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doi: 10.4049/jimmunol.1400059

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Blown Away: The Unexpected Role of Lymphotoxin in Lymphoid Organ Development

Jason G. Cyster

Lymph nodes (LNs), Peyer’s patches (PPs), and the spleen are command centers for the adaptive immune system, bringing together Ags and immune cells for the initiation of antipathogen offensives. The anatomy of these organs has been studied for over 100 years, and their organization into thymus-dependent (T) and -independent (B) regions was appreciated even before T and B cells had been named (1–3). It is therefore perhaps a surprise to realize that a mere 20 years ago nothing was known about the molecular requirements for lymphoid organ development. All that changed for good when Chaplin and colleagues (4) dissected the first lymphotoxin (LT) knockout mice and discovered that they entirely lacked LNs and PPs and had disorganized spleens. Since that seminal finding, and the simultaneous discovery of the organizing activities defined.

The moniker “lymphotoxin” conjures images of cell death and tissue destruction, not organ development. What is the backstory here? The term lymphotoxin was first applied during studies in the 1960s to describe an activity made by activated lymphocytes that could kill transformed cell lines (6, 7). Another tumor cell killing activity, TNF, identified in serum of animals injected with Mycobacterium or endotoxin, was made predominantly by activated macrophages (8, 9). In addition to differing in cellular origin, there were notions at that time that LT could be distinct from TNF through involvement in a membrane-associated cytotoxicity mechanism. These important cellular studies suggesting that LT and TNF may be antitumor agents led to concerted efforts in the early days of DNA cloning to isolate their genes. A B lymphoblastoid cell line (RPMI 1788) was identified as a potent source of TNF. The factors were biochemically purified, with active fractions identified based on their cytotoxicity for a fibroblast cell line, and small amounts of protein sequence were obtained by Edman degradation. This allowed the cloning and sequencing in 1984 of cDNAs for LT and TNF, reported in back-to-back Nature articles (10, 11). The sequences showed that LT and TNF were closely related.

Work on TNF (also known as TNF-α), a soluble homotrimer, moved quickly and included identification of two receptors, TNFR1 and TNFR2 (12). Although this research helped explain the proapoptotic signaling by TNF in some transformed cell types, it also led to the realization that the predominant physiological role of TNF was as a proinflammatory mediator (12, 13). The subsequent development of a range of TNF antagonists for treatment of inflammatory diseases, in particular rheumatoid arthritis, is now legendary. The rate of advance in understanding LT was slower, and this turned out to reflect the more interesting biochemical property of this cytokine, which was realized to have distinct membrane and secreted forms (12). The type of LT expressed as a complex on lymphocyte membranes included a second polypeptide chain. Browning and Ware and their colleagues (14) cloned this new chain and found it was related in sequence to TNF and LT, and thus was another member of the growing TNF family. To reflect the biochemical complex, the new chain was called LTβ and the older chain was renamed LTα.

LTβ was a transmembrane protein, and the predominant membrane form of LT was an LTα1β2 heterotrimer. In contrast, the form detected in lymphoid cell supernatants, and that was particularly abundant in the RPMI 1788 cell supernatant, was an LTα2 homotrimer (12). The homotrimer, it turned out, engaged the same receptors as TNF, accounting for the striking similarity in activities of soluble LT and TNF in many of the early assays (so similar, in fact, that LTα for a time also went by the name TNF-β). The membrane LTα1β2 heterotrimer did not engage the TNF receptors, leading to the hunt for an LT-specific receptor. Focus and persistence paid off for Ware, Browning, Goodwin, Smith, and collaborators who, by screening several TNFR–Fc fusion proteins for binding to LTα1β2hi T cells, identified a single receptor that bound selectively to LTα1β2 (5). They called this new TNFR family member LTβR.

Following from the Chaplin et al. (4) discoveries for LTα, the first in vivo evidence that LTβR was likely the crucial receptor for lymphoid organogenesis came when Hochman and colleagues (15) showed that in utero delivery of a soluble form of the receptor, LTβR–Ig, blocked development of PPs and most LNs. Satisfyingly, when LTβR–deficient and LTβR-deficient mice were generated, they suffered very similar lymphoid organ deficiencies to LTα-deficient mice (16, 17). An exception was that mesenteric, sacral, and cervical LNs were retained in LTβR-deficient mice, perhaps because LTα3 homotrimer signaling is exaggerated in the absence of LTβR and can rescue a subset of mucosal LNs (18).
In studies that began before the Chaplin laboratory report, Ruddle and colleagues (19) had shown using transgenic mice that LTα was sufficient to promote lymphocyte-rich infiltrates in nonlymphoid tissue. Later, using more refined anatomical assessments, they observed that the infiltrates took on many of the organized features of a lymphoid tissue, indicating that LTα was sufficient to induce lymphoid neogenesis (20). Shortly thereafter, an agonistic LTβR-specific Ab was used to rescue genesis of all LNs in LTα-deficient mice, although antagonists of LTβR and TNFR1 together were needed to inhibit all LNs in wild-type mice (21). The consensus view that emerged from these and many other studies was that LTαβ2 and LTβR had the dominant role in lymphoid organ development, whereas engagement of TNFR1 by LTα3 or TNF under noninflammatory conditions could promote some aspects of lymphoid tissue genesis (18).

An important advance in studying lymphoid organogenesis came from Nishikawa and colleagues (22), who used whole-mount staining of the embryonic mouse intestine to visualize the earliest steps in PP development. This led to the finding that CD45+IL-7RαCD4+ cells clustered at intervals along the intestine and induced VCAM1+ “spots.” Although up to this point lymphocytes had been highlighted as the main cell types expressing LTαβ2, this did not mesh well with the cytokine’s role in lymphoid organogenesis, as B and T lymphocytes were not needed for the earliest steps in PP or LN development. An answer to this mystery emerged when Mebius et al. (23) described the presence of CD45+IL-7RαCD4+CD3+ cells in developing LNs that expressed LT. These cells, which were later termed lymphoid tissue inducer (LTI) cells (and more recently innate lymphoid cells type 3, or ILC3) (24), were confirmed to be a crucial source of LTαβ2 for LN and PP development when it was found that LTI-deficient mice failed to develop most lymphoid organs (25). A model emerged in which LTI cells, recruited to nascent sites of lymphoid tissue development, engaged LTβR on mesenchymal “organizer” cells to induce VCAM1 and other factors needed for lymphoid organogenesis (25).

If LTI cells needed to be recruited to provide LTαβ2 for lymphoid organogenesis, what was doing the recruiting? The first hint at an answer was the 1996 discovery that the chemokine receptor CXCR5 (BLR1) was needed for development of most PPs and LNs (26). LTI cells were later shown to express CXCR5, and its ligand, CXCL13, was expressed in the earliest lymphoid organ anlage (25). However, CXCR5- and CXCL13-deficient mice are not like lymphoid organ deficient as LTα−/− mice or LTI-deficient mice (26, 27). Defining the full set of chemoattractants mediating LTI cell recruitment remains an ongoing area of investigation (25).

With LTαβ2 and LTβR playing such crucial roles in lymphoid organogenesis, an obvious challenge was to identify the full cast of downstream players. In vitro signaling studies showed that, similar to other TNFR family members, LTβR activated the NF-κB pathway, particularly the alternative NF-κB pathway (28). This realization helped another early observation fall into place: a spontaneous mutant mouse strain that lacked LNs and PPs, termed almphalopsia (aly) (29), was found to have a defect in NF-κB–inducing kinase (30). NF-κB–inducing kinase was required for LTβR-mediated activation of NF-κB in organizer cells and for induction of VCAM1 (31). Further study of factors functioning downstream of the LTβR was facilitated by the observation of Chaplin and colleagues (4) that whereas spleen development did not require LTαβ2, organization of the lymphoid regions, or white-pulp cords, did. The finding that CXCR5- and CXCL13-deficient mice suffered disruptions of splenic B cell follicles led to the hypothesis, subsequently supported, that LTαβ2 was required for adequate expression of this chemokine by stromal cells (32). LTαβ2 was also necessary for full expression of the T zone chemokines CCL21 and CCL19. In a satisfying throwback to the original cloning of LTα from a B lymphoblastoid cell line, B cells were discovered to be a necessary source of LTαβ2 for follicular stromal cell induction and CXCL13 expression (27, 33).

The “build out” from the seminal studies of Chaplin, Ware, and coworkers continues to this day. An important therapeutic implication of the discoveries is that in any condition in which ectopic lymphoid tissues arise, such as in the joint synovia of rheumatoid arthritis patients, the thyroid of thyroiditis patients, or the intestine of Crohn’s disease patients, LTαβ2 is implicated as a driver of the chronic inflammation and thus of pathology. Moreover, in B lymphomas, LTαβ2 may function in a positive feedback loop to stimulate trophic support of the cancer by stromal cells. This thinking rightly led to the view that blockade of LTαβ2 might have therapeutic benefit in the treatment of some chronic inflammatory diseases. Valiant efforts to explore this idea were spearheaded by workers at Biogen Idec, and although this effort has not yet yielded a drug, the potential remains real. Looking to the future, perhaps in a Pillars article 20 years from now, we will read about the successful engineering of artificial LNs to provide extra command centers for immunity against emergent diseases. Although we do not yet know all the components that will be needed, we can be sure that the starter kit will contain LTαβ2 and LTβR.

Disclosures
The author has no financial conflicts of interest.

References


