4

THE FUNCTION OF LOCAL LYMPHOID TISSUES IN PULMONARY IMMUNE RESPONSES

Juan Moyron-Quiroz, Javier Rangel-Moreno, Damian M. Carragher, and Troy D. Randall*

1. LYMPH NODE STRUCTURE AND DEVELOPMENT

Primary adaptive immune responses are initiated in secondary lymphoid organs, such as spleen, lymph nodes, and Peyer's patches. These lymphoid organs recruit naive lymphocytes[1] as well as activated antigen-presenting cells (APCs)[2], and facilitate lymphocyte activation, expansion, and differentiation. For example, infection of the lung with influenza virus leads to activation of pulmonary dendritic cells, which engulf local antigens and traffic to the draining mediastinal lymph node (MLN)[3], where they home to the T cell area surrounding the high endothelial venules (HEVs) (Figure 1). Naive B and T cells are constantly recruited into the lymph node via these HEVs and rapidly become activated as they encounter cognate antigen on APCs. Activated lymphocytes subsequently expand and differentiate into effector cells. For T cells, this differentiation primarily occurs in the T cell zone. In contrast, B cells rapidly expand and are selected for high-affinity variants in the germinal centers (GCs) that develop on the border between the T cell area and the B cell follicle. As the immune response progresses, effector B and T cells leave the lymph node via the efferent lymphatics, which drain into the blood via the thoracic duct. Once in the blood, activated effector cells recirculate to sites of inflammation, including the original site of infection in the lung, and use their effector functions to combat infection. An important point of this model is that, while infection occurs locally in non-lymphoid organs, primary immune responses are initiated centrally in secondary lymphoid organs. This scheme is outlined in Figure 1.

*From the Trudeau Institute, Saranac Lake, New York 12983, USA.

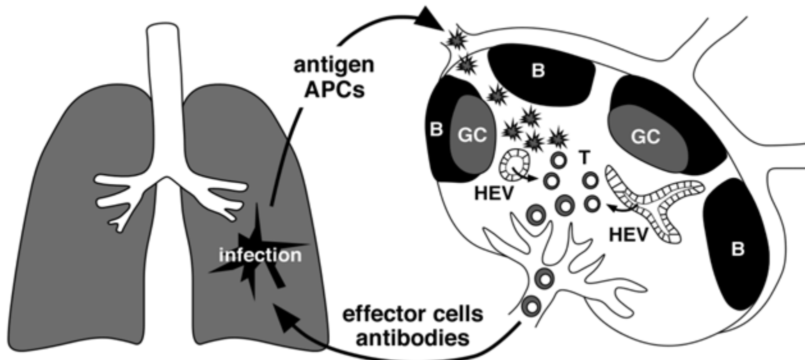

Figure 1. Secondary lymphoid organs acquire antigen and activated APCs from regional tissues and initiate primary immune responses. APCs enter the lymph node via afferent lymphatics and home to the T cell zone. Naive lymphocytes enter the lymph node via the HEVs and encounter antigen displayed on APCs. Effector lymphocytes leave the lymph node via efferent lymphatics.

Although there are similarities in the architecture of all secondary lymphoid organs, their structure differs depending on their location and function[4]. For example, the spleen samples antigens directly from the blood[5], while regional lymph nodes sample antigens from afferent lymphatics that drain regional tissues[4]. In contrast, mucosal lymphoid tissues, such as Peyer's patches and Nasal Associated Lymphoid Tissue (NALT) have no afferent lymphatics and sample antigens directly across the mucosal epithelium[6]. Therefore, each secondary lymphoid organ is specialized to acquire antigen from the regional tissues that it drains and to generate immune responses appropriate to those antigens.

Lymph node and Peyer's patch development occurs between day E11 of gestation and birth[7]. The development of these organs is dependent on the lymphotoxin (LT) signaling pathway and mice lacking LTα[8], LTβ[9], LTβR[10], or NFκB-inducing kinase[11] fail to develop most lymph nodes and Peyer's patches. Embryonic lymph node development is initiated by Lymphoid Tissue inducer cells (LTi cells), which provide lymphotoxin to local LTβR-expressing mesenchymal cells at sites of future lymph node development[12]. In turn, signals from the LTβR trigger mesenchymal cells to differentiate into mature stromal cells that express the chemokines and adhesion molecules necessary to recruit and maintain lymphocytes in mature lymph nodes[12]. In the absence of these chemokines, particularly CXCL13[13,14], LTi cells fail to migrate to sites of future lymph node formation and lymph nodes do not develop. Once formed, the architecture of secondary lymphoid organs is maintained by a positive feedback loop between lymphotoxin and the homeostatic chemokines[14]. In fact, lymphotoxin-dependent CXCL13 expression is required for maintenance of B cell follicles, formation of germinal centers, and differentiation of follicular dendritic cells[14]. In contrast, CCL21 is expressed primarily on HEVs and promotes rolling arrest

and migration across the vascular endothelium[15], while CCL19 expression on stromal cells is important for recruitment of T cells and activated APCs into the T cell area[16]. Like the expression of CXCL13, the expression of CCL19 and CCL21 is dependent on lymphotoxin[17]. Thus, lymphotoxin and homeostatic chemokines are essential for both the development of secondary lymphoid organs and for maintenance of proper lymphoid architecture.

2. ROLE OF LOCAL LYMPHOID ORGANS IN PULMONARY IMMUNITY

Despite the important role of secondary lymphoid organs in initiating primary immune responses, there are some indications that naive lymphocytes can be primed locally in non-lymphoid tissues. For example, adoptively transferred naive OTII TCR transgenic CD4 T cells can be primed by intranasal challenge with OVA in $LT\alpha^{-/-}$ hosts[18]. Since $LT\alpha^{-/-}$ mice lack all lymph nodes and Peyer's patches, these data suggest that the transferred T cells are primed directly in the lung tissue by pulmonary DCs. We also used $LT\alpha^{-/-}$ mice in our initial studies to address whether conventional secondary lymphoid organs were required for immune responses to influenza[19]. As expected based on studies showing that $LT\alpha^{-/-}$ mice are unable to generate primary immune responses to a variety of infectious agents[20–23], we found that $LT\alpha^{-/-}$ mice are more susceptible than normal mice to influenza. However, even though the onset of immunity is slightly delayed, $LT\alpha^{-/-}$ mice make robust immune responses to influenza[19]. For example, although the initial CD8 T cell response is slightly delayed, normal numbers of influenza-specific CD8 T cells are eventually generated that have normal cytotoxic activity and make normal levels of IFNγ upon restimulation. The B cell response is also slightly delayed, but ultimately produces above-normal levels of influenza-specific IgM and slightly below normal levels of influenza-specific IgG. Together, the T and B cell responses of $LT\alpha^{-/-}$ mice clear influenza virus from the lungs with only a slight delay[19]. Thus, primary immune responses to influenza are intact in $LT\alpha^{-/-}$ mice, despite their lack of lymph nodes.

2.1. Structure and Function of Nasal-Associated Lymphoid Tissue (NALT)

Given that $LT\alpha^{-/-}$ mice are also able to make an immune response to another respiratory virus, γHerpesvirus-68[24], we hypothesized that there is something unique about the respiratory tract that allows primary immune responses to occur in the absence of lymph nodes. One possibility is that some secondary lymphoid organs remain in the respiratory tract of $LT\alpha^{-/-}$ mice. Since the loss of all conventional lymph nodes is extensively documented in $LT\alpha^{-/-}$ mice[8], we examined whether the development of NALT is disrupted by the loss of LTα. Surprisingly, we showed that, unlike all other lymph nodes and Peyer's patches,

NALT develops independently of LTα signaling[25]. However, despite the ability of NALT to bypass LTα for its development, the structure and function of NALT is severely compromised in the absence of LTα[25,26]. The NALT of LTα$^{-/-}$ mice is lymphopenic, lacks B cell follicles, follicular dendritic cells and HEVs, and is unable to initiate B and T cell responses to intranasal antigens. However, both the structure and immune function of NALT can be restored by reconstitution of LTα$^{-/-}$ mice with normal bone marrow[25]. Since conventional lymph nodes and Peyer's patches are not restored by reconstitution with normal cells[7], this suggests either that the basic scaffolding of NALT is formed independently of LTα, or that the development of NALT is not confined to an early developmental window. In either case, the development of NALT is unlike that of other secondary lymphoid organs.

The severely compromised structure and function of NALT in LTα$^{-/-}$ mice also suggests that LTα may control the proper expression of homeostatic chemokines in NALT as it does in the spleen. We investigated this possibility and found that, indeed, LTα is required for the expression of CXCL13, CCL19, and CCL21 in NALT and that the loss of these chemokines is responsible for the disrupted architecture and impaired function of NALT in LTα$^{-/-}$ mice[26]. For example, the architecture of NALT is severely disrupted and both B cell and T cell responses are impaired in LTα$^{-/-}$ mice. In contrast, only B cell responses are impaired in CXCL13$^{-/-}$ mice and only T cell responses are impaired in *plt/plt* mice, which lack both CCL19 and CCL21[26]. Thus, the structural and functional defects in LTα$^{-/-}$ NALT can be directly traced to impaired chemokine expression. These data are consistent with the severely reduced expression of CXCL13, CCL19, and CCL21[17], the compromised lymphoid architecture[27], and impaired function[28] of spleens in LTα$^{-/-}$ mice.

2.2. Pulmonary Immune Responses in the Absence of Secondary Lymphoid Organs

Although it is clear that NALT can develop in the absence of LTα, the inability of NALT to support B and T cell responses in LTα$^{-/-}$ mice suggest that immune responses to influenza can be initiated in a location other than NALT. To test this possibility, we next examined whether immune responses are intact in mice that lacked secondary lymphoid organs, but express LTα[29]. In these experiments, we splenectomized and irradiated LTα$^{-/-}$ mice and reconstituted them with normal bone marrow. These mice, which lack spleen, all lymph nodes, and Peyer's patches, are referred to as Spleen, Lymph node, and Peyer's patch-deficient mice (SLP mice). As controls, we reconstituted C57BL/6 mice with C57BL/6 bone marrow to generate WT mice (Figure 2A). After reconstitution, these mice were infected with influenza and assayed for T and B cell responses. As shown in Figure 2B, CD8 cells specific for influenza nucleoprotein$_{366-374}$ (NP) are easily identified in the lungs of WT animals on day 9, the peak of the CD8 T cell response to influenza in normal mice[19]. Influenza-specific CD8 cells are also de-

tected in the lungs of SLP mice, although at a lower frequency than in WT mice (Figure 2B). To determine whether influenza-specific CD8 T cells ever accumulated to normal levels in SLP mice, we followed the kinetics of the CD8 T cell response. As expected, the number of influenza-specific CD8 T cells peaked in the lungs of WT mice on day 9 and then declined (Figure 2C). Despite the delayed appearance of influenza-specific CD8 T cells in the lungs of SLP mice, the number of influenza-specific CD8 T cells in the lungs of SLP mice reached WT levels by day 14 (Figure 2C). Thus, influenza-specific CD8 T cells are primed in the absence of conventional lymphoid organs, albeit with a slight delay.

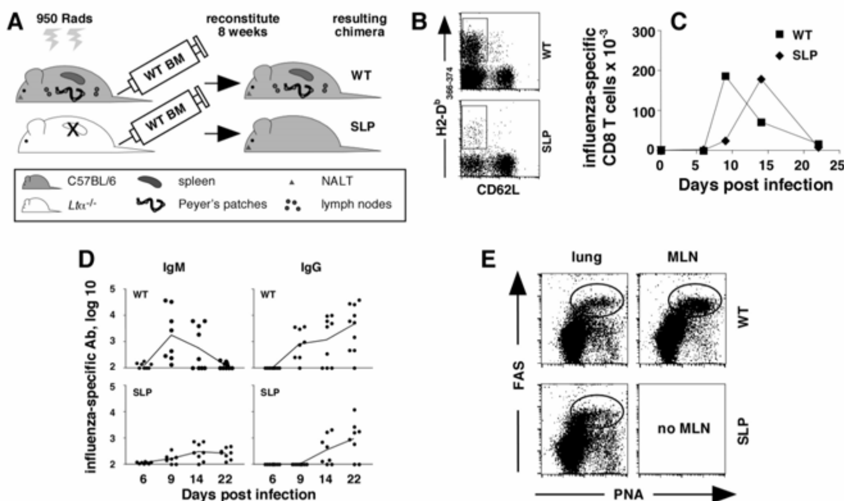

Figure 2. Respiratory immune responses are intact in the absence of conventional lymphoid organs. (A) C57BL/6 mice or splenectomized $LT\alpha^{-/-}$ recipient mice were lethally irradiated and reconstituted with bone marrow from C57BL/6 donors. Reconstituted C57BL/6 mice are referred to as WT mice. Splenectomized and reconstituted $LT\alpha^{-/-}$ mice lack Spleen, Lymph nodes and Peyer's patches and are referred to as SLP mice. (B) Chimeric mice were infected with influenza and nucleoprotein (NP)-specific CD8 cells were identified by tetramer binding. (C) The combined number of $CD8^{+}CD62L^{lo}H–2D^{b}PA_{224-233}$ and $CD8^{+}CD62L^{lo}H–2D^{b}NP_{366-374}$ tetramer-binding T cells in the lungs was determined by flow cytometry. (D) The serum titers of influenza-specific IgM and IgG were determined by influenza-specific ELISA. (E) $CD19^{+}FAS^{+}PNA^{+}$ germinal center B cells were identified in the lungs and draining lymph nodes (MLNs). The plots shown were gated on $CD19^{+}$ cells and the $FAS^{hi}PNA^{hi}$ germinal center B cells are circled.

We also determined whether humoral immune responses were generated in SLP mice[29]. As shown in Figure 2D, influenza-specific IgM and IgG are produced in SLP mice, although the titers of IgM are reduced and the appearance of IgG is delayed. Since isotype switching in B cells usually occurs in germinal centers, which facilitate the proliferation and selection of B cells that produce

high-affinity neutralizing antibody[30], we tested whether B cells with a germinal center phenotype could be found in SLP mice. As shown in Figure 2E, germinal center B cells are found in the lungs of both WT and SLP mice on day 14 after infection. This was somewhat surprising, as germinal centers are normally found in highly organized lymphoid tissues, such as lymph nodes or spleen. These data demonstrate that the formation of germinal center B cells and the production of influenza-specific isotype-switched antibody are not dependent on the presence of peripheral lymphoid tissues, and suggest that the lung itself is competent to initiate and maintain influenza-specific B cell responses.

2.3. Structure and Function of Bronchus-Associated Lymphoid Tissue (BALT)

The presence of B cells with a germinal center phenotype in the lungs of WT and SLP mice suggests that organized lymphoid tissues can be formed in the lung. Since the lungs of normal uninfected mice typically do not contain organized lymphoid tissue, we wanted to know whether influenza infection induces the formation of organized lymphoid structures. To test this we infected mice with influenza and analyzed thick sections of lungs for the presence of organized lymphoid tissues on day 7 post-infection. As shown in Figure 3A–B, organized lymphoid tissues are found in the lungs of mice previously infected with influenza. The majority of the cells in these areas are B cells, which are organized into follicles and are surrounded by T cells (Figure 3A–B). In areas where several B cell follicles are clustered, the interfollicular regions develop T cell zones that contain both CD4 and CD8 T cells as well as CD11c expressing dendritic cells[31]. Our published studies also show that the B cell follicles in the lung are centered on CD21-expressing follicular dendritic cells and that some B cell follicles even have well-defined germinal centers[29]. Proliferating CD8 T cells are found in the interfollicular T cell areas, while proliferating CD4 T cells are observed in the interfollicular area and in the germinal center in close proximity to rapidly proliferating B cells[29]. These data demonstrate that infection triggers the development of organized lymphoid tissues in the lung that have all of the characteristics of secondary lymphoid organs. We have termed these structures "inducible Bronchus Associated Lymphoid Tissue" or iBALT.

Although it is clear that organized lymphoid tissues can be formed in the lung and that these tissues are capable of supporting immune responses, it is not clear whether these tissues represent true secondary lymphoid organs or whether they are tertiary lymphoid tissues that are formed only in response to inflammation or infection. Bronchus Associated Lymphoid Tissue (BALT) was originally described as a mucosal lymphoid tissue found along the upper bronchi of the respiratory tract in pigs and rabbits[32,33]. In these studies, BALT appears very similar to Peyer's patches, with prominent lymphoid follicles underneath a specialized dome epithelium[33,34]. However, unlike classically defined BALT, which

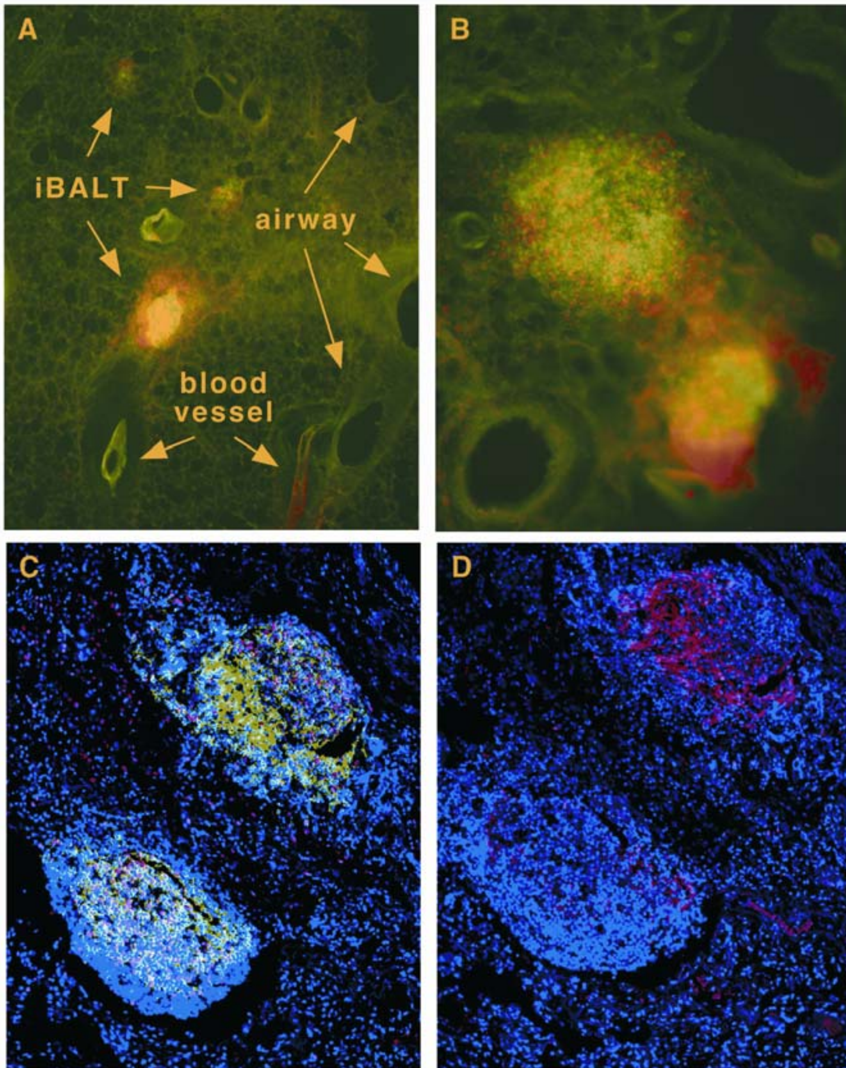

Figure 3 (see color insert). The structure of iBALT in murine and human lungs. (A–B) C57BL/6 mice were infected with influenza and 100-μM sections were prepared from lungs 3 weeks after infection and stained with antibodies to B220 (green) and CD3 (red). The brightness of the green channel was increased so that the autofluorescence of the airways and blood vessels would be visible. (C–D) Sections of a lung biopsy from a patient with follicular bronchiolitis associated with Rheumatoid Arthritis were stained with antibodies to CD20 (green) and PCNA (red) to identify proliferating B cells (C) and with antibodies to CD21 (red) to identify follicular dendritic cell networks (D). Sections C and D are counterstained with DAPI (blue).

develops during embryogenesis and is generated and maintained in the absence of antigen[35], iBALT in both mouse and humans appears to be formed only after infection or inflammation[36–38] and is found in numerous places along the upper and lower bronchi and even in the interstitial areas of the lung. In fact, the sporadic appearance of BALT-like areas in humans and mice has led some investigators to doubt whether BALT is an important secondary lymphoid tissue in these species[36]. However, transgenic mice that express various cytokines in the lung develop iBALT[39,40], as do mice and humans prone to autoimmune diseases[41,42] or humans with recurrent or chronic respiratory infections[43,44]. For example, B cell follicles are easily observed in the lungs of a patient with follicular bronchiolitis associated with Rheumatoid Arthritis (Figure 3D–E). These B cell follicles support B cell proliferation and are centered on CD21-expressing follicular dendritic cells just as they are in murine lungs[29]. Thus, iBALT has a similar structure in both murine and human lungs, and its formation is probably triggered by similar mechanisms.

Although the cellular and molecular mechanisms that lead to iBALT formation are not known, similar ectopic lymphoid tissues often form at sites of chronic inflammation, such as in rheumatoid joints[45–47]. Some investigators have termed this process “lymphoid neogenesis” and suggest that chronic inflammation is responsible for the appearance of lymphoid tissues in a variety of sites[46,47]. Thus, iBALT is probably another example of an ectopic lymphoid tissue that develops upon local inflammation triggered by infection or autoimmunity. As in the development of conventional lymphoid organs, lymphotoxin and the homeostatic chemokines appear to play a role in the development of ectopic lymphoid tissues[47]. For example, the transgenic expression of CXCL13, CCL21, or LTα in the pancreas results in the development of lymphoid tissue in this organ[48–50], complete with B and T cell areas, follicular dendritic cells (FDCs), and high endothelial venules (HEVs). Since homeostatic chemokines are clearly important for both the development and the organization of ectopic lymphoid tissues, we tested whether the expression of these chemokines is induced upon influenza infection of the lung. As expected, CXCL13 mRNA is not expressed in normal uninfected lungs[29]. However, it is rapidly induced upon infection with influenza virus and is maintained after infection is resolved. We also demonstrated that the expression of CXCL13 and CCL21 proteins co-localize with the B and T cell areas observed in the lung[29]. The majority of CXCL13 protein expression localizes to the follicular area that contains B cells and follicular dendritic cells. In contrast, CCL21 expression is observed on vascular endothelium as well as on reticular cells around the edge of the follicles and in the interfollicular areas[29]. These areas are similar to where $CD11c^+$ dendritic cells and T cells are found[31]. Finally, we observed that PNAd, the peripheral lymph node addressin, is expressed on vascular endothelial cells in the areas of iBALT surrounding the B cell follicles[29]. These data demonstrate that the lymphoid areas of the lung that form after infection are not simply accumulations of effector cells that are primed in conventional lymphoid organs. Instead, these newly formed lymphoid

tissues express chemokines and homing receptors necessary to recruit naive lymphocytes and to organize them into structures that support lymphocyte priming and differentiation.

2.4. Does iBALT Confer Antiinflammatory Properties on Local Immune Responses?

The data above showed that B and T cell immune responses to influenza can be primed in iBALT in mice that lacked conventional lymphoid organs[29]. However, the delayed B and T cell responses observed in these mice also suggest that immunity in these mice may not be as effective in the absence of normal secondary lymphoid organs. In fact, viral clearance is effective, but somewhat delayed in mice lacking lymphoid tissues[29]. Interestingly, the delayed viral clearance did not result in increased morbidity and mortality, and SLP mice actually survived higher doses of influenza significantly better than did WT mice[29]. Furthermore, when infected with lower doses of virus that both WT and SLP mice could survive (240 and 50 EIU), the SLP mice exhibited substantially less morbidity throughout the experiment. Thus, the lack of peripheral lymphoid organs does not result in increased susceptibility to influenza but, instead, significantly increases the ability of these mice to survive infection with normally lethal doses of virus. These data are very surprising and somewhat difficult to explain. If the SLP mice make delayed immune responses and are less efficient at clearing virus, why do they exhibit less morbidity and mortality? One possibility is that, because the overall magnitude of the immune response is smaller in SLP mice, there is less immunopathology. Another possibility is that lymphocytes primed exclusively in iBALT produce antiinflammatory cytokines. These possibilities are currently being investigated.

3. CONCLUSIONS AND FUTURE DIRECTIONS

The data presented here suggest that the current paradigm, in which primary adaptive immune responses are generated exclusively in conventional lymphoid organs, such as spleen, lymph node and Peyer's patches, should be modified to include a role for locally induced lymphoid tissues, such as iBALT. In this new model, infection and inflammation activate local APCs, which traffic to preexisting lymphoid organs and initiate primary immune responses. At the same time, infection and inflammation trigger the formation of local lymphoid tissues, such as iBALT, at the site of inflammation (Figure 4). These local lymphoid tissues are fully capable of initiating primary immune responses and of expanding effector cells that were primed in conventional lymphoid organs. The generation of primary immune responses in these local tissues may be slightly delayed relative to that in conventional lymphoid organs, because it takes time for the local tissues to develop the appropriate architecture that will recruit and

prime naive lymphocytes. However, once local lymphoid tissues are formed, we predict that they will initiate primary immune responses to new antigens even faster than conventional lymphoid organs due to their close proximity to antigen. In the case of immune responses to infectious agents, this faster response will likely be beneficial. However, in the case of immune responses to local autoantigens, faster responses may be harmful and may exacerbate local pathology.

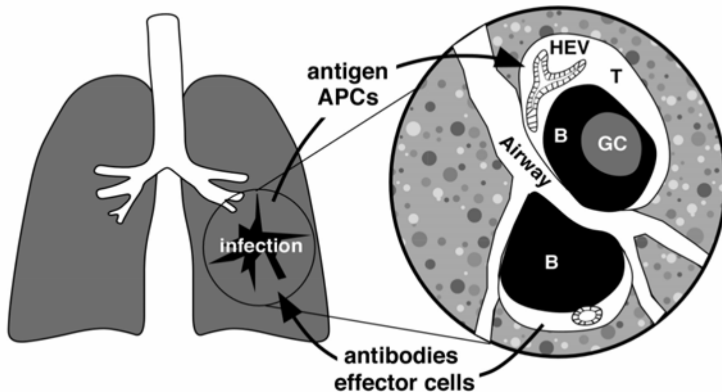

Figure 4. Local infection in the lung triggers the development of iBALT. iBALT has the structure of an ectopic lymphoid tissue, with B cell follicles, germinal centers, HEVs, and T cell areas. In concert with conventional lymphoid organs, iBALT acts as a secondary lymphoid tissue that facilitates local immune responses.

Despite the demonstration that local lymphoid tissues like iBALT are functional, numerous questions remain about how they actually work. For example, do lymphocytes primed in iBALT have different effector functions than those primed in the lymph nodes that drain the lung? Can APCs traffic from the airways directly to areas of iBALT? Do lymphocytes primed in iBALT traffic directly into the airways, or do they have to exit iBALT via efferent lymphatics and re-enter the lung from the blood? What are the mechanisms that trigger iBALT formation? How long does iBALT persist in the lung after the initial inflammation is resolved? Finally, does the presence of iBALT in human lungs provide an overall benefit or detriment to the individual?

4. ACKNOWLEDGMENTS

The authors would like to thank Dr. Frances Lund for her critical review of this manuscript and for helpful discussions during the course of this work. We grate-

fully acknowledge financial support for this work from NIH grants HL-69409, HL63925, the Sandler Program for Asthma Research, and the Trudeau Institute.

5. REFERENCES

1. E.C. Butcher and L.J. Picker. Lymphocyte homing and homeostasis. *Science* **272**:60–62 (1996).
2. J.G. Cyster. Chemokines and the homing of dendritic cells to the T cell areas of lymphoid organs. *J Exp Med* **189**:447–450 (1999).
3. K.L. Legge and T.J. Braciale. Accelerated migration of respiratory dendritic cells to the regional lymph nodes is limited to the early phase of pulmonary infection. *Immunity* **18**:265–277 (2003).
4. C.C. Goodnow. Chance encounters and organized rondezvous. *Immunol Rev* **156**:5–10 (1997).
5. M. Balazs, F. Martin, T. Zhou and J. Kearney. Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. *Immunity* **17**:341–352 (2002).
6. M.R. Neutra, A. Frey and J.P. Kraehnenbuhl. Epithelial M cells: gateways of mucosal infection and immunization. *Cell* **86**:345–348 (1996).
7. P.D. Rennert, J.L. Browning, R.E. Mebius, F. Mackay and P.S. Hochman. Surface lymphotoxin a/b complex is required for the development of peripheral lymphoid organs. *J Exp Med* **184**:1999–2006 (1996).
8. P. de Togni, J. Goellner, N.H. Ruddle, P.R. Streeter, A. Fick, S. Mariathasan, S.C. Smith, R. Carlson, L.P. Shornick and J. Strauss–Schoenberger. Abnormal development of peripheral lymphoid organs in mice deficient in lymphotoxin. *Science* **264**:703–707 (1994).
9. P.A. Koni, R. Sacca, P. Lawton, J.L. Browning, N.H. Ruddle and R.A. Flavell. Distinct roles in lymphoid organogenisis for lymphotoxins a and b revealed in lymphotoxin b deficient mice. *Immunity* **6**:491–500 (1997).
10. A. Futterer, K. Mink, A. Luz, M.H. Kosco-Vilbois and K. Pfeffer. The lymphotoxin b receptor controls organogenisis and affinity maturation in peripheral lymphoid tissues. *Immunity* **9**:59–70 (1998).
11. S. Miyawaki, Y. Nakamura, H. Suzuka, M. Koba, R. Yasumizu, S. Ikehara and Y. Shibata. A new mutation, aly, that induces a generalized lack of lymph nodes accompanied by immunodeficiency in mice. *Eur J Immunol* **24**:429–434 (1994).
12. K. Honda, H. Nakano, H. Yoshida, S. Nishikawa, P. Rennert, K. Ikuta, M. Tamechika, K. Yamaguchi, T. Fukumoto, T. Chiba and S.I. Nishikawa. Molecular basis for hematopoietic/mesenchymal interaction during initiation of Peyer's patch organogenesis. *J Exp Med* **193**:621–630 (2001).
13. S.A. Luther, K.M. Ansel and J.G. Cyster. Overlapping roles of CXCL13, interleukin 7 receptor alpha, and CCR7 ligands in lymph node development. *J Exp Med* **197**:1191–1198 (2003).
14. K.M. Ansel, V.N. Ngo, P.L. Hayman, S.A. Luther, R. Forster, J.D. Sedgwick, J.L. Browning, M. Lipp and J.G. Cyster. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* **406**:309–314 (2000).

15. M.D. Gunn, K. Tangemann, C. Tam, J.D. Cyster, S.D. Rosen and L.T. Williams. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc Natl Acad Sci* **95**:258–263 (1998).
16. M.D. Gunn, S. Kyuwa, C. Tam, T. Kakiuchi, A. Matsuzawa, L.T. Williams and H. Nakano. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. *J Exp Med* **189**:451–460 (1999).
17. V.N. Ngo, H. Korner, M.D. Gunn, K.N. Schmidt, D.S. Riminton, M.D. Cooper, J.L. Browning, J.D. Sedgewick and J.G. Cyster. Lymphotoxin a/b and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. *J Exp Med* **189**:403–412 (1999).
18. S.L. Constant, J.L. Brogdon, D.A. Piggott, C.A. Herrick, I. Visintin, N.H. Ruddle and K. Bottomly. Resident lung antigen-presenting cells have the capacity to promote Th2 T cell differentiation in situ. *J Clin Invest* **110**:1441–1448 (2002).
19. F.E. Lund, S. Partida-Sanchez, B.O. Lee, K.L. Kusser, L. Hartson, R.J. Hogan, D.L. Woodland and T.D. Randall. Lymphotoxin-alpha-deficient mice make delayed, but effective, t and b cell responses to influenza. *J Immunol* **169**:5236–5243 (2002).
20. F. Amiot, P. Vuong, M. Desfontaines, C.D. Pater and F.M. Liance. Secondary alveolar echinococcosis in lymphotoxin-a and tumour necrosis factor-a deficient mice: exacerbation of *Echinococcus multilocularis* larval growth is associated with cellular changes in the periparasitic granuloma. *Parisite Immunol* **21**:475–483 (1999).
21. T.A. Banks, B.T. Rouse, M.K. Kerley, P.J. Blair, V.L. Godfrey, N.A. Kuklin, D.M. Bouley, J. Thomas, S. Kanangat and M.L. Mucenski. Lymphotoxin a deficient mice: effects on secondary lymphoid organ development and humoral immune responsiveness. *J Immunol* **155**:1685–1693 (1995).
22. D.P. Berger, D. Naniche, M.T. Crowley, P.A. Koni, R.A. Flavell and M.B.A. Oldstone. Lymphotoxin-b-deficient mice show defective antiviral immunity. *Virology* **260**:136–147 (1999).
23. U. Kumaraguru, I.A. Davis, S. Deshpande, S.S. Tevethis and B.T. Rouse. Lymphotoxin $a^{-/-}$ mice develop functionally impaired CD8+ t cell responses and fail to contain virus infection of the central nervous system. *J Immunol* **166**:1066–1074 (2001).
24. B.J. Lee, S. Santee, S. Von Gesjen, C.F. Ware and S.R. Sarawar. Lymphotoxin-a-deficient mice can clear a productive infection with murine gammaherpesvirus 68 but fail to develop spenomegaly or lymphocytosis. *J Virol* **74**:2786–2792 (2000).
25. A. Harmsen, K. Kusser, L. Hartson, M. Tighe, M.J. Sunshine, J.D. Sedgwick, Y. Choi, D.R. Littman and T.D. Randall. Organogenesis of Nasal Associated Lymphoid Tissue (NALT) occurs independently of lymphotoxin-α (LTα) and retinoic acid receptor-related orphan receptor-γ, but the organization of NALT is LTα-dependent. *J Immunol* **168**:986–990 (2002).
26. J. Rangel-Moreno, J. Moyron-Quiroz, K. Kusser, L. Hartson, H. Nakano and T.D. Randall. Role of CXC chemokine ligand 13, CC chemokine ligand (CCL) 19, and CCL21 in the organization and function of nasal-associated lymphoid tissue. *J Immunol* **175**:4904–4913 (2005).
27. M. Matsumoto, S. Mariathasan, M.H. Nahm, F. Baranyay, J.J. Peschon and D.D. Chaplin. Role of lymphotoxin and the type 1 TNF receptor in the formation of germinal centers. *Science* **271**:1289–1291 (1996).

28. M. Matsumoto, S.F. Lo, C.J.L. Carruthers, J. Min, S. Mariathasan, G. Huang, D.R. Plas, S.M. Martin, R.S. Geha, M.H. Nahm and D.D. Chaplin. Affinity maturation without germinal centers in lymphotoxin a deficient mice. *Nature* **382**:462–466 (1996).
29. J.E. Moyron-Quiroz, J. Rangel-Moreno, K. Kusser, L. Hartson, F. Sprague, S. Goodrich, D.L. Woodland, F.E. Lund and T.D. Randall. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. *Nat Med* **10**:927–934 (2004).
30. G. Kelsoe. The germinal center: a crucible for lymphocyte selection. *Semin Immunol* **8**:179–184 (1996).
31. D.L. Woodland and T.D. Randall. Anatomical features of anti-viral immunity in the respiratory tract. *Semin Immunol* **16**:163–170 (2004).
32. J. Bienenstock, N. Johnston and D.Y. Perey. Bronchial lymphoid tissue, I: morphologic characteristics. *Lab Invest* **28**:686–692 (1973).
33. J. Bienenstock and N. Johnston. A morphologic study of rabbit bronchial lymphoid aggregates and lymphoepithelium. *Lab Invest* **35**:343–348 (1976).
34. T. Sminia, G.J. van der Brugge-Gamelkoorn and S.H. Jeurissen. Structure and function of bronchus-associated lymphoid tissue (BALT). *Crit Rev Immunol* **9**:119–150 (1989).
35. S. Delventhal, A. Hensel, K. Petzoldt and R. Pabst. Effects of microbial stimulation on the number, size and activity of bronchus-associated lymphoid tissue (BALT) structures in the pig. *Int J Exp Path* **73**:351–357 (1992).
36. R. Pabst and I. Gehrke. Is the bronchus-associated lymphoid tissue (BALT) an integral structure of the lung in normal mammals, including humans? *Am J Respir Cell Mol Biol* **3**:131–135 (1990).
37. I. Richmond, G.E. Pritchard, T. Ashcroft, A. Avery, P.A. Corris and E.H. Walters. Bronchus associated lymphoid tissue (BALT) in human lung. its distribution in smokers and non-smokers:*Thorax* **48**, 1130–1134 (1993).
38. T. Tshering and R. Pabst. Bronchus associated lymphoid tissue (BALT) is not present in normal adult lung but in different diseases. *Pathobiology* **68**:1–8 (2000).
39. J. J. Lee, M.P. McGarry, S.C. Farmer, K.L. Denzler, K.A. Larson, P.E. Carrigan, I.E. Brenneise, M.A. Horton, A. Haczku, E.W. Gelfan, G.D. Leikauf and N.A. Lee. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med* **185**:2143–2156 (1997).
40. S. Goya, H. Matsuoka, M. Mori, H. Morishita, H. Kida, Y. Kobashi, T. Kato, Y. Taguchi, T. Osaki, I. Tachibana, N. Nishimoto, K. Yoshizaki, I. Kawase, and S. Hayashi. Sustained interleukin-6 signalling leads to the development of lymphoid organ-like structures in the lung. *J Pathol* **200**:82–87 (2003).
41. B. Xu, N. Wagner, L.N. Pham, V. Magno, Z. Shan, E.C. Butcher and S.A. Michie. Lymphocyte homing to bronchus-associated lymphoid tissue (BALT) is mediated by L-selectin/PNAd, alpha4beta1 integrin/VCAM-1, and LFA-1 adhesion pathways. *J Exp Med* **197**:1255–1267 (2003).
42. R.K. Chin, J.C. Lo, O. Kim, S.E. Blink, P.A. Christiansen, P. Peterson, Y. Wang, C. Ware and Y.X. Fu. Lymphotoxin pathway directs thymic Aire expression. *Nat Immunol* **4**:1121–1127 (2003).
43. H.D. Chen, A.E. Fraire, I. Joris, M.A. Brehm, R.M. Welsh and L.K. Selin. Memory CD8+ T cells in heterologous antiviral immunity and immunopathology in the lung. *Nat Immunol* **2**:1067–1076 (2001).

44. J.H. Vernooy, M.A. Dentener, R.J. van Suylen, W.A. Buurman and E.F. Wouters. Long-term intratracheal lipopolysaccharide exposure in mice results in chronic lung inflammation and persistent pathology. *Am J Respir Cell Mol Biol* **26**:152–159 (2002).
45. N.H. Ruddle. Lymphoid neo-organogenesis: lymphotoxin's role in inflammation and development. *Immunol Res* **19**:119–125 (1999).
46. A. Kratz, A. Campos-Neto, M.S. Hanson and N.H. Ruddle. Chronic inflammation caused by lymphotoxin is lymphoid neogenesis. *J Exp Med* **183**:1461–1472 (1996).
47. P. Hjelmstrom, J. Fjell, T. Nakagawa, R. Sacca, C. Cuff and N. Ruddle. Lymphoid tissue homing chemokines are expressed in chronic inflammation. *Am J Pathol* **156**:1133–1138 (2000).
48. S.A. Luther, T. Lopez, W. Bai, D. Hanahan and J.G. Cyster. BLC expression in pancreatic islets causes b cell recruitment and lymphotoxin-dependent lymphoid neogenesis. *Immunity* **12**:471–481 (2000).
49. S.A. Luther, A. Bidgol, D.C. Hargreaves, A. Schmidt, Y. Xu, J. Paniyadi, M. Matloubian and J.G. Cyster. Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis. *J Immunol* **169**:424–433 (2002).
50. R. Sacca, C.A. Cuff, W. Lesslauer and N.H. Ruddle. Differential activities of secreted lymphotoxin-alpha3 and membrane lymphotoxin-alpha1beta2 in lymphotoxin-induced inflammation: critical role of TNF receptor 1 signaling. *J Immunol* **160**:485–491 (1998).