

To be clear, the fact that protein S deficiency is a risk factor for venous thrombosis is not a matter of controversy.<sup>3</sup> Based on my reading of the study, Buil et al are now proposing that the bonding between C4BP and protein S, compelling as it has been due to the function in blood coagulation, may have led to the impression that further investigations into any additional genetic factors with direct ties to this specific pathway were unnecessary.

In the broadest sense, genome-wide association studies liberate basic researchers from preexisting hypotheses so that even if particular loci have dominated discussions in the context of a disease, the genome-wide association approach provides an unbiased method to interrogate the genome for other significant loci. This approach has paid off; their results suggest that C4BP merits its own discussion, apart from protein S binding, as another avenue of investigation into the risk for venous thrombosis.<sup>1</sup>

While the C4BP locus emerged as an independent player on the stage of venous thrombosis from this study, its prominent molecular liaison, protein S, has dropped out of the picture, which feels a bit uncomfortable given the appreciation of the importance of this bond between the 2 proteins.<sup>4</sup> Not only was protein S not present in the genome-wide association studies presented in the paper, but the authors went to some length to confirm that the SNPs were not associated with protein S levels. However, because each of the 4 study components was rather independent, with its own strengths and limitations, the evidence *against* protein S perhaps could have been stronger. Moreover, the authors rely heavily on the interpretation of linkage disequilibrium to connect study components that relied on different sets of SNPs, but in the end, leave the entire CBPB locus standing as a candidate (see figure). Linkage disequilibrium within and between the genes at the CBPB locus likely will render further fine-scale mapping attempts tricky.<sup>2</sup> Finally, attempts to replicate or expand on this study need to consider that linkage disequilibrium between key SNPs might vary between study cohorts drawn from general populations of various genomic backgrounds.<sup>7</sup>

The work by Buil et al should set the stage for basic research into the unknown pathways that contribute to the risk of venous thrombosis in the general population. It is common for the variances in complex

phenotypic trait values that can be explained by candidate SNPs to be underwhelming.<sup>2</sup> Thus, the ability to account for approximately 11% of the variance in expression levels of C4BPA and plasma levels of unbound  $\alpha_7\beta_0$  should be looked upon as great success that is consistent with the authors' view that the study of the unbound C4BP  $\alpha_7\beta_0$  isoform could be a productive field for investigation on its own. However, although compelling, results by others indicate that the study of the interaction between protein S and C4BP continues to yield results of interest with regard to quantitative variation in protein S,<sup>8</sup> and thus, that it may be premature to throw out the baby with the bath water.

In all, the result that the up-regulation of unbound C4b-complementary binding protein (C4BP), but not protein S, emerged from these analyses as a genetic risk factor for venous thrombosis is an unexpected but needed impetus for an expanded view of the factors involved in venous thrombosis. It also merits a shift from protein S-centric research. Despite the potential fallacies of array-based genome-wide association studies,<sup>2</sup> in this and a few other cases a bird's-eye view of the genome has enabled Buil et al to spot a new target well worth pursuing on the ground.<sup>1,9,10</sup>

## ● ● ● IMMUNOBIOLOGY

Comment on Kumar et al, page 4725

# Bulging glands? Blame it on B cells

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Gross lymph node morphologic changes often accompany infections. In this issue of *Blood*, Kumar and colleagues show that B cells contribute to lymphoid remodeling via LTs following viral insults.<sup>1</sup>

**A** crucial function of the lymph node (LN) is to facilitate physical interactions among rare immune cells arriving from various tissue compartments. The unique positioning of the LN at the interface between the blood and lymphatic systems allows tissue-derived antigen and antigen-presenting cells (APCs) to congregate in close proximity to blood-derived lymphocytes. Increased lymphocyte recruitment, decreased egress, and

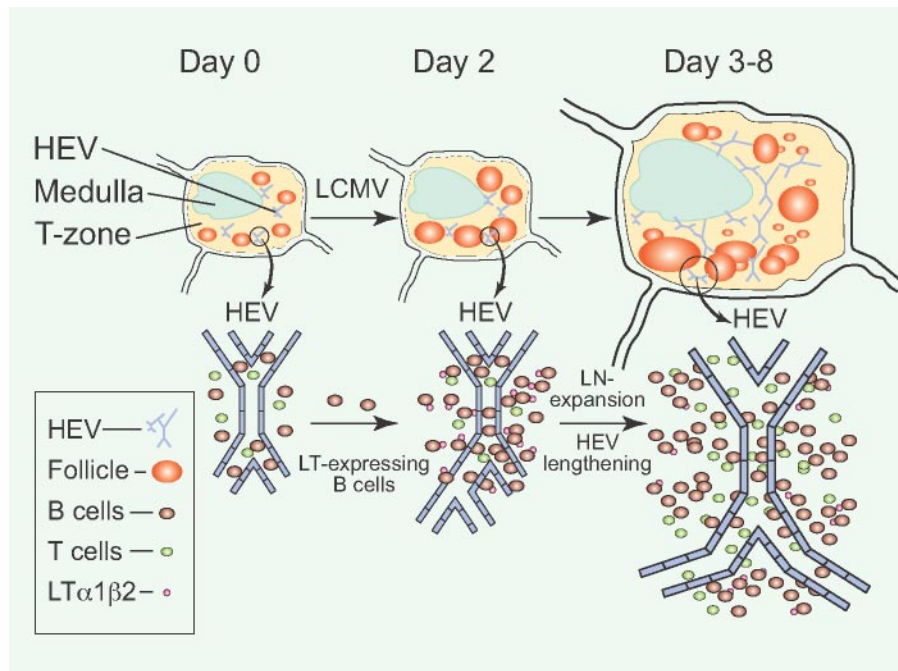
*Conflict-of-interest disclosure:* The author declares no competing financial interests. ■

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enhanced cytokine/chemokine communication network, together with changes in LN architecture to accommodate the massive cellular influx, all conspire to accomplish one goal: to enhance physical encounters between relevant lymphocytes and APCs that lead to timely and efficient immune activation.<sup>2,3</sup>

In recent years, there has been an increased appreciation of the role of B cells in lymphoid tissue organogenesis and immune response



**LT $\alpha$ -expressing B cells contribute to lymph node remodeling after LCMV infection.** Circulating B cells are induced to express membrane-bound LT $\alpha$  upon entering the LN early during LCMV infection on day 0. A large influx of B cells occurs during the first 2 days, accompanied by increasing B-cell follicle size and HEV branch formation. Enlargement of LN size, lengthening of HEV, and new B-cell follicle formation then ensue from day 3 to day 8 after LCMV infection.<sup>1</sup> Professional illustration by Paulette Dennis.

modulation. Specifically, B cell–derived lymphotoxin (LT) plays a vital role in the development of B-cell follicles, T-cell zones, follicular dendritic cells (FDC), splenic stromal cell subsets, and ectopic lymphoid tissue in autoimmune target organs.<sup>4–10</sup> Studies have shown that LTs, together with tumor necrosis factor (TNF) and LIGHT (LT-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells), compose an integrated signaling network necessary for efficient innate and adaptive immune responses.<sup>11</sup> There are 2 major forms of LT molecules with distinct receptor utilizations. While the homo-trimeric LT $\alpha$ 3 is secreted as a soluble molecule and binds to TNFR1 and TNFR2 but not LT $\beta$ R, only the membrane-bound hetero-trimeric LT $\alpha$ 1 $\beta$ 2 (LT $\alpha$  $\beta$ ) binds to LT $\beta$ R, which is constitutively present on stromal fibroblasts, epithelial cells, and myeloid cells. Cytokines (eg, IL-4, IL7) and chemokines (eg, CCL19, CCL21) can induce membrane-bound LT $\alpha$  $\beta$  on splenic T cells; however, most naive follicular B cells and naive CD4<sup>+</sup> T cells constitutively express LT $\alpha$  $\beta$ . As they migrate to the blood, B cells rapidly lose LT $\alpha$  $\beta$  expression. Upon entering the LN, however, CXCR5<sup>+</sup> B cells re-express membrane-bound LT $\alpha$  $\beta$  upon sensing a CXCL13 gradient produced by

LT $\beta$ R<sup>+</sup> stromal cells. Direct stromal cell–B cell interaction through the LT $\alpha$  $\beta$ –LT $\beta$ R axis further promotes production of CXCL13 by stromal cells, thereby completing the circuit.<sup>8,10</sup> B-cell expression of LT $\alpha$  $\beta$  is further induced by CD40–CD40L interaction.<sup>12</sup> The LT $\alpha$  $\beta$ –LT $\beta$ R signaling therefore allows unidirectional communication between lymphocytes and surrounding stromal and parenchymal cells in a cell–cell contact–dependent manner.

Kumar et al investigated the global 3D LN structural remodeling phenomenon during infection with lymphocytic choriomeningitis virus (LCMV) by using a novel mesoscopic imaging tool, the optical projection tomography (OPT).<sup>1</sup> This new imaging technique bridges the resolution gap between whole-animal imaging techniques (such as magnetic resonance imaging [MRI] and computed tomography [CT]) and microscopic confocal/2-photon cellular imaging modalities. The OPT technique captures static images of the whole-organ with resolution at the follicle and high endothelial venule (HEV) level, while allowing quantitative fluorescence analyses of structural data. Using this and other techniques, the authors provide evidence that LN enlargement and remodeling following LCMV infection is also dependent on the ac-

tion of LT $\alpha$  $\beta$ -expressing B cells for the induction and maintenance of LN volume and HEV expansion (see figure). Transfer of wild-type B cells into B cell–deficient JHT recipient mice demonstrates a dose-dependency of B cells on LN growth. This LN expansion is independent of VEGF-A, as administration of a VEGFR inhibitor, sunitinib, and anti-VEGFR2/anti-VEGF-A neutralizing antibodies did not alter LN growth in the LCMV infection model. Interestingly, although B cells constitute a major source of LT, approximately 30% of post-LCMV LN enlargement and HEV lengthening is unaccounted for by B cells alone. Thus, the search for other sources of LT continues.

Unresolved questions remain to fully depict the process of infection-related LN remodeling. The precise LT-mediated molecular and cellular network responsible for inducing LN growth needs further study. Scandella et al showed that LN mRNA levels of LT $\alpha$  and LT $\beta$ R were elevated early in LCMV infection.<sup>13</sup> As LT $\beta$ R is expressed by stromal cells including HEV and FRC, cross-linking LT $\beta$ R by LT has been shown to induce VEGF-A expression. Although the current data presented by Kumar et al do not support a role for VEGF-A in LN enlargement and HEV growth in LCMV infection, its role in LN enlargement associated with other infections needs to be clarified. The role of T cells, particularly constitutive LT-expressing CD4<sup>+</sup> T cells also needs to be further defined. The use of inducible, tissue-restricted LT-deficient animal models will help elucidate these issues. It is yet to be shown whether the dependence on LT and B cells is a general phenomenon in other non-LCMV infection-associated lymphoid remodeling processes. Indeed, the authors provide evidence suggesting that VSV-associated LN remodeling is not accompanied by HEV lengthening or changes in total LN volume.<sup>1</sup> It is therefore likely that the molecular mechanisms reported by Kumar et al do not fully describe all the molecular and cellular rules of LN morphogenesis.

The authors in the current study observed an association between HEV length and the absolute number of lymphocyte recruitment in the LN. This implies a direct correlation between the length of the HEV to the number of available port-of-entry sites for lymphocyte extravasations from general circulation.<sup>14</sup> This raises the debate of whether specific HEV “hot

spots” truly exist for lymphocyte extravasations, and future investigation will be needed to resolve this issue. Additionally, Bajenoff and Germain recently reported that the remodeling of the conduit system that allows efficient delivery of soluble antigens from the periphery to FDC accompanies B-cell follicle development. It remains to be determined what role, if any, LT $\alpha$  $\beta$  expression by B cell may contribute to this process.<sup>15</sup>

Finally, the observation presented in this report also poses the possibility that a similar paradigm may exist to accomplish LN resolution following viral clearance. As mRNA levels of LT $\alpha$  and LT $\beta$  diminish after day 4 after LCMV infection, there is a concomitant decrease in the number of B cells in the LN.<sup>13</sup> Understanding molecular and cellular identities involved in the restoration of LN architecture and lymphocyte homeostasis following viral insults will add to our knowledge base in understanding the complex communication network regulating immune responses in vivo. The current work by Kumar et al furthers our understanding in a rapidly evolving cutting-edge field and highlights the symbiotic relationship between anatomy and function in immunobiology.<sup>1</sup>

*Conflict-of-interest disclosure: The author declares no competing financial interests.* ■

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## ● ● ● LYMPHOID NEOPLASIA

Comment on Chen et al, page 4778

# Hitting the sweet spot for lymphoma

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Anyone who has ever played a racket sport, baseball, cricket, and/or golf can attest to experiencing the “sweet spot”—that place where the least jolt is felt on contact. In this issue of *Blood*, Chen et al show how sugar-decorated liposomes can be used to deliver doxorubicin in a targeted manner to malignant B cells, thus limiting the systemic jolt of this potent cytotoxic agent.<sup>1</sup>

As envisioned by scientists as far back as the 1800s, the ideal chemical therapy would possess exquisite selectivity, thereby restricting, if not eliminating, collateral cellu-

lar damage (eg, Ehrlich’s “magic bullet”). In cancer therapeutics, selectivity has been achieved to varying degrees of success by employing mAb, with (eg, gemtuzumab ozogami-

cin) or without (eg, rituximab) toxic conjugates. Although the advent of liposomal particles that carry an encapsulated chemotherapy cargo has reduced adverse effects typically associated with the chemotherapeutic agent and commensurately allows for higher drug dose levels, side effects of these agents have generally been attenuated, but not eliminated. Antibody-covered liposomes (immunoliposomes) incorporating relevant chemotherapeutic agents have been developed to further improve selectivity and, hence, the therapeutic index of encapsulated agents, but these agents are still not without significant toxicities.<sup>2</sup>

As an alternative to immunoliposomes, the work of Chen et al offers targeted delivery of liposomal doxorubicin to B cells by exploiting the ligand activity of a B cell-specific cell-surface protein, CD22 (also known as Siglec-2).<sup>3,4</sup> CD22 is a lectin, that is, a protein that binds carbohydrate structures, that belongs to the family of lectins known as Siglecs that recognize sialylated glycans (sialosides).<sup>4</sup> CD22 has specificity for sialic acid in  $\alpha(2,6)$ -linkage to galactose.<sup>5</sup> Importantly, CD22 displays highly efficient, constitutive endocytosis, which is accelerated by ligation.<sup>6</sup> Thus, binding to CD22 by cognate ligands (ie, sialosides) or by mAb would be expected to lead to rapid internalization of the relevant structure(s).<sup>7</sup>

The authors report that doxorubicin-loaded liposomal nanoparticles incorporating  $\alpha(2,6)$ -sialosides show significantly higher cytotoxicity to Daudi cells (a human Burkitt lymphoma cell line) in vitro compared with those using nontargeted doxorubicin-loaded liposomes. In vivo studies using immunocompromised mice injected with Daudi cells showed heightened cytotoxicity when using the sialoside-bearing doxorubicin-liposome preparation, with prolonged survival of tumor-bearing mice. Furthermore, the authors demonstrate that drug-loaded liposomes incorporating sialosides bind to B cells obtained from blood of patients with chronic lymphocytic leukemia, hairy cell leukemia, and marginal zone lymphoma, with binding efficiency correlating with expression of CD22. Notably, in vitro studies show that the capacity of sialoside-bearing drug-loaded liposomes to kill B cells is not proportional to expression of CD22, suggesting that the high efficiency of CD22-mediated drug internalization is sufficient to induce cell death even when surface CD22 levels are relatively low.