Lymphotoxin and the amazing technicolor circus of intestinal homeostasis

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Recently a new lymphocyte subset called innate lymphoid cells has emerged and it includes key producers of interleukin (IL)-17 and IL-22 in the mucosal environment. Using *Citrobacter rodentium* infection to deliver a pathogenic insult to the colon, two studies have revealed an underlying role for lymphotoxin-β receptor signaling in the generation of IL-22 by these cells. This observation links a system well known for its ability to organize lymphoid microenvironments into a basic mucosal response.

The circus thrills the senses with unimaginable spectacles. By analogy, mucosal immunologists could identify the menagerie of participants going in and out of the circus tent, but have longed to view the show in its whole splendor. Over the last decade, the tent has been opening its doors revealing the amazing equilibrium between the immune system and commensal flora at the mucosal interface. The intestinal microenvironments need to establish homeostasis with the gut contents, yet respond, usually aggressively, to an imbalance or pathogen while preserving the ability of the gut to harvest nutrients. Having a key role in this Herculean task is a new class of lymphocytes called innate lymphoid cells (ILCs).[1,2] One branch of this family is dependent upon the transcription factor RORγt for the development and includes the lymphoid tissue inducer cell (LTi), an "adult" LTi-like cell and a natural killer (NK) cell (NK22 or 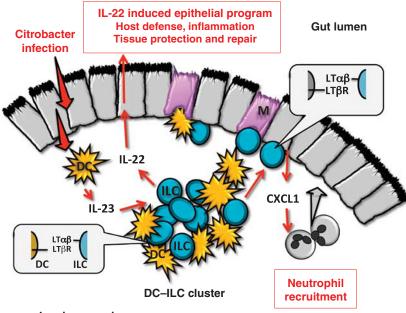NKp46 + NK cell). Robust interleukin (IL)-17 or IL-22 secretion can be a trademark of these cells, yet they lack surface T- or B-cell receptors and hence are considered "innate". Both cytokines trigger complex spectra of activity with both protective and proinflammatory components that appear to vary from organ to organ.[2] A second branch of RORγt-independent ILC has been described on the basis of Th2-type cytokine secretion. Along with these discoveries, substantial progress has occurred in the understanding of the development and function of organized lymphoid environments. Adaptive immunological processes do not occur efficiently without such structures and in the gut they include the developmentally determined draining lymph nodes and Peyer's patches as well as the non-predetermined tertiary lymphoid tissues (TLTs), i.e., isolated lymphoid follicles (ILF), cryptopatches, and cecal and colonic lymphoid patches (CLP, historically referred to simply as colonic patches).[3] It was into the intersection of these exciting systems that the membrane form of lymphotoxin (LT) was recently inserted (**Figure 1**).

In general, the LT system is a mechanism by which lymphocytes can instruct stromal cells to differentiate into specialized reticular networks and vasculature. These elements form the portals that gate lymphocyte entry into organized sites and the frameworks that position cells for optimal encounters with antigen.[4] The LT motif is used in the gut during mesenteric lymph node and Peyer's patch development as well as to drive the generation and maturation of mucosal TLTs. Over a decade of work has linked the LT system to aspects of mucosal immunology, including M-cell differentiation, γδ T-cell differentiation, ILFs, mucosal immunoglobulin (Ig)A production, oral tolerance, and pathology in rodent gastritis and colitis models.[5,6]

The current story warmed up in 2010 when a team led by Alexei Tumanov and Yang-Xin Fu followed up on an observation made by Thomas Spahn and colleagues in 2004 that mice lacking the surface LT (the LTα/β trimeric ligand) or its receptor, LTβR, readily succumbed to a *Citrobacter rodentium* infection.[7,8] *C. rodentium* infection is often used to explore the inflammatory effects of an enteric pathogen and the host's immune response. They found that LT expressed by RORγt + lymphocytes, i.e., ILC, was critical for lymphocyte communication to the epithelium and the recruitment of neutrophils to the damaged barrier. Direct signaling of LT ligand on lymphocytes to receptor-positive epithelial cells is certainly plausible based on the studies of lymphotoxin-β receptor (LTβR) agonism in colorectal carcinoma cells or hepatocytes in hepatitis.[9,10] An important prequel to this saga was written in 2008 by Wenjun Ouyang's group at Genentech

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Figure 1 Schematic illustration of the proposed events following *C. rodentium* infection. Infection is sensed by dendritic cells (DCs) causing them to secrete interleukin (IL)-23. IL-23 triggers the innate lymphoid cells (ILCs) to make the IL-22 that can directly engage receptors on epithelial cells and initiate an epithelial response program. This program includes host defense mechanisms such as anti-microbial protein release, e.g., REG IIIβ and γ, secretion of inflammatory cytokines and chemokines as well as various tissue protection and repair elements. Host defense to this pathogen absolutely relies on the induction of this IL-22-initiated epithelial response. Selective deletion of lymphotoxin-β receptor expression (LTβR) in DC or in epithelial cells revealed roles for the receptor in both cell types. Therefore, direct ILC–DC and ILC–epithelial dialogs have been postulated. ILCs exist as clusters with DC in the villi in organized structures such as isolated lymphoid follicles and colonic follicles with M-cell links to the lumen.

in San Francisco, where they showed that early secretion of IL-22 was critical for survival following *C. rodentium* infection, and, moreover, IL-23 drove the expression of IL-22.[11] IL-22 triggers a complex gene expression program in epithelial cells with proinflammatory, chemokine secretion, production of anti-microbial proteins, and tissue repair components. Release of anti-microbial proteins such as RegIIIγ helps to control the initial bacterial invasion. It was a quick leap in subsequent papers by both groups to show that IL-22 production was impaired in mice deficient in the LT pathway.[12–14]

In both studies, the LTα/β ligand and not the alternative LTβR ligand, LIGHT, was found to be responsible for the IL-23/IL-22 effects and, moreover, LT ligand expression by RORγt + ILC was essential. While these rodent events were clearly LIGHT-independent, LIGHT has considerable history in the gut and its involvement in other mucosal settings may be

more dramatic such as seen in the recent observations in the lung.[15] IL-22 is made by multiple cell types; however, the LTi is the dominant source in this setting.[2,16] LTβR is typically not present on lymphocytes, indicating indirect regulation of IL-22 production from LTi. Upon dissection, LTβR signaling was required to induce the IL-23 that triggers the IL-22 response.[12] As dendritic cells are dominant producers of IL-23, an LT-dependent dialog between dendritic cells (DC) and ILC was postulated. Whether the role of LTβR signaling is strictly limited to modulating DC production of IL-23 is less certain. A DC selective deletion of LTβR resulted only in a partial exacerbation of *C. rodentium* infection and a modest twofold decrease in IL-23 production.[12] Moreover, the earlier work showed that LTβR on both epithelium and hematopoietic cells was critical.[8] The work by Ota *et al.*[13] focused on the organized lymphoid architecture in the colon, i.e., CLP

and ILF and the hypertrophy of these tissues that occurs following *C. rodentium* infection. Blockade of LTβR signaling in mice infected with *C. rodentium* reduced the size and organization of these structures, an observation also made previously in another colitis setting.[17] Therefore, the role of LTβR is likely a combination of maintaining TLT organization along with both ILC–DC and ILC–epithelial communication. These events could be intertwined with the control of chemokine-induced positioning, perhaps involving CCL20 production.

The ability of LTβR signaling to orchestrate cell trafficking and positioning is well defined in both secondary and TLTs in normal and reactive states. It appears that there are several routes to TLT formation and organization including RORγt + ILC dependent and independent as well as LT dependent and independent mechanisms. In general, the formation of large TLT is LT-dependent, but recently, and of relevance to the gut, Th17 cells were shown to trigger TLT formation in the lung.[18] Somewhat surprisingly, in the Ouyang study, IL-22 administration was able to restore robust TLT in the colon even in the presence of LTβR blockade, suggesting that IL-22 can directly impact organization. It will be interesting to see how these IL-22-mediated events translate in colitis settings as infection models have considerable additional complexity. For example, the net impact of *C. rodentium* on host survival is a gemisch of colitis, effective host immune response, systemic spread of the infection and the kinetics of bacterial clearance. Pathogen clearance in LT-deficient mice is impaired in many studies, presumably reflecting alterations in basic adaptive immunology or efficient antigen sensing. In general, the questions of whether the lack of LTβR signaling led to an inefficient or slowed immune response and/or an unchecked inflammatory response remain poorly explored. Second, LTβR signaling is central to the differentiation of high endothelial venules and display of addressins in both secondary and tertiary settings. Therefore, the trafficking of various cells into the reactive sites should be altered in the absence of LTβR signaling. Reduced trafficking could be anti-inflammatory,

yet also impair the host response to the pathogen. Finally, in several lymphoid tissues, the fibroblastoid reticular networks are affected by LTβR signaling, and this element could be an important factor in the host response to *C. rodentium*. Indeed, LTβR expression on mucosal non-hematopoietic cells is required for the development of gut IgA production.[19]

These data point toward a more intimate relationship between the ILC and DC in the mucosal niches, and there were some clues from prior studies. In what was a rather perplexing body of work; DC homeostasis or the maintenance of normal numbers of DCs in some secondary lymphoid organs was at least partially LT-dependent.[20] Recently, the differentiation of a DC subset in the spleen and gut was found to require Notch2 receptor signaling in an LTβR-dependent manner.[21] Intriguingly, the differentiation of RORγt + ILC in the periphery is also Notch2-dependent and it will be interesting to learn if these two events are related.[22,23] More poignantly, the idea of a DC–ILC dialog resonates well with a dissection of the roles of LTβR and CD40 signaling in DC during adaptive T-cell responses, as elucidated in the work of Jen Gommerman and colleagues[24] in Toronto. In fact, their work started with the observation that DC-T cell clusters were disrupted following pharmacological blockade of LTβR signaling. DC licensing for an adaptive T-cell response was found to require LTβR signaling, and this function was non-redundant with CD40 signaling even though CD40 signaling is classically associated with DC licensing/maturation. Does the gut simply represent a similar dialog between more constitutively LT-positive ILC and DC? Non-canonical nuclear factor-κB signaling is crucial for DC maturation/licensing and it is reasonable that LTβR signaling could provide this element.[25]

Human inflammatory bowel disease (IBD) is viewed as a deviation from the normal balance between the microbiome and the mucosal immune system with a continuing cycle of inflammation-triggered epithelial damage and excessive immune activation. How would an LTβR inhibitory agent fare in this setting? It has been posited that many experimental therapies for IBD have been limited in efficacy because a larger swath of the immunology needs to be brought under control.[26] The exaggerated dextran sulfate sodium colitis in the genetic absence of RORγt and hence lack of ILC certainly highlights the many roads to TLT formation and inflammation. LTβR inhibition attenuates disease in a wide range of mouse colitis models including an RORγt-independent setting.[4,27] It can affect a broad range of immunological events, including the production of certain homeostatic chemokines, neutrophil-attracting chemokines released by epithelial cells, vascular addressin expression, reticular network specialization, the maintenance of large TLT, and now modulation of IL-22-triggered epithelial defense/repair programs. The consequences of IL-22 inhibition are difficult to forecast as IL-22 has both proinflammatory and repair roles depending on the organ and even differs between different IBD models.[2] Thus, there appears to be some pieces missing in this puzzle, such as a temporal component to the expression of IL-17/IL-22 or perhaps the expression of the soluble IL-22 inhibitory receptor, Il22ra2, can resolve this conundrum. Whatever the exact details surrounding IL-22's protective effects, LTβR signaling appears to have a fundamental organizing role in the center ring of this circus. It will be exciting to see whether this LTβR core function reflects control of the spatial organization of the DC–ILC clusters or more direct ILC–DC or ILC–epithelial signaling events. It is possible in IBD that LTβR inhibition could reduce the amount of gut immunological activity at multiple levels, break the pathological cycle of commensal imbalance and barrier breach, and allow a "time-out" for the immune system to reset.

DISCLOSURE

JLB is employed by Biogen Idec, which has had an interest in the LTβR pathway.

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