Wouldn’t you like to know: are tertiary lymphoid structures necessary for lung defence?

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A key principle of emergency management is establishing an incident command centre. That’s where information is gathered, plans are formulated, responsibilities assigned, and resources deployed. The immune system deals with microbial threats by this same approach, using organised lymphoid tissues. At the early stages of infections, conventional dendritic cells (cDCs) transmit intelligence on the nature of the threat to regional lymph nodes, which along with the spleen, tonsils and Peyer’s patches, constitute the secondary lymphoid organs. Within lymph nodes, immune responses are optimised as large numbers of effector T cells search for their cognate antigens on the surfaces of cDCs, under the supervision of regulatory T cells. However, widespread and chronic threats require additional command centres closer to the action. “Tertiary lymphoid structures” is one name for these de novo frontline assemblages, along with “lymphoid follicles”. The process, termed lymphoid neogenesis, occurs within many organs in infectious, autoimmune and inflammatory disorders, and in transplanted organs and malignancies [1]. Because tertiary lymphoid structures (TLSs) develop in multiple lung diseases (table 1), defining their roles in pathogenesis is a crucial unmet goal that experimental models are well-suited to address.

In the lungs, TLSs stereotypically arise adjacent to bronchovascular bundles. This localisation has been proposed to reflect recruitment of the cells that comprise TLSs, principally lymphocytes, across the perivascular capillary bed of the pulmonary arterial system [2]. Accordingly, TLSs in the lung have also been termed inducible bronchus-associated lymphoid tissue (iBALT) [3, 4]. However, the analogy of iBALT with mucosal-associated lymphoid tissue (MALT) in the gut is undercut by the absence in lung TLS of epithelial M cells or expression of the mucosal vascular addressin by their high endothelial venules (HEV) [5, 6]. Experimentally, lung TLSs can be induced rapidly in mice by a single strong intratracheal antigenic or infectious challenge [7–9], but they generally require more persistent exposures [10]. TLSs are minimally present in the human lung at birth [11], then develop during early childhood; their prevalence is driven by cigarette smoking [12] and likely by particulate air pollution [13].

TLSs are distinguished from simple lymphocytic infiltrates by their organisation into distinct T cell zones containing HEV and B cell zones containing germinal centres and follicular dendritic cells. This organisation is shared with lymph nodes, and as is also true for them, recruitment of lymphocytes to TLSs
depends on the recognition of the chemokines CXCL13 by CXCR5 and CCL19, plus CCL21 by CCR7 [14]. cDCs elaborate those chemokines and appear to be essential to maintain lung TLSs [8, 15]. However, unlike lymph nodes (but like MALT), TLSs in the lungs and elsewhere generally are neither encapsulated nor supplied by afferent lymphatics [1]. Instead, TLSs appear to have direct contact to adjacent antigenic and chemotactic signals. Nonetheless, prolonged inflammation can induce efferent lymphatics from lung TLSs via signalling involving vascular endothelial growth factor receptor (VEGFR)-2/VEGFR-3; these lymphatics may not regress when inflammation wanes [16]. Recent elegant experiments employing gene-targeted mice showed that, relative to lymphatics in other organs, pulmonary lymphatics in mice and humans uniquely lack coverage by smooth muscle and pericytes [17]. That same study also linked defective lung lymphatic function to TLS development, impaired leukocyte emigration, and eventual

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### TABLE 1: Associations of tertiary lymphoid structure (TLS) development with human lung diseases and experimental models

<table>
<thead>
<tr>
<th>Human lung diseases</th>
<th>Association with disease stage</th>
<th>Effect on outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>Present late, development not well-studied</td>
<td>Unknown</td>
<td>[39, 51, 52]</td>
</tr>
<tr>
<td>Non-CF bronchiectasis</td>
<td>May develop in areas of relatively mild pathology</td>
<td>Associated with adjacent loss of elastic fibres</td>
<td>[39, 53]</td>
</tr>
<tr>
<td>COPD</td>
<td>Increases with spirometric severity, generally prominent only late</td>
<td>Causal role suspected but unproven</td>
<td>[10, 41, 54–56]</td>
</tr>
<tr>
<td>Severe asthma</td>
<td>Late manifestation</td>
<td>Unknown</td>
<td>[57]</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>Appears to develop early</td>
<td>Beneficial to pathogen clearance</td>
<td>[18]</td>
</tr>
<tr>
<td>Lung transplant</td>
<td>Appears soon after surgery</td>
<td>Graft tolerance may be promoted by stimulating development of efferent lymphatics</td>
<td>[29, 30]</td>
</tr>
<tr>
<td>Idiopathic pulmonary hypertension</td>
<td>Chronology unstudied</td>
<td>Lung TLS are a site of autoantibody production</td>
<td>[20, 58]</td>
</tr>
<tr>
<td>Idiopathic pulmonary fibrosis</td>
<td>Chronology unstudied</td>
<td>Significance unproven</td>
<td>[21, 22]</td>
</tr>
<tr>
<td>NSIP</td>
<td>Chronology unstudied</td>
<td>Numbers of follicular B cells greater in fibroblasts than cellular NSIP</td>
<td>[23]</td>
</tr>
<tr>
<td>IgG4-related disease</td>
<td>Chronology unstudied</td>
<td>Correlated with improved FVC after 1 year of therapy but not survival</td>
<td></td>
</tr>
<tr>
<td>Bronchogenic carcinoma</td>
<td>Frequently present in low TNM stage tumours</td>
<td>Unknown</td>
<td>[59, 60]</td>
</tr>
</tbody>
</table>

**Selected experimental models of lung disease**

| CFTR−/− mice                | Develop with ageing in the absence of infection                     | Lung TLS B cells spontaneously express BAFF and increased MHC class II, and produce more IL-6 on stimulation | [51]       |
| Murine TB infection         | Lung TLS are smaller in infected male mice                          | TLS size correlates with improved survival                                        | [62]       |
| Murine cigarette smoke exposure | Develops within 14 weeks but not 8 weeks; persists and expands on cessation for 120 days | Associated with local production of anti-nuclear antibodies                        | [25, 63, 64] |
| Murine lung transplant      | Lung TLS larger in female mice                                      | Differences in TLS size not seen in mice ovariotomised before exposure            | [31–33]   |
| BeO exposure of HLA-DP2 transgenic mice | Develops within 21 days Associated with appearance of IgG-secreting B cells absent from lung-draining lymph nodes | Site of FOXP3+ T regulatory cells essential for survival of tolerised grafts | [65]       |

Adapted from [1, 18, 50]. BAFF: B cell-activating factor of tumor necrosis factor family; BeO: beryllium oxide; CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; FVC: forced vital capacity; GPCR: G-protein coupled receptor; IL: interleukin; MHC: major histocompatibility complex; NSIP: nonspecific interstitial pneumonitis; TB: tuberculosis; TNM: tumour, nodal, metastases.

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emphysematous changes [17]. It appears that surprisingly much remains to be learned about lung ultrastructural anatomy and physiology.

Thus, the presence of lung TLSs implies that focal immune responses are occurring, but what is their significance to lung diseases and hence to pulmonologists? The answer appears to be highly contextual (table 1), though the primary reliance on resected lung tissue to investigate lung TLSs potentially biases judgment about the pace of their development and associations with clinical outcomes. Lung TLSs are prominent in chronic airway diseases, including cystic fibrosis (CF), non-CF bronchiectasis, COPD and severe asthma [10]. The strong connection of lung TLSs in these diseases with an altered lung microbiome suggests that TLSs might in some cases be important for local host defence, which is arguably best demonstrated in tuberculosis (reviewed in [18]). On the other hand, TLSs are also linked to autoimmunity, in which they facilitate epitope spreading [19], and they are found in specific vascular and interstitial lung diseases with autoimmune features [20–23]. In advanced COPD, lung TLSs are a site of production of the pro-inflammatory cytokine IL-18 [24], which can drive destructive T1 responses. Ablating lung TLSs in mice reduced emphysema but not airway remodelling [25], and has very recently been suggested to facilitate lung regeneration in smoking-induced injury [26]. By contrast, in several malignancies, including nonsmall cell lung cancer, B cell-enrichment of TLSs is a highly favourable prognostic factor, regardless of the proportions of T cells (reviewed in [27, 28]). Moreover, in contrast to the uniformly detrimental involvement of TLSs in most transplanted organs, both beneficial and harmful effects of TLSs have been shown in lung transplantation (table 1) [29–33]. So, are lung TLSs a good thing or not [18, 34–37]?

In this issue of the European Respiratory Journal, Regard et al. [38] provide novel insights on a key aspect of that question, the role of TLSs in lung host defence. Those authors used their established murine model of chronic bronchial infection, in which its standard form [39, 40] employs agarose beads impregnated with viable Staphylococcus aureus to induce an airway infection that persists without the need for immunosuppression or repeated challenges. Importantly, such persistent infection (but not an identical dose of bacteria without beads) reproducibly induces TLS development. More recently, this group showed that persistent airway infection in mice (in that case using Pseudomonas aeruginosa beads) increased total IgA and anti-Pseudomonas-specific IgA, which were not observed when TLSs were induced by 8-week exposure of mice to cigarette smoke [41]. They also identified TLS in the lungs of COPD patients as the site of immunoglobulin class-switching to IgA, the isotype crucial for mucosal protection [41]. Collectively, these results suggest that lung TLSs contribute to local mucosal defences, a point supported by murine models of several other types of lung pathogens [3, 42–45] but untested for the bacteria that plague patients with advanced CF or bronchiectasis of other origin.

In the current study [38], the authors address that question by first using monoclonal antibodies to selectively deplete mice of B cells, CD4+ or CD8+ T cells, or all three lymphocyte subsets; an isotype control group was also included. They next infected mice with S. aureus-containing beads by the intratracheal route, then examined the lungs 14 days later. As anticipated, lymphocyte depletion resulted in marked TLS disorganisation, but there were three unanticipated and important findings. First, blocking lung accumulation of either B cells or T cells did not increase mortality or lung bacterial loads. As the authors discuss, this finding differs from that seen in murine models of pulmonary infection with influenza or Mycobacterium tuberculosis [3, 42–44], but is congruent with results in a Pneumocystis model [45]. The disparity likely reflects the immune requirements to clear S. aureus versus pathogens whose elimination depends more on a robust adaptive immune response, and thus on the “command centre” environment provided by TLSs. Nevertheless, the current result is an informative advance, given the prominence of both pathogenic bacteria and lung TLSs in chronic airway diseases.

Second, Regard et al. [38] show that depletion of both CD4+ and CD8+ T cells markedly reduced lung TLS size and number, whereas B cell depletion did not impact T cell accumulation. This dependence on CD8+ T cells is unexpected. CD4+ T cells, but not CD8+ T cells, are required for lymphocyte recruitment to the lungs in response to a non-infectious particulate antigen [46, 47]. Perhaps this finding in the S. aureus bead model reflects crucial involvement by mucosal-associated invariant T cells, about half of which are CD8+ in humans (though this innate cell type is less prominent in laboratory mice) [48]. The lack of effect of B cell depletion contrasts somewhat with the clinical success of rituximab monotherapy in several human diseases involving lung TLSs (reviewed in [49]), but that may be due to the differences in end-points (symptoms and imaging versus histological analysis). Third, both CD4+ and CD8+ T cells were needed for germinal centre formation. Here too, the requirement for CD8+ T cells is unexpected; their role, possibly indirect, merits further study.

Thus, this interesting and carefully performed study implies that inhibiting lung TLS might not run the risk of serious bacterial lung infections, even if the contrasting murine results cited above suggest that the outcome to respiratory viruses might differ. After decades of being limited largely to suppressing the
immune system with the cudgel of corticosteroids, we now have the ability to manipulate it much more selectively, but to do so safely will require much greater understanding. This article is an example of how the results of carefully designed experiments in animal models can advance that understanding, making it noteworthy to practitioners who seek to stay abreast of the latest clinically relevant science.

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