpression [22]. By influencing lipid levels, the LT β R pathway could thus influence the generation of atherosclerotic plaques, and in this way this study also established the link between an elevated basal inflammatory response and atherogenesis (Figure 2A-B).

Atherosclerotic lesions are areas of intense inflammation, and building upon this data regarding dyslipidemia in LIGHT-transgenic mice, it was also appreciated that the LT-pathway contributed to the inflammatory remodeling of atherosclerotic lesions in *Apoe*^{-/-} mice [82]. Specifically, medial smooth muscle cells become activated to express the chemokines CXCL13 and CCL21 through LT β R agonism [82]. Not unlike what occurs within lymphoid tissues where the LT-pathway serves a similar purpose, expression of these

chemokines resulted in the formation of tertiary lymphoid structure within the plaques themselves (Figure 2A-B) [82].

Reexamining the connections between the LT-pathway in glucose homeostasis and obesity, two later studies revealed LT-deficient mice resist weight gain despite similar feed consumption (Figure 2C) [68,83]. In two separate studies, it was determined that *Lta*^{-/-} mice were resistant to diet induced obesity while mice lacking the TNF-pathway were susceptible to weight gain as wild type mice [83,84]. Deficiency in the LT-pathway resulted in greater infiltration of inflammatory cells into adipose tissue [83]. Contrary to the widely held role of inflammation and obesity, *Lta*^{-/-} mice were less glucose intolerant despite having a greater number of inflammatory cells within their adipose tissue (Figure 2C) [83]. However, the magnitude of inflammatory infiltrate did not correlate completely with body size, suggesting that the LT-pathways role in weight gain was through another mechanism.

Simultaneous work revealed that mice lacking the LT-pathway resisted weight gain through a mechanism dependent on commensal microorganisms [68]. The commensal microbiota contributes to host growth and has been implicated in obesity [85,86]. In response to high fat diet feeding, wild-type mice repress the growth of SFB, foster the growth of Erysipelotrichi class members, and experience overall reductions in commensal diversity; mice lacking the LT-pathway are unable to undergo these similar changes in their commensal microbiota [68]. Furthermore, cecal transplantation confers differences in weight gain between *Ltbr*^{+/-} and *Ltbr*^{-/-} mice to germ free recipients, demonstrating the contribution of these changes to the microbiota in weight gain [68]. Housing normalized the microbial communities of *Ltbr*^{-/-} animals and restored weight gain [68]. Similar to the work on *C. rodentium* it appears as though RORγ⁺ cells, IL-23, and IL-22 also contribute to LT-mediated weight gain, implicating ILC3s as potential contributors to weight gain and metabolic syndrome (Figure 2C) [68]. Restoring downstream elements of this ILC3 controlled pathway into *Ltbr*^{-/-} mice rescued SFB clearance and weight gain, demonstrating the importance of mucosal immunity as participating in normal diet induced changes to the microbiota that enable obesity [68].

The role of microbial dysbiosis and metabolic disease is quite complex and has been linked to other immune pathways. This notion that a microbial dysbiosis could drive metabolic illness is driven in large part by the discovery of the obesity-associated microbiome, which demonstrated microbial changes correlated with the obese state and could contribute to obesity [85,87]. Turnbaugh et al. demonstrated that the composition of the microbial communities of the distal gut vary between lean and obese hosts [85,88]; furthermore this study demonstrates that transplantation of the microbiota associated with obesity is sufficient to confer greater weight gain than that of the lean microbiota [85]. This study built upon the findings of Ley et al. and demonstrate that the obese microbiome was characterized by a less diverse community and had a greater proportion of Firmicute phyla members [85,89]. It was later validated that these changes occurred in wild-type mice in response to high calorie diets, that a subgroup of bacteria from the Erysipelotrichi class expanded in response to such diets [90]. Given the role of the microbiota in adapting to the obese state in a way that contributes to disease, elucidation of the factors that contribute to alterations in the microbiota will provide insight into both obesity and metabolic illness.

It is intriguing to note that the LT-pathway is linked to various components of metabolic disease, and that the deficiency of LT β R results in an overgrowth of SFB and a lack of overgrowth of Erysipelotrichi class members who have been correlated to the obese state. It is currently speculated that the LT-pathway contributes to microbial community composition through the regulation of antimicrobial peptides. It is intriguing to note that mice lacking RELM β resist weight gain and have altered microbiota [91], however whether the LT-pathway regulates RELM β is unappreciated. RELM β appears to regulate the expression of RegIII β and RegIII γ [92], which are also regulated by the LT-pathway and may explain the lack of growth seen in mice lacking RELM β . Given the role of the microbiota in adapting to the obese state in a way that contributes to disease, elucidation of the immune pathways and other factors that contribute to alterations in the microbiota will provide insight into the pathogenesis of metabolic diseases including obesity and metabolic syndrome.

Conclusions & Future Directions

The biology of the LT-pathway has developed greatly over the past 3 decades. It appears that this pathway is essential for LT α i cells to initiate the development of organized lymphoid structure, and the LT-pathway then becomes a key effector pathway for closely related ILC3s. Given the multiple roles of the LT-pathway underlying the pathology of metabolic syndrome, it will be exciting to see how manipulation of this pathway will both unearth new mechanisms behind metabolic syndrome and be a potential therapeutic target.

Biographies

Vaibhav Upadhyay is an MSTP student at the University of Chicago Pritzker School of Medicine. He has pursued his PhD work in the laboratory of Dr. Yang-Xin Fu where he studied host-microbial interactions in weight gain through the lymphotoxin pathway. He completed his PhD training in early 2013.

Yang-Xin Fu is a professor of Pathology and Immunology at the University of Chicago. He completed his MD at Shanghai Medical University in 1983. He completed his PhD studies at the University of Miami in 1990 and then pursued a pathology resident training in Washington University Medical school and also additional research training in the laboratory of David Chaplin in 1994. He started his independent laboratory in the University of Chicago since 1998 and become full professor in 2005. He studies the cellular and molecular mechanisms of lymphotoxin biology, diseases and its potential

immunotherapeutic applications. He has published more than 100 papers, mostly related to TNF superfamily, lymphotoxin and LIGHT in particular.

References

1. Kelker HC, Oppenheim JD, Stone-Wolff D, Henriksen-DeStefano D, Aggarwal BB, Stevenson HC, et al. Characterization of human tumor necrosis factor produced by peripheral blood monocytes and its separation from lymphotoxin. *Int J Cancer*. 1985; 36:69–73. [PubMed: 3874835]
2. Ruddle NH. Tumor necrosis factor (TNF- α) and lymphotoxin. *Current Opinion in Immunology*. 1992; 4:327–32. [PubMed: 1329840]
3. Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, et al. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature*. 1984; 312:724–9. [PubMed: 6392892]
4. Eck MJ, Ultsch M, Rinderknecht E, de Vos AM, Sprang SR. The structure of human lymphotoxin (tumor necrosis factor-beta) at 1.9-Å resolution. *J Biol Chem*. 1992; 267:2119–22. [PubMed: 1733919]
5. Gommerman JL, Browning JL. Lymphotoxin/LIGHT, lymphoid microenvironments and autoimmune disease. *Nature Reviews Immunology*. 2003; 3:642–55.
6. Crowe PD, VanArsdale TL, Walter BN, Ware CF, Hession C, Ehrenfels B, et al. A lymphotoxin-beta-specific receptor. *Science*. 1994; 264:707–10. [PubMed: 8171323]
7. Fütterer A, Mink K, Luz A, Kosco-Vilbois MH, Pfeffer K. The Lymphotoxin β Receptor Controls Organogenesis and Affinity Maturation in Peripheral Lymphoid Tissues. *Immunity*. 1998; 9:59–70. [PubMed: 9697836]
8. Browning JL, Ngam-ek A, Lawton P, DeMarinis J, Tizard R, Chow EP, et al. Lymphotoxin beta, a novel member of the TNF family that forms a heteromeric complex with lymphotoxin on the cell surface. *Cell*. 1993; 72:847–56. [PubMed: 7916655]
9. De Togni P, Goellner J, Ruddle NH, Streeter PR, Fick A, Mariathasan S, et al. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. *Science*. 1994; 264:703–7. [PubMed: 8171322]
10. Fu Y-X, Chaplin DD. Development and Maturation of Secondary Lymphoid Tissues. *Annu Rev Immunol*. 1999; 17:399–433. [PubMed: 10358764]
11. Ware CF, VanArsdale TL, Crowe PD, Browning JL. The ligands and receptors of the lymphotoxin system. *Curr Top Microbiol Immunol*. 1995; 198:175–218. [PubMed: 7774281]
12. Dejardin E, Droin NM, Delhase M, Haas E, Cao Y, Makris C, et al. The Lymphotoxin- β Receptor Induces Different Patterns of Gene Expression via Two NF- κ B Pathways. *Immunity*. 2002; 17:525–35. [PubMed: 12387745]
13. Schneider K, Potter KG, Ware CF. Lymphotoxin and LIGHT signaling pathways and target genes. *Immunological Reviews*. 2004; 202:49–66. [PubMed: 15546385]
14. Ohshima Y, Yang LP, Avicé MN. Naive human CD4⁺ T cells are a major source of lymphotoxin α . *The Journal of ...* 1999
15. Cuff CA, Lesslauer W, Ruddle NH. Differential activities of secreted lymphotoxin- α 3 and membrane lymphotoxin- α 1 β 2 in lymphotoxin-induced inflammation: critical role of TNF receptor 1 signaling. *The Journal of ...* 1998
16. Steffen M, Ottmann OG, Moore MA. Simultaneous production of tumor necrosis factor-alpha and lymphotoxin by normal T cells after induction with IL-2 and anti-T3. *The Journal of Immunology*. 1988
17. Tumanov AV, Koroleva EP, Guo X, Wang Y, Kruglov A, Nedospasov S, et al. Lymphotoxin Controls the IL-22 Protection Pathway in Gut Innate Lymphoid Cells during Mucosal Pathogen Challenge. *Cell Host and Microbe*. 2011; 10:44–53. [PubMed: 21767811]
18. Yoshida H, Naito A, Inoue J-I, Satoh M, Santee-Cooper SM, Ware CF, et al. Different Cytokines Induce Surface Lymphotoxin- α β on IL-7 Receptor- α Cells that Differentially Engender Lymph Nodes and Peyer's Patches. *Immunity*. 2002; 17:823–33. [PubMed: 12479827]

19. Browning JL, French LE. Visualization of lymphotoxin- β and lymphotoxin- β receptor expression in mouse embryos. *The Journal of Immunology*. 2002
20. Murphy M, Walter BN, Pike-Nobile L, Fanger NA, Guyre PM, Browning JL, et al. Expression of the lymphotoxin beta receptor on follicular stromal cells in human lymphoid tissues. *Cell Death Differ*. 1998; 5:497–505. [PubMed: 10200501]
21. Wang Y, Koroleva EP, Kruglov AA, Kuprash DV, Nedospasov SA, Fu Y-X, et al. Lymphotoxin Beta Receptor Signaling in Intestinal Epithelial Cells Orchestrates Innate Immune Responses against Mucosal Bacterial Infection. *Immunity*. 2010; 32:403–13. [PubMed: 20226692]
22. Lo JC, Wang Y, Tumanov AV, Bamji M, Yao Z, Reardon CA, et al. Lymphotoxin β Receptor-Dependent Control of Lipid Homeostasis. *Science*. 2007; 316:285–8. [PubMed: 17431181]
23. Girard J-P, Moussion C, Förster R. HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. *Nature Reviews Immunology*. 2012; 12:762–73.
24. Moussion C, Girard J-P. Dendritic cells control lymphocyte entry to lymph nodes through high endothelial venules. *Nature*. 2011; 479:542–6. [PubMed: 22080953]
25. Upadhyay V, Fu Y-X. Lymphotoxin signalling in immune homeostasis and the control of microorganisms. *Nature Reviews Immunology*. 2013; 13:270–9.
26. Zhai Y, Guo R, Hsu TL, Yu GL, Ni J, Kwon BS, et al. LIGHT, a novel ligand for lymphotoxin beta receptor and TR2/HVEM induces apoptosis and suppresses in vivo tumor formation via gene transfer. *J Clin Invest*. 1998; 102:1142–51. [PubMed: 9739048]
27. Kabashima K, Banks TA, Ansel KM, Lu TT, Ware CF, Cyster JG. Intrinsic lymphotoxin-beta receptor requirement for homeostasis of lymphoid tissue dendritic cells. *Immunity*. 2005; 22:439–50. [PubMed: 15845449]
28. Phan TG, Green JA, Gray EE, Xu Y, Cyster JG. Immune complex relay by subcapsular sinus macrophages and noncognate B cells drives antibody affinity maturation. *Nature Immunology*. 2009; 10:786–93. [PubMed: 19503106]
29. Shui J-W, Larange A, Kim G, Vela JL, Zahner S, Cheroute H, et al. HVEM signalling at mucosal barriers provides host defence against pathogenic bacteria. *Nature*. 2012; 488:222–5. [PubMed: 22801499]
30. Murphy KM, Nelson CA, Šedý JR. Balancing co-stimulation and inhibition with BTLA and HVEM. *Nature Reviews Immunology*. 2006
31. Granger SW, Rickert S. LIGHT-HVEM signaling and the regulation of T cell-mediated immunity. *Cytokine Growth Factor Rev*. 2003; 14:289–96. [PubMed: 12787566]
32. Alimzhanov MB, Kuprash DV, Kosco-Vilbois MH, Luz A, Turetskaya RL, Tarakhovsky A, et al. Abnormal development of secondary lymphoid tissues in lymphotoxin β -deficient mice. *Proceedings of the* 1997:1–6.
33. Cupedo T, Jansen W, Kraal G, Mebius RE. Induction of secondary and tertiary lymphoid structures in the skin. *Immunity*. 2004; 21:655–67. [PubMed: 15539152]
34. Eberl G, Marmon S, Sunshine M-J, Rennert PD, Choi Y, Littman DR. An essential function for the nuclear receptor ROR γ t in the generation of fetal lymphoid tissue inducer cells. *Nature Immunology*. 2003; 5:64–73. [PubMed: 14691482]
35. van de Pavert SA, Mebius RE. New insights into the development of lymphoid tissues. *Nature Reviews Immunology*. 2010; 10:664–74.
36. Pavert SA, Olivier BJ, Goverse G, Vondenhoff M. Chemokine CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation. 2009
37. Eberl G, Lochner M. The development of intestinal lymphoid tissues at the interface of self and microbiota. *Mucosal Immunology*. 2009; 2:478–85. [PubMed: 19741595]
38. Lorenz RG, Chaplin DD, McDonald KG, McDonough JS, Newberry RD. Isolated lymphoid follicle formation is inducible and dependent upon lymphotoxin-sufficient B lymphocytes, lymphotoxin β receptor, and TNF receptor I function. *J Immunol*. 2003; 170:5475–82. [PubMed: 12759424]
39. Golovkina TV, Shlomchik M, Hannum L, Chervonsky A. Organogenic Role of B Lymphocytes in Mucosal Immunity. *Science*. 1999
40. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews Immunology*. 2009; 9:313–23.

41. Hapfelmeier S, Lawson MAE, Slack E, Kirundi JK, Stoel M, Heikenwalder M, et al. Reversible Microbial Colonization of Germ-Free Mice Reveals the Dynamics of IgA Immune Responses. *Science*. 2010; 328:1705–9. [PubMed: 20576892]
42. Tsuji M, Suzuki K, Kitamura H, Maruya M, Kinoshita K, Ivanov II, et al. Requirement for Lymphoid Tissue-Inducer Cells in Isolated Follicle Formation and T Cell-Independent Immunoglobulin A Generation in the Gut. *Immunity*. 2008; 29:261–71. [PubMed: 18656387]
43. Lochner M, Ohnmacht C, Presley L, Bruhns P, Si-Tahar M, Sawa S, et al. Microbiota-induced tertiary lymphoid tissues aggravate inflammatory disease in the absence of ROR γ t and LT α cells. *Journal of Experimental Medicine*. 2011; 208:125–34. [PubMed: 21173107]
44. Furtado GC, Pacer ME, Bongers G, Zech CBENE, He Z, Chen L, et al. TNF α -dependent development of lymphoid tissue in the absence of ROR γ t lymphoid tissue inducer cells. *Mucosal Immunology*. 2013:1–13.
45. Sonnenberg GF, Artis D. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. *Immunity*. 2012; 37:601–10. [PubMed: 23084357]
46. Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JKM, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature*. 2008; 457:722–5. [PubMed: 18978771]
47. Eberl G, Littman DR. Thymic origin of intestinal $\alpha\beta$ T cells revealed by fate mapping of ROR γ t+ cells. *Science Signaling*. 2004; 305:248–51.
48. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med*. 2005; 201:233–40. [PubMed: 15657292]
49. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The Orphan Nuclear Receptor ROR γ t Directs the Differentiation Program of Proinflammatory IL-17+ T Helper Cells. *Cell*. 2006; 126:1121–33. [PubMed: 16990136]
50. Takatori H, Kanno Y, Watford WT, Tato CM, Weiss G, Ivanov II, et al. Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. *Journal of Experimental Medicine*. 2009; 206:35–41. [PubMed: 19114665]
51. Sawa S, Cherrier M, Lochner M, Satoh-Takayama N, Fehling HJ, Langa F, et al. Lineage relationship analysis of ROR γ t+ Innate Lymphoid Cells. *Science*. 2010; 330:665–9. [PubMed: 20929731]
52. Cherrier M, Sawa S, Eberl G. Notch, Id2, and ROR γ t sequentially orchestrate the fetal development of lymphoid tissue inducer cells. *Journal of Experimental Medicine*. 2012; 209:729–40. [PubMed: 22430492]
53. Vonarbourg C, Mortha A, Bui VL, Hernandez PP, Kiss EA, Hoyler T, et al. Regulated Expression of Nuclear Receptor ROR γ t Confers Distinct Functional Fates to NK Cell Receptor-Expressing ROR γ t+ Innate Lymphocytes. *Immunity*. 2010; 33:736–51. [PubMed: 21093318]
54. Satoh-Takayama N, Lesjean-Pottier S, Sawa S, Voshchenrich CAJ, Eberl G, Di Santo JP. Lymphotoxin- β receptor-independent development of intestinal IL-22-producing NKp46+ innate lymphoid cells. *Eur J Immunol*. 2011; 41:780–6. [PubMed: 21341264]
55. Tumanov AV, Grivennikov SI, Shakhov AN, Rybtsov SA, Koroleva EP, Takeda J, et al. Dissecting the role of lymphotoxin in lymphoid organs by conditional targeting. *Immunological Reviews*. 2003; 195:106–16. [PubMed: 12969314]
56. Ota N, Wong K, Valdez PA, Zheng Y, Crellin NK, Diehl L, et al. IL-22 bridges the lymphotoxin pathway with the maintenance of colonic lymphoid structures during infection with *Citrobacter rodentium*. *Nature Immunology*. 2011; 12:941–8. [PubMed: 21874025]
57. Liang SC, Tan X-Y, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med*. 2006; 203:2271–9. [PubMed: 16982811]
58. Dumoutier L, Louahed J, Renauld JC. Cloning and characterization of IL-10-related T cell-derived inducible factor (IL-TIF), a novel cytokine structurally related to IL-10 and inducible by IL-9. *J Immunol*. 2000; 164:1814–9. [PubMed: 10657629]

59. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat Med*. 2008; 14:282–9. [PubMed: 18264109]
60. Satoh-Takayama N, Vosshenrich CAJ, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, et al. Microbial Flora Drives Interleukin 22 Production in Intestinal NKp46+ Cells that Provide Innate Mucosal Immune Defense. *Immunity*. 2008; 29:958–70. [PubMed: 19084435]
61. Spahn TW, Maaser C, Eckmann L, Heidemann J, Lügering A, Newberry R, et al. The lymphotoxin-beta receptor is critical for control of murine *Citrobacter rodentium*-induced colitis. *Gastroenterology*. 2004; 127:1463–73. [PubMed: 15521015]
62. Kinnebrew MA, Buffie CG, Diehl GE, Zenewicz LA, Leiner I, Hohl TM, et al. Interleukin 23 Production by Intestinal CD103+CD11b+ Dendritic Cells in Response to Bacterial Flagellin Enhances Mucosal Innate Immune Defense. *Immunity*. 2012; 36:276–87. [PubMed: 22306017]
63. Spits H, Cupedo T. Innate Lymphoid Cells: Emerging Insights in Development, Lineage Relationships, and Function. *Annu Rev Immunol*. 2012; 30:647–75. [PubMed: 22224763]
64. Aujla SJ, Kolls JK. IL-22: A critical mediator in mucosal host defense. *Journal of Molecular Medicine*. 2009; 87:451–4. [PubMed: 19219418]
65. Sherlock JP, Joyce-Shaikh B, Turner SP, Chao C-C, Sathe M, Grein J, et al. IL-23 induces spondyloarthritis by acting on ROR- γ + CD3+CD4-CD8-entheseal resident T cells. *Nature Medicine*. 2012; 18:1069–76.
66. Kiss EA, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C, et al. Natural Aryl Hydrocarbon Receptor Ligands Control Organogenesis of Intestinal Lymphoid Follicles. *Science*. 2011; 334:1561–5. [PubMed: 22033518]
67. Qiu J, Guo X, Chen Z-ME, He L, Sonnenberg GF, Artis D, et al. Group 3 Innate Lymphoid Cells Inhibit T-Cell-Mediated Intestinal Inflammation through Aryl Hydrocarbon Receptor Signaling and Regulation of Microflora. *Immunity*. 2013:1–14.
68. Upadhyay V, Poroyko V, Kim T-J, Devkota S, Fu S, Liu D, et al. Lymphotoxin regulates commensal responses to enable diet-induced obesity. *Nature Immunology*. 2012; 13:947–53. [PubMed: 22922363]
69. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will All Americans Become Overweight or Obese? Estimating the Progression and Cost of the US Obesity Epidemic. *Obesity*. 2008; 16:2323–30. [PubMed: 18719634]
70. Wilson PWF, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and Obesity as Determinants of Cardiovascular Risk: The Framingham Experience. *Arch Intern Med*. 2002; 162:1867–72. [PubMed: 12196085]
71. Willett WC, Manson JE, Stampfer MJ, Colditz GA, Rosner B, Speizer FE, et al. Weight, Weight Change, and Coronary Heart Disease in Women. *JAMA: the Journal of the American Medical Association*. 1995; 273:461–5. [PubMed: 7654270]
72. Field AE, Willett WC, Lissner L, Colditz GA. Dietary Fat and Weight Gain Among Women in the Nurses' Health Study[ast]. *Obesity*. 2007; 15:967–76. [PubMed: 17426332]
73. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA: the Journal of the American Medical Association*. 2002; 287:356–9. [PubMed: 11790215]
74. Leroy P, Dessolin S, Villageois P, Moon BC, Friedman JM, Ailhaud G, et al. Expression of ob Gene in Adipose Cells. *Journal of Biological Chemistry*. 1996; 271:2365–8. [PubMed: 8576190]
75. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nature Reviews Immunology*. 2011; 11:85–97.
76. Norman RBCRE. Linkage Between Obesity and a Marker Near the Tumor Necrosis Factor- α Locus in Pima Indians. *J Clin Invest*. 1995; 96:158–62. [PubMed: 7615786]
77. Hamid YH, Urhammer SA, Gl mer C, Borch-Johnsen K, J rgensen T, Hansen T, et al. The common T60N polymorphism of the lymphotoxin- α gene is associated with type 2 diabetes and other phenotypes of the metabolic syndrome. *Diabetologia*. 2005; 48:445–51. [PubMed: 15729581]

78. Mahajan A, Tabassum R, Chavali S, Dwivedi OP, Chauhan G, Tandon N, et al. Obesity-dependent association of TNF-LTA locus with type 2 diabetes in North Indians. *Journal of Molecular Medicine*. 2010; 88:515–22. [PubMed: 20177654]
79. Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T, et al. Functional SNPs in the lymphotoxin-[alpha] gene that are associated with susceptibility to myocardial infarction. *Nature Genetics*. 2002; 32:650–4. [PubMed: 12426569]
80. Yamada A, Ichihara S, Murase Y, Kato T, Izawa H, Nagata K, et al. Lack of association of polymorphisms of the lymphotoxin α gene with myocardial infarction in Japanese. *Journal of Molecular Medicine*. 2004; 82
81. Upadhyay V, Fu YX. Linking the microbiota and metabolic disease with lymphotoxin. *International Immunology*. 2013
82. Grabner R, Lotzer K, Dopping S, Hildner M, Radke D, Beer M, et al. Lymphotoxin β receptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of aged ApoE $^{-/-}$ mice. *Journal of Experimental Medicine*. 2009; 206:233–48. [PubMed: 19139167]
83. Pamir N, McMillen TS, Edgel KA, Kim F, LeBoeuf RC. Deficiency of Lymphotoxin-alpha does not exacerbate high fat diet induced obesity but does enhance inflammation in mice. *AJP: Endocrinology and Metabolism*. 2012; 302:E961–71.
84. Pamir N, McMillen TS, Kaiyala KJ, Schwartz MW, LeBoeuf RC. Receptors for Tumor Necrosis Factor- Play a Protective Role against Obesity and Alter Adipose Tissue Macrophage Status. *Endocrinology*. 2009; 150:4124–34. [PubMed: 19477937]
85. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006; 444:1027–131. [PubMed: 17183312]
86. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences*. 2007; 104:979–84.
87. Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. Diet-Induced Obesity Is Linked to Marked but Reversible Alterations in the Mouse Distal Gut Microbiome. *Cell Host and Microbe*. 2008; 3:213–23. [PubMed: 18407065]
88. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: Human gut microbes associated with obesity. *Nature*. 2006; 444:1022–3. [PubMed: 17183309]
89. Ley RE, Backhed F, Turnbaugh P. Obesity alters gut microbial ecology. *Proceedings of the* 2005
90. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice. *Science Translational Medicine*. 2009; 1:6ra14–4.
91. Hildebrandt MA, Hoffmann C, Mix SAS, Keilbaugh SA, Hamady M, Chen YY, et al. High-Fat Diet Determines the Composition of the Murine Gut Microbiome Independently of Obesity. *Gastroenterology*. 2009; 137:1716–1724.e2. [PubMed: 19706296]
92. Hogan SP, Seidu L, Blanchard C, Groschwitz K, Mishra A, Karow ML, et al. Resistin-like molecule β regulates innate colonic function: Barrier integrity and inflammation susceptibility. *Journal of Allergy and Clinical Immunology*. 2006; 118:257–68. [PubMed: 16815164]

Figure 1. Closely related LTis and ILC3s utilize the LT-pathway for their critical effector functions during development and in the adult host

(A) LTi function during development. LTi cells which express the transcription factor ROR γ t are recruited to the lymph node anlagen by retinoic acid released by local neurons. Within the gut, ILF formation is stimulated by peptidoglycan produced by local bacteria; NOD1 is essential for peptidoglycan recognition but it is unclear if it is expressed within LTi cells for this function. Once LTi cells are recruited to either body site, they stimulate chemokine production by $LT\alpha_1\beta_2$ mediated agonism of $LT\beta$ R on stromal cells, which results in chemokine recruitment for lymphoid structure formation (B) ILC3 function in the adult host. ILC3s exist in ILFs and colocalize with local DC populations. A TLR dependent signal and an $LT\beta$ R dependent signal from ILC3 produced $LT\alpha_1\beta_2$ triggers release of IL-23 from local DCs. Such IL-23 in conjunction with a MyD88 dependent signal in ILC3s induces IL-22 production. IL-22 production results in antimicrobial peptide production from intestinal epithelial cells, which contributes to defense from *C. rodentium*. ILC3s also express $LT\alpha_1\beta_2$, which is recognized by $LT\beta$ R on intestinal epithelial cells which results in CXCL1/CXCL2 mediated neutrophil recruitment.

Figure 2. The LT-pathway contributes to metabolic illness through several distinct mechanisms

(A) Atherogenesis is influenced by the LT-pathway. T cells expressing $LT\alpha_1\beta_2$ induce the expression of CXCL13 and CCL21 from medial smooth muscle cells in the initial phases of atherogenesis. As a result, tertiary lymphoid structure formation, which is characteristic of atherosclerotic plaques can occur through the LT-mediated cellular recruitment (B) Hepatic lipid homeostasis can be regulated by the LT-pathway. T-cells transgenically expressing LIGHT, a ligand for $LT\beta R$, down regulate hepatic lipase production and contribute to hyperlipidemia. (C) Weight gain and glucose intolerance are influenced by the LT-pathway. HFD induces agonism of the LT pathway (VU and YXF unpublished data). $ROR\gamma t^+$ cells expressing LT induce production of IL-23. The IL-23 and IL-22 pathway results in antimicrobial peptide production, which changes the composition of the microbiome and contributes to host weight gain.