Published in final edited form as:

Curr Opin Immunol. 2013 April ; 25(2): 222–229. doi:10.1016/j.coi.2013.01.001.

Lymphotoxin Network Pathways Shape the Tumor Microenvironment

Ryan L. Bjordahl^a, Christian Steidl^b, Randy D. Gascoyne^b, and Carl F. Ware^{a,c}

^aInfectious and Inflammatory Diseases Center, Sanford Burnham Medical Research Institute, 10901 N. Torrey Pines Road, La Jolla, CA 92037 USA

^bDepartment of Pathology, University of British Columbia; Centre for Lymphoid Cancer, Department of Experimental Therapeutics, British Columbia Cancer Research Centre, 675 W 10th Avenue, Vancouver, BC V5Z 1L3, Canada

Abstract

Accumulating evidence indicates that Lymphotoxin (LT)- β related cytokines directly contribute to the phenotype of cancer cells and alter the tumor microenvironment. Lymphotoxins are part of a cytokine network well known in controlling the development and homeostasis of secondary lymphoid organs. In the adult, the LT network takes on the responsibility of generating inflammatory microenvironments that control innate and adaptive immune responses involved in host defense. This review provides a perspective of the emerging evidence implicating the LT Network in the development and progression of various cancers including lymphoma. Redirecting the LT Network to alter tumor microenvironments may provide a specific approach to therapeutically target tumor-permissive microenvironments and cancer progression.

Introduction

Substantial evidence indicates that the development and progression of cancer is aided by inflammation in the tumor microenvironment [1,2]. This transformative hypothesis leads to the prediction that therapeutic targeting of specific inflammation-associated phenotypes may improve therapy and ultimately patient survival. Indeed, associations between autoimmune diseases and lymphomagenesis are well recognized, but with limited mechanistic understanding. However, the complexity of “inflammation” in the cancer setting is not well understood, limiting the number of therapeutic targets and the efficacy of existing approaches. The cellular chaos of lymphoid cancers is reflected by variable numbers of malignant cells intermixed in a matrix of connective tissue cells, endothelium with nascent blood and lymphatic vessel formation, and often prominent lymphocyte and macrophage infiltration [3]. These cellular features are strikingly similar to the formation of nascent lymphoid tissue observed at sites of persistent inflammation. Expressed genes encoding cytokines, chemokines and growth factors can be detected in tumor microenvironments, but the cellular infiltrates are not typically considered “organized”, and vary substantially when

© 2013 Elsevier Ltd. All rights reserved.

^cCorresponding author: cware@sanfordburnham.org.

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.

Conflict of Interest Statement: RB, RCG, CS declare no conflicts of interest; CFW has licensed patents to several TNFSF/TNFRSF molecules.

compared to normal secondary lymphoid tissues. It is a widely accepted concept that intercellular communication pathways between tumor cells and the surrounding stroma have evolved with the tumor to create permissive or selective microenvironments allowing malignant cells to mutate, grow, and resist immune recognition and destruction, or survive therapeutic assaults [4,5]. In particular, Hodgkin lymphoma can serve as a paradigm how the malignant cells shape their inflammatory microenvironments to their selective advantage (reviewed in [6]).

Cancer inflammation represents discontinuous phases of new lymphoid tissue formation, lymphoid neogenesis [7]. This perspective of cancer inflammation and neogenesis points directly to the cytokine network formed by Lymphotoxin (LT)- $\alpha\beta$, LIGHT (TNFSF14) and their receptors [8]. The LT $\alpha\beta$ -LIGHT cytokine network directs innate and adaptive host defense mechanisms providing strong selective pressures on viral pathogens to evolve countermeasures. Recent evidence identifies the LT $\alpha\beta$ -LIGHT network as a contributing factor in several chronic inflammatory diseases (Table 1). Importantly, emerging evidence indicates that solid tumors and hematopoietic malignancies manipulate components in the LT $\alpha\beta$ -LIGHT network to modify their tumor microenvironment, suggesting these cytokines provide strong selective pressure for tumor evolution and tumor immune evasion.

The LT $\alpha\beta$ -LIGHT Network

The LT-related cytokines include TNF α , lymphotoxin (LT)- α , LT- β , LIGHT (TNFSF14), and their cognate superfamily of receptors, TNF receptor (TNFR)-1, TNFR2, LT β R, herpesvirus entry mediator (HVEM; *TNFRSF14*) and decoy receptor-3 (DcR3) (Figure 1). The LT $\alpha\beta$ -LIGHT Network provides communication between lymphoid cells and surrounding tissue cells. The different ligand-receptor systems can activate proinflammatory pathways (eg., TNF α -TNFR1), homeostatic signaling (eg., LT $\alpha\beta$ -LT β R) or inhibitory signaling responses (eg., HVEM-BTLA). The LT $\alpha\beta$ -LT β R system serves as a developmental pathway required for the formation of secondary lymphoid organs and maintenance of lymphoid tissue architecture. In the neonate, B lymphocytes assume responsibility for the maturation and homeostasis of secondary lymphoid organs including the spleen through the LT $\alpha\beta$ -LT β R pathway [9]. In the adult, LT $\alpha\beta$ in B cells provide key differentiation signals to LT β R expressing stromal cells and macrophages to initiate type 1 interferon production to viral pathogens [10,11]. Additionally, LT $\alpha\beta$ orchestrates formation of tertiary lymphoid tissues at extra lymphatic sites of chronic inflammation. Importantly, components of the TNFRSF signaling pathway are frequently mutated in cancer including components of the ubiquitin E3 ligase (TRAF3, TRAF2, and cIAP) that regulates expression of the NF κ B inducing kinase, NIK [12].

The LT β R-defined Microenvironment

LT β R signaling in stromal cells forms the microarchitecture of the lymphoid organ. Scaffolds of highly differentiated stromal cells, reticular fibrocytes, lymphatic endothelium, and follicular dendritic cells, all responsive to LT β R signaling, partition the lymphoid organ into specialized niches where T and B lymphocytes congregate [13]. Distinct lineages of myeloid cells require LT β R signaling for positioning and differentiation to populate these niches [14]. LT $\alpha\beta$ expressing marginal zone B cells form a critical barrier to pathogens by inducing differentiation of macrophages and stromal cells that produce type 1 interferons (IFN $\alpha\beta$) in response to viral pathogens [10,11]. These LT-differentiated cells produce high levels of IFN $\alpha\beta$, but allow the pathogen to replicate, amplifying antigens available to stimulate effective T and B cell responses [15]. T and B cells segregate into separate niches created by LT $\alpha\beta$ differentiated stromal cells secreting distinct patterns of chemokines. CXCL13 is the chemoattractant forming the B cell-rich follicles, and CCL21 recruits T cells

around the arteriole. CXCL13 through its cognate receptor CXCR5 stimulates LT $\alpha\beta$ expression in B cells forming a reciprocal signaling pathway. Inflammatory cells organize at extra lymphatic sites during persistent inflammation into tertiary lymphoid tissues through the expression of LT $\alpha\beta$ expressed in a variety of activated cells, such as T and B cells, NK cells, DC and LTi cells [16]. T and B cells, and LT β R signature chemokines (CCL21 and CXCL13) and integrins are detected at these ectopic sites [17–19]. The LT $\alpha\beta$ -LIGHT Network provides an essential component for generating inflammatory environments, from the initial innate response to full blown adaptive immune responses.

The LIGHT-HVEM-BTLA component of the Network provides inhibitory and proinflammatory signaling initiated by dual action of HVEM [20,21]. HVEM-mediated signaling is an important mechanism suppressing inflammation in mucosal tissue [22]. For example, experimental colitis is dramatically accelerated in *Hvem*-deficient mice. This immune regulation mechanism involves *trans* signaling between HVEM expressed in intestinal epithelia cells and BTLA in T cells to activate inhibitory signaling. In lymphoid cells, intrinsic HVEM expression in T cells is important for the survival of effector memory T cells during chronic lung inflammation [23] and graft *vs* host disease [24–26]. BTLA expression in B cells is thought to play a similar role in limiting cellular activation [27]. The loss or amplification of HVEM may act intrinsically in cancer cells or modulate the microenvironment.

Lymphotoxins in the Cancer Microenvironment

Emerging evidence in animal models and human genetic data implicates the LT $\alpha\beta$ -LIGHT Network as a key mechanism creating microenvironments suitable for cancer development and progression. Two recent studies showed that the LT $\alpha\beta$ pathway can drive carcinogenesis, with LT $\alpha\beta$ -LT β R linking malignant cells to the inflammatory, tumor permissive environment. In the mouse, transgenic expression of LT α and LT β under control of the liver-specific albumin promoter caused chronic hepatitis in all transgenic animals [28]. By 18 months of age, around 35% of these mice developed liver tumors closely resembling human hepatocellular carcinoma (HCC). The development of HCC was dependent on lymphocytes and NF κ B signaling (RelA dependent) in hepatocytes. As expected, administration of LT β R-Fc decoy to block LT $\alpha\beta$ and LIGHT also prevented tumor formation. LT $\alpha\beta$ -dependent carcinoma failed to develop in mice lacking T and B cells (Rag1^{-/-}). This result suggested that the LT $\alpha\beta$ -LT β R system did not have direct oncogenic properties in hepatocytes, but rather LT β R signaling reshapes the hepatic microenvironment through secretion of chemokines resulting in recruitment of inflammatory cells that drive oncogenesis. Robust upregulation of LT α , LT β , and LT β R transcripts were found in the livers of HCC patients consistent with an influx of inflammatory cells during cancer progression. The link between LT β R and HCC is anticipated from data showing that stimulation of LT β R in hepatocytes is critical for liver regeneration after partial hepatectomy, and this process depends on production of IL-6 induced by LT β R signaling [29]. Intriguingly, hepatic LT production can be induced by IL-6 [30], suggesting that hepatocyte LT β R forms the center of an IL-6–LT $\alpha\beta$ inflammatory loop that contributes to both liver regeneration and liver cancer. The connection between IL-6/Stat3 and LT β R/NF κ B are particularly intriguing in view of the cooperation between Stat3 and NF κ B in cancer [31].

Expression of LT $\alpha\beta$ in B cells drives the rapid re-emergence of prostate cancer (CaP) following androgen deprivation [32]. In this model, CaP cells transplanted into mice, which are then castrated, causing progression to androgen independence. However, androgen-independent tumor progression was significantly delayed by treatment with LT β R-Fc decoy or in B cell specific deletion of LT $\alpha\beta$. The authors demonstrate that expression of LT β in B

cells depends on the expression of IKK β in those cells, while expression of LT β R and IKK α in CaP cells was required for rapid androgen-independent tumor emergence. The dependence of prostate cancer progression on LT β in B cells is remarkably similar to the innate B cell host defense mechanism to viral pathogens.

HVEM–BTLA Pathway in Cancer

The HVEM–BTLA pathway is intimately linked with the LT β R pathway [33] and emerging evidence indicates mutations in these components may alter the tumor and its microenvironment. The GISTIC somatic gene copy number dataset [34] (www.broadinstitute.org/tumorscape) revealed deletions in *BTLA* are frequent in human hematologic malignancies. A frequent (5.1%) and narrow deletion peak at chr3q13.2 encompassing human *BTLA* is associated with acute lymphoblastic leukemia and in all hematologic malignancies. *HVEM* also shows significant and frequent deletion in epithelia tumors including lung, breast and renal tumors, and is also found amplified in epithelia tumors, perhaps revealing dual action of HVEM in promoting and inhibiting inflammation. Moderate to high HVEM expression was observed in melanoma, perhaps serving as a potential suppressive mechanism for anti-tumor CD8 $^{+}$ T cells. In contrast to virus-specific CD8 $^{+}$ effector cells, which downregulate BTLA upon activation, tumorspecific CD8 $^{+}$ effectors maintain high BTLA levels and remain susceptible to inhibition through binding of HVEM [35,36]. CD160, in contrast to BTLA, was unexpectedly identified as a tumor specific marker with significant amplification in acute lymphoblastic leukemia [37,38]. The frequency of mutations in *HVEM*, *CD160* and *BTLA* in epithelial and hematologic malignancies adds substantial significance to the idea that selective pressures promote clonal dominance of these mutations in cancer.

HVEM in Lymphoma

The most common B-cell lymphoma subtypes are diffuse large B-cell lymphoma (DLCL) and follicular lymphoma (FL). Classical Hodgkin lymphoma (CHL) is less common, but the most frequent lymphoma in younger patients and thus represents a significant healthcare issue. The role of non-neoplastic immune cells in the microenvironment is increasingly recognized as an important contributor to lymphoma pathogenesis [39]. Importantly, the abundance of non-cancerous cells infiltrating the tumor correlates with the survival of patients with FL, DLCL and CHL [40–42]. Experimental models of lymphoma reinforce the tumor-stroma dialog via LT α β –LT β R-regulated chemokine pathways [43]. However, the cellular interactions between immune cells and B cell lymphoma are complex, underscored in a recent study that revealed a tumor-promoting role for innate NKT cells through inhibition of the CD8 cytotoxic T cell response to the B cell lymphoma by opposing the effects of type II NKT cells [44].

HVEM has emerged as a recognized mutational target in lymphoma, which is not surprising in that HVEM is subject to strong selective pressures as target of viral immune evasion strategies [45]. These data raise the possibility that HVEM-dependent pathways are critical for immune reactions against tumor cells. The dominance of somatic *HVEM* mutations was revealed in FL and DLCL [46,47] and recurrent deletions of *HVEM* in Reed-Sternberg cells microdissected from Hodgkin lymphoma [6]. Together these results implicate *HVEM* as a universal tumor suppressor gene across a broad range of lymphoma subtypes.

Somatic *HVEM* Mutations in B-cell Lymphoma

The incidence of non-synonymous mutations in the HVEM gene is high. Cheung et. al. [47] identified 47 cases (18%) with mutations affecting *HVEM* in a cohort of 251 FL patients. These mutations were associated with inferior outcome and high-risk clinical features in

addition to an increased risk of transformation to DLBCL. Another study showed similar results with *HVEM* mutations in FL, but with an even higher frequency (44%). However, the latter cohort was not associated with diminished survival [48]. Frequent deletion of chromosome 1p36.22–36.33 involving *HVEM* occurred in 25% of FL patients and were associated with poor outcome [46]. More recently, deep-sequencing approaches in DLBCL uncovered recurrent mutations in *HVEM*, and of interest, all of the reported mutations were exclusive to the germinal center B cell type [49,50]. Since the cell-of-origin in follicular lymphoma and Hodgkin Reed Sternberg cells are believed to be a (post-)germinal center B cell, *HVEM* inactivation might be exclusively associated with germinal center-driven lymphomagenesis.

Mutational Spectrum in *HVEM*

Targeted DNA re-sequencing of the *TNFRSF14* coding exons found 47 mutations in total from 251 FL patients [47]. Furthermore, genome-wide RNA sequencing revealed additional mutations in FL and DLBCL clinical samples [50]. The mutational spectrum indicated some “hot spot” areas (Figure 2). Several mutations overlap with mutations in *HVEM* reported by Launay *et al* [48], suggesting that these recurrently altered sites might be functionally relevant. Although gene inactivating mutations are predominant in *HVEM*, the majority of point mutants are almost exclusively located in the extracellular domain suggesting the potential to alter ligand binding. In FL and DLBCL, where *HVEM* expression is frequently deleted, this mutational pattern could reflect different selective pressures at work to override the intrinsic (*cis*) inhibitory *HVEM*-*BTLA* signaling pathway. The *HVEM*-*BTLA* cancer genotype is consistent with the dual roles of *BTLA* and *HVEM* in activation of $\text{NF}\kappa\text{B}$ survival pathways and as an inhibitory pathway. We also suggest that mutations in *HVEM* or *BTLA*, owing to its counter regulatory actions [33], will likely lead to significant perturbations in the $\text{LT}\alpha\beta$ - $\text{LT}\beta\text{R}$ network, thus altering local microenvironments. Together with human genetic data these results implicate *HVEM**BTLA* as a possible control point in B cell lymphoma development and progression.

Targeting the $\text{LT}\alpha\beta$ -*LIGHT* Network

Therapeutic rewiring of the $\text{LT}\alpha\beta$ -*LIGHT* Network can provide microenvironments suitable to induce immune rejection overriding intrinsic mutations in cancer cells. Most instructive in this regard is the demonstration that *LIGHT* can induce a strong anti-tumor response *in vivo*. Ectopic expression of *LIGHT* in tumor cells leads to a robust and sustained anti-tumor immune response dependent on NK cells, $\text{IFN}\gamma$, and CD8^+ T cells [51]. *LIGHT* expression in tumor cells led to an increase in $\text{LT}\beta\text{R}$ -regulated chemokines and cytokines within the tumor, which may form a more lymphoid-like environment that allows for increased priming of naïve T cells within the tumor site. The anti-tumor effects of *LIGHT* likely depend on both *HVEM* and $\text{LT}\beta\text{R}$, as *HVEM* is necessary for NK cell activation [51]. Further work has demonstrated *LIGHT* delivered to tumors through adenoviral vectors or engineered mesenchymal stem cells [52] are effective at inducing meaningful anti-tumor responses and tumor regression, demonstrating that the antitumor effects of *LIGHT* do not depend on tumor-specific *LIGHT* expression.

Conclusions

The foregoing discussion provides strong evidence supporting the idea that the $\text{LT}\alpha\beta$ -*LIGHT* Network is involved in cancer development and progression. Principles emerging from investigating these cytokines in infectious and inflammatory diseases have provided a framework to understand the selective pressures that are operative in cancer (Figure 3). In the cancer setting, the expression of *HVEM* in melanoma cells would likely provide a

selective advantage by suppressing immune rejection via BTLA, and cell survival signaling via HVEM (bidirectional mode) in the tumor cell. However, the loss of HVEM observed in other solid tumors may lead to enhanced inflammation, perhaps via increased LT $\alpha\beta$ -LT β R signaling or loss of lymphocyte inhibition through BTLA, creating tumor-promoting microenvironments, as observed in the hepatocellular and prostate models [28,32]. In FL and DLBCL, HVEM loss suggests a strong selective pressure from environmental interactions.

The linkage of tumor cells with the inflammatory microenvironment through LT $\alpha\beta$ -LT β R and LIGHT/HVEM/BTLA pathways offers potential for significant therapeutic intervention. The specificity inherent in receptor and antibody therapeutics provides a direct route to rewiring these pathways to achieve efficacy in controlling cancer progression. Antagonism of the LT β R pathway utilizing LT β R-Fc (bamnercept) may deprive the microenvironment of key inflammation-driven survival factors required by the HCC cells. TNF α is a contributing inflammation factor in the tumor milieu suggesting that antagonism of TNFR signaling with anti-TNF may be warranted. In HVEM-deficient FL or DLCL agonist activation of BTLA could attenuate signals required for continued lymphoma progression. However, the efficacy of these approaches will clearly depend on knowledge of the mutational spectrum contained in an individual patient's tumor. For example, mutations affecting signaling components downstream of the receptor (such as deletions in TRAF3 leading to constitutively active NF κ B mediated survival signaling) could nullify the effects of antagonizing these cytokines produced by inflammatory cells in the tumor microenvironment. Accumulating evidence indicates the LT $\alpha\beta$ -LIGHT Network contributes strong selective pressures for tumor evolution and immune evasion in both solid tumor and hematologic malignancies. We suggest that effective therapy utilizing these pathways will need to consider both targeting of tumor cells and the microenvironment to achieve optimal effectiveness.

Acknowledgments

The authors would like to recognize grant support from the US National Institutes of Health (AI033068, CA164679 and AI48073 to CFW), and support from the Terry Fox Foundation New Frontiers Program Project (Grant 019001) and Genome Canada/BC Grant Competition III (To RDG). CS is supported by a Career Investigator Award from the Michael Smith Foundation for Health Research and RB is supported by an NIH Postdoctoral Fellowship (T32CA121949). The bioinformatics and artwork assistance of Fong Chun Chan and Vasileios Bekiaris is gratefully appreciated.

References

1. Porta C, Larghi P, Rimoldi M, Totaro MG, Allavena P, Mantovani A, Sica A. Cellular and molecular pathways linking inflammation and cancer. *Immunobiology*. 2009; 214:761–777. [PubMed: 19616341]
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–674. [PubMed: 21376230] . Often cited as the blueprint for cancer research.
3. Kuppers R. The biology of Hodgkin's lymphoma. *Nat Rev Cancer*. 2009; 9:15–27. [PubMed: 19078975]
4. Staudt LM. Oncogenic activation of NF-kappaB. *Cold Spring Harb Perspect Biol*. 2010; 2:a000109. [PubMed: 20516126]
5. Gascoyne RD, Rosenwald A, Poppema S, Lenz G. Prognostic biomarkers in malignant lymphomas. *Leuk Lymphoma*. 2010; 51(Suppl 1):11–19. [PubMed: 20658955]
6. Steidl C, Connors JM, Gascoyne RD. Molecular pathogenesis of Hodgkin's lymphoma: increasing evidence of the importance of the microenvironment. *J Clin Oncol*. 2011; 29:1812–1826. [PubMed: 21483001]

7. Hjelmstrom P. Lymphoid neogenesis: de novo formation of lymphoid tissue in chronic inflammation through expression of homing chemokines. *J Leukoc Biol.* 2001; 69:331–339. [PubMed: 11261778]
8. Ware CF. The TNF receptor super family in immune regulation. *Immunol Rev.* 2011; 244:5–8. [PubMed: 22017427]
9. Cyster JG. Lymphoid organ development and cell migration. *Immunol Rev.* 2003; 195:5–14. [PubMed: 12969306]
10. Schneider K, Loewendorf A, De Trez C, Fulton J, Rhode A, Shumway H, Ha S, Patterson G, Pfeffer K, Nedospasov SA, et al. Lymphotoxin-mediated crosstalk between B cells and splenic stroma promotes the initial type I interferon response to cytomegalovirus. *Cell Host Microbe.* 2008; 3:67–76. [PubMed: 18312841]
11. Moseman EA, Iannacone M, Bosurgi L, Tonti E, Chevrier N, Tumanov A, Fu YX, Hacohen N, von Andrian UH. B Cell Maintenance of Subcapsular Sinus Macrophages Protects against a Fatal Viral Infection Independent of Adaptive Immunity. *Immunity.* 2012; 36:415–426. [PubMed: 22386268]
12. Sun SC. Non-canonical NF-kappaB signaling pathway. *Cell Res.* 2011; 21:71–85. [PubMed: 21173796]
13. den Haan JM, Mebius RE, Kraal G. Stromal cells of the mouse spleen. *Front Immunol.* 2012; 3:201. [PubMed: 22807924]
14. Yi T, Wang X, Kelly LM, An J, Xu Y, Sailer AW, Gustafsson JA, Russell DW, Cyster JG. Oxysterol Gradient Generation by Lymphoid Stromal Cells Guides Activated B Cell Movement during Humoral Responses. *Immunity.* 2012; 37:535–548. [PubMed: 22999953]
15. Honke N, Shaabani N, Cadeddu G, Sorg UR, Zhang DE, Trilling M, Klingel K, Sauter M, Kandolf R, Gailus N, et al. Enforced viral replication activates adaptive immunity and is essential for the control of a cytopathic virus. *Nat Immunol.* 2012; 13:51–57. [PubMed: 22101728]
16. Ruddle NH, Akirav EM. Secondary lymphoid organs: responding to genetic and environmental cues in ontogeny and the immune response. *J Immunol.* 2009; 183:2205–2212. [PubMed: 19661265]
17. Mounzer RH, Svendsen OS, Baluk P, Bergman CM, Padera TP, Wiig H, Jain RK, McDonald DM, Ruddle NH. Lymphotoxin-alpha contributes to lymphangiogenesis. *Blood.* 2010; 116:2173–2182. [PubMed: 20566898]
18. Liao S, Bentley K, Lebrun M, Lesslauer W, Ruddle FH, Ruddle NH. Transgenic LacZ under control of Hec-6st regulatory sequences recapitulates endogenous gene expression on high endothelial venules. *Proc Natl Acad Sci U S A.* 2007; 104:4577–4582. [PubMed: 17360566]
19. Nasr IW, Reel M, Oberbarnscheidt MH, Mounzer RH, Baddoura FK, Ruddle NH, Lakkis FG. Tertiary lymphoid tissues generate effector and memory T cells that lead to allograft rejection. *Am J Transplant.* 2007; 7:1071–1079. [PubMed: 17359505]
20. Steinberg MW, Cheung TC, Ware CF. The signaling networks of the herpesvirus entry mediator (TNFRSF14) in immune regulation. *Immunol Rev.* 2011; 244:169–187. [PubMed: 22017438]
21. Murphy TL, Murphy KM. Slow down and survive: Enigmatic immunoregulation by BTLA and HVEM. *Annu Rev Immunol.* 2010; 28:389–411. [PubMed: 20307212]
22. Steinberg MW, Turovskaya O, Shaikh RB, Kim G, McCole DF, Pfeffer K, Murphy KM, Ware CF, Kronenberg M. A crucial role for HVEM and BTLA in preventing intestinal inflammation. *J Exp Med.* 2008; 205:1463–1476. [PubMed: 18519647]
23. Soroosh P, Doherty TA, So T, Mehta AK, Khorram N, Norris PS, Scheu S, Pfeffer K, Ware C, Croft M. Herpesvirus entry mediator (TNFRSF14) regulates the persistence of T helper memory cell populations. *J Exp Med.* 2011; 208:797–809. [PubMed: 21402741]
24. Hurchla MA, Sedy JR, Murphy KM. Unexpected role of B and T lymphocyte attenuator in sustaining cell survival during chronic allostimulation. *J Immunol.* 2007; 178:6073–6082. [PubMed: 17475832]
25. Sakoda Y, Park JJ, Zhao Y, Kuramasu A, Geng D, Liu Y, Davila E, Tamada K. Dichotomous regulation of GVHD through bidirectional functions of the BTLAHVEM pathway. *Blood.* 2011; 117:2506–2514. [PubMed: 21220749]

26. Del Rio ML, Jones ND, Buhler L, Norris P, Shintani Y, Ware CF, Rodriguez-Barbosa JI. Selective Blockade of Herpesvirus Entry Mediator-B and T Lymphocyte Attenuator Pathway Ameliorates Acute Graft-versus-Host Reaction. *J Immunol.* 2012; 188:4885–4896. [PubMed: 22490863]
27. Vendel AC, Calemine-Fenaux J, Izrael-Tomasevic A, Chauhan V, Arnott D, Eaton DL. B and T lymphocyte attenuator regulates B cell receptor signaling by targeting Syk and BLNK. *J Immunol.* 2009; 182:1509–1517. [PubMed: 19155498]
28. Haybaeck J, Zeller N, Wolf MJ, Weber A, Wagner U, Kurrer MO, Bremer J, Iezzi G, Graf R, Clavien PA, et al. A lymphotoxin-driven pathway to hepatocellular carcinoma. *Cancer Cell.* 2009; 16:295–308. [PubMed: 19800575] . This work provided key evidence for a LT $\alpha\beta$ driven link between inflammation and cancer through changes in the tumor microenvironment.
29. Tumanov AV, Koroleva EP, Christiansen PA, Khan MA, Ruddy MJ, Burnette B, Papa S, Franzoso G, Nedospasov SA, Fu YX, et al. T cell-derived lymphotoxin regulates liver regeneration. *Gastroenterology.* 2009; 136:694–704. e694. [PubMed: 18952083]
30. Subrata LS, Lowes KN, Olynyk JK, Yeoh GC, Quail EA, Abraham LJ. Hepatic expression of the tumor necrosis factor family member lymphotoxin-beta is regulated by interleukin (IL)-6 and IL-1beta: transcriptional control mechanisms in oval cells and hepatoma cell lines. *Liver Int.* 2005; 25:633–646. [PubMed: 15910501]
31. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, et al. IL-6 and stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell.* 2009; 15:103–113. [PubMed: 19185845]
32. Ammirante M, Luo JL, Grivennikov S, Nedospasov S, Karin M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature.* 2010; 464:302–305. [PubMed: 20220849] . This model identified B cell expressed LT $\alpha\beta$ mediates prostate cancer progression.
33. De Trez C, Ware CF. The TNF receptor and Ig superfamily members form an integrated signaling circuit controlling dendritic cell homeostasis. *Cytokine Growth Factor Rev.* 2008; 19:277–284. [PubMed: 18511331]
34. Beroukhi R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, Barretina J, Boehm JS, Dobson J, Urashima M, et al. The landscape of somatic copynumber alteration across human cancers. *Nature.* 2010; 463:899–905. [PubMed: 20164920]
35. Fourcade J, Sun Z, Pagliano O, Guillaume P, Luescher IF, Sander C, Kirkwood JM, Olive D, Kuchroo V, Zarour HM. CD8(+) T cells specific for tumor antigens can be rendered dysfunctional by the tumor microenvironment through upregulation of the inhibitory receptors BTLA and PD-1. *Cancer Res.* 2012; 72:887–896. [PubMed: 22205715]
36. Derre L, Rivals JP, Jandus C, Pastor S, Rimoldi D, Romero P, Michielin O, Olive D, Speiser DE. BTLA mediates inhibition of human tumor-specific CD8+ T cells that can be partially reversed by vaccination. *J Clin Invest.* 2010; 120:157–167. [PubMed: 20038811]
37. Farren TW, Giustiniani J, Liu FT, Tsitsikas DA, Macey MG, Cavenagh JD, Oakervee HE, Taussig D, Newland AC, Calaminici M, et al. Differential and tumor-specific expression of CD160 in B-cell malignancies. *Blood.* 2011
38. Chabot S, Jabrane-Ferrat N, Bigot K, Tabiasco J, Provost A, Golzio M, Noman MZ, Giustiniani J, Bellard E, Brayer S, et al. A novel antiangiogenic and vascular normalization therapy targeted against human CD160 receptor. *J Exp Med.* 2011; 208:973–986. [PubMed: 21482699]
39. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, Fisher RI, Braziel RM, Rimsza LM, Grogan TM, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N Engl J Med.* 2004; 351:2159–2169. [PubMed: 15548776]
40. Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, Delaney A, Jones SJ, Iqbal J, Weisenburger DD, et al. Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. *N Engl J Med.* 2010; 362:875–885. [PubMed: 20220182]
41. Farinha P, Al-Tourah A, Gill K, Klasa R, Connors JM, Gascoyne RD. The architectural pattern of FOXP3-positive T cells in follicular lymphoma is an independent predictor of survival and histologic transformation. *Blood.* 2010; 115:289–295. [PubMed: 19901260]

42. Lenz G, Wright G, Dave SS, Xiao W, Powell J, Zhao H, Xu W, Tan B, Goldschmidt N, Iqbal J, et al. Stromal gene signatures in large-B-cell lymphomas. *N Engl J Med*. 2008; 359:2313–2323. [PubMed: 19038878]
43. Rehm A, Mensen A, Schradi K, Gerlach K, Wittstock S, Winter S, Buchner G, Dorken B, Lipp M, Hopken UE. Cooperative function of CCR7 and lymphotoxin in the formation of a lymphoma-permissive niche within murine secondary lymphoid organs. *Blood*. 2011; 118:1020–1033. [PubMed: 21586747]
44. Bjordahl RL, Gapin L, Marrack P, Refaeli Y. iNKT cells suppress the CD8+ T cell response to a murine Burkitt's-like B cell lymphoma. *PLoS ONE*. 2012; 7:e42635. [PubMed: 22880059] . This model revealed unexpected complexity in cells involved in immune rejection of lymphoma.
45. Sedy JR, Spear PG, Ware CF. Cross-regulation between herpesviruses and the TNF superfamily members. *Nat Rev Immunol*. 2008; 8:861–873. [PubMed: 18949019]
46. Cheung KJ, Shah SP, Steidl C, Johnson N, Relander T, Telenius A, Lai B, Murphy KP, Lam W, Al-Tourah AJ, et al. Genome-wide profiling of follicular lymphoma by array comparative genomic hybridization reveals prognostically significant DNA copy number imbalances. *Blood*. 2009; 113:137–148. [PubMed: 18703704]
47. Cheung KJ, Johnson NA, Affleck JG, Severson T, Steidl C, Ben-Neriah S, Schein J, Morin RD, Moore R, Shah SP, et al. Acquired TNFRSF14 mutations in follicular lymphoma are associated with worse prognosis. *Cancer Res*. 2010; 70:9166–9174. [PubMed: 20884631] . This work identified the clinical importance of HVEM (TNFRSF14) mutations in human lymphoma.
48. Launay E, Pangault C, Bertrand P, Jardin F, Lamy T, Tilly H, Tarte K, Bastard C, Fest T. High rate of TNFRSF14 gene alterations related to 1p36 region in de novo follicular lymphoma and impact on prognosis. *Leukemia*. 2012; 26:559–562. [PubMed: 21941365]
49. Lohr JG, Stojanov P, Lawrence MS, Auclair D, Chapuy B, Sougnez C, Cruz-Gordillo P, Knoechel B, Asmann YW, Slager SL, et al. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. *Proc Natl Acad Sci U S A*. 2012; 109:3879–3884. [PubMed: 22343534]
50. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, Johnson NA, Severson TM, Chiu R, Field M, et al. Frequent mutation of histonemodifying genes in non-Hodgkin lymphoma. *Nature*. 2011; 476:298–303. [PubMed: 21796119]
51. Fan Z, Yu P, Wang Y, Fu ML, Liu W, Sun Y, Fu YX. NK-cell activation by LIGHT triggers tumor-specific CD8+ T-cell immunity to reject established tumors. *Blood*. 2006; 107:1342–1351. [PubMed: 16223768]
52. Zou W, Zheng H, He TC, Chang J, Fu YX, Fan W. LIGHT delivery to tumors by mesenchymal stem cells mobilizes an effective antitumor immune response. *Cancer Res*. 2012; 72:2980–2989. [PubMed: 22511579]
53. Bolstad AI, Le Hellard S, Kristjansdottir G, Vasaitis L, Kvarnstrom M, Sjowall C, Johnsen SJ, Eriksson P, Omdal R, Brun JG, et al. Association between genetic variants in the tumour necrosis factor/lymphotoxin alpha/lymphotoxin beta locus and primary Sjogren's syndrome in Scandinavian samples. *Ann Rheum Dis*. 2012; 71:981–988. [PubMed: 22294627]
54. Fu Z, Li D, Jiang W, Wang L, Zhang J, Xu F, Pang D. Association of BTLA gene polymorphisms with the risk of malignant breast cancer in Chinese women of Heilongjiang Province. *Breast Cancer Res Treat*. 2010; 120:195–202. [PubMed: 19585237]
55. Tello-Ruiz MK, Curley C, DelMonte T, Giallourakis C, Kirby A, Miller K, Wild G, Cohen A, Langelier D, Latiano A, et al. Haplotype-based association analysis of 56 functional candidate genes in the IBD6 locus on chromosome 19. *Eur J Hum Genet*. 2006; 14:780–790. [PubMed: 16570073]
56. Cheung TC, Coppieters K, Sanjo H, Osborne LM, Norris PS, Coddington A, Granger SW, Elewaut D, Ware CF. Polymorphic variants of LIGHT (TNF superfamily-14) alter receptor avidity and bioavailability. *J Immunol*. 2010; 185:1949–1958. [PubMed: 20592286]
57. Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L, Dilthey A, Su Z, Freeman C, Hunt SE, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*. 2011; 476:214–219. [PubMed: 21833088]

58. Dubois PC, Trynka G, Franke L, Hunt KA, Romanos J, Curtotti A, Zhernakova A, Heap GA, Adany R, Aromaa A, et al. Multiple common variants for celiac disease influencing immune gene expression. *Nat Genet.* 2010; 42:295–302. [PubMed: 20190752]
59. Folseraas T, Melum E, Rausch P, Juran BD, Ellinghaus E, Shiryayev A, Laerdahl JK, Ellinghaus D, Schramm C, Weismuller TJ, et al. Extended analysis of a genomewide association study in primary sclerosing cholangitis detects multiple novel risk loci. *J Hepatol.* 2012; 57:366–375. [PubMed: 22521342]
60. Kurreeman FA, Stahl EA, Okada Y, Liao K, Diogo D, Raychaudhuri S, Freudenberg J, Kochi Y, Patsopoulos NA, Gupta N, et al. Use of a multiethnic approach to identify rheumatoid- arthritis-susceptibility loci, 1p36 and 17q12. *Am J Hum Genet.* 2012; 90:524–532. [PubMed: 22365150]
61. Coenen MJ, Trynka G, Heskamp S, Franke B, van Diemen CC, Smolonska J, van Leeuwen M, Brouwer E, Boezen MH, Postma DS, et al. Common and different genetic background for rheumatoid arthritis and coeliac disease. *Hum Mol Genet.* 2009; 18:4195–4203. [PubMed: 19648290]
62. Bassols J, Moreno JM, Ortega F, Ricart W, Fernandez-Real JM. Characterization of herpes virus entry mediator as a factor linked to obesity. *Obesity (Silver Spring).* 2010; 18:239–246. [PubMed: 19680232]

Highlights

- Lymphotoxin and LIGHT mediate inflammation in a range of human autoimmune diseases.
- The Lymphotoxin-LIGHT network mediates cancer inflammation in hematologic and solid tumors.
- Frequent somatic mutations implicate *HVEM* as a universal tumor suppressor gene in lymphoma
- Rewiring Lymphotoxin LIGHT Network can alter tissue microenvironments to promote tumor immunity.

Figure 1. The LTαβ-LIGHT Network

The diagram depicts the binding interactions between cytokines and receptors related to Lymphotoxins. The arrows define the specificity of the ligand-receptor interactions. Arrowheads define the directionality of signaling, with dual arrowheads defining bidirectional signaling. The TNF-related ligands include TNFα, LTα, LTαβ₂, LIGHT (TNFSF14), and are shown as trimers in their membrane-bound form and expressed in lymphoid cells. Their cognate receptors, TNFR I, TNFR II, LTβR, and HVEM (TNFRSF14) are expressed in stromal and myeloid cells. Decoy receptor-3 is secreted and also binds Fas Ligand and TL1A (TNFSF25)(not shown). HVEM binds the Ig superfamily members BTLA and CD160, which form bidirectional signaling pathways. BTLA and HVEM are

coexpressed in lymphocytes forming a complex *in cis*. Not shown in this diagram are herpesvirus proteins gD and UL144 that signal via HVEM or BTLA.

Figure 3. LTαβ-LIGHT Network in Lymphoma

Depicted are the signaling interactions between B cells, T cells and stromal cells via the LTαβ-LIGHT Network. HVEM binding in *cis* or *trans* with BTLA confers inhibitory signaling in T cells or B cells via recruitment of the tyrosine phosphatases, SHP1/2 to the immunotyrosine inhibitory motif in BTLA. LIGHT can disrupt the HVEM-BTLA *cis* complex to activate NFκB and survival gene expression. BTLA binding HVEM *in trans* can also activate NFκB. LTαβ activation in stromal cells induces chemokine secretion recruiting T cells to the microenvironment. Mutations in HVEM suggest HVEM-BTLA serves as a putative checkpoint limiting lymphoma development. The checkpoint is created via intrinsic HVEM-BTLA *cis* complex or by HVEM expressing cells in microenvironment (*trans* signaling). With functional loss of HVEM, inhibitory signaling is lost and may contribute to lymphoma progression.

Table 1Genetic Linkage of the LT α β -LIGHT Network to Human Disease

Gene	Disease Association	Reference
<i>LT</i> β	Sjögren's Syndrome	[53]
<i>BTLA</i>	Breast Cancer	[54]
<i>LIGHT</i> (<i>TNFSF14</i>)	Inflammatory bowel diseases	[55,56]
<i>LIGHT</i>	Multiple sclerosis	[57]
<i>HVEM</i> (<i>TNFRSF14</i>)	Multiple sclerosis	[57]
<i>HVEM</i> (<i>TNFRSF14</i>)	Celiac disease	[58]
<i>HVEM</i> (<i>TNFRSF14</i>)	Sclerosing cholangitis	[59]
<i>HVEM</i> (<i>TNFRSF14</i>)	Rheumatoid arthritis	[60,61]
<i>HVEM</i> (<i>TNFRSF14</i>)	Obesity	[62]
<i>HVEM</i> (<i>TNFRSF14</i>)	Follicular lymphoma	[47]