Pulmonary dendritic cells: thinking globally, acting locally

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The phrase "think globally, act locally" was coined in the early 1970s and directed individuals to clean up their local environment with the ultimate goal of improving the health of the entire planet. Several recent studies indicate that similar considerations apply to the immune system, in which small numbers of leukocytes, such as pulmonary dendritic cells, can modify the local immune environment in the lung and promote a positive outcome for the organism.

Conventional wisdom suggests that when pulmonary dendritic cells (DCs) are activated by influenza infection, they phagocytose local antigens and migrate to the draining lymph node (LN). Once in the LN, they either present antigen directly (Ballesteros-Tato et al., 2010) or hand off antigen to LN-resident DCs (Belz et al., 2004), which then present antigen to naive CD8 T cells. Effective antigen presentation leads to the activation, proliferation, and differentiation of effector CD8 T cells, which then migrate to the lung and kill influenza-infected epithelial cells. However, more recent studies show that pulmonary DCs are also important in the lung itself to maintain CD8 T cell effectors (McGill et al., 2008). In fact, mice depleted of pulmonary (but not systemic) DCs via intranasally delivered chlodronate-filled liposomes are unable to maintain CD8 T cell responses in the lung and do not effectively clear influenza virus (McGill et al., 2008). Thus, influenza-specific effector CD8 T cells require continued stimulation from DCs, even after the T cells have arrived in the lung.

In this issue, McGill et al. build on these findings and show that CD8 T cells continue to proliferate in the lungs of influenza-infected mice, regardless of whether pulmonary DCs are depleted or not (McGill et al., 2010). However, despite normal rates of proliferation in DC-depleted mice, the numbers of CD8 T cells in the lungs are down and the markers of apoptosis are up. CD8 T cell numbers are restored and apoptosis is reversed by intranasal reconstitution with pulmonary DCs, implicating DCs in the local survival of CD8 T cells in the lung. Importantly, the transferred DCs must present antigen and trans-present interleukin-15 (IL-15) to restore the pulmonary CD8 T cell response.

Although IL-15 is known to play a role in the maintenance of memory CD8 T cells (Schluns and Lefrançois, 2003; Surh et al., 2006), the ability of IL-15 to support the survival of effector CD8 T cells is not well appreciated. Nevertheless, the results reported here are consistent with previous data showing that the administration of exogenous IL-15 to mycobacteria-infected mice up-regulates Bcl-2 expression and inhibits the apoptosis of effector CD8 T cells in the lung (Tang et al., 2009). Similar studies suggest that IL-15 promotes the survival of a subset of KLRG-1$^+$CD27$^-$ CD8 T cells, which are thought to represent short-lived effectors (Stonier et al., 2008). IL-15 is also important for the survival of *Listeria monocytogenes*–specific CD44$^+$CD62L$^-$CD127$^-$ effector CD8 T cells during the contraction phase of the CD8 T cell response (Yajima et al., 2006). Given the finding that depletion of pulmonary DCs leads to poor viral clearance (McGill et al., 2008), it is tempting to speculate that the absence of IL-15–presenting DCs leads to poor CD8 T cell effector activity in the lung. However, direct evidence that IL-15 maintains functional CD8 T cell effectors, rather than simply promoting CD8 T cell survival during the transition from effector to memory cells, is lacking.

The identity of the cells that trans-present IL-15 to CD8 T cells is also somewhat controversial. Some studies show that expression of the IL-15R on macrophages, but not on DCs, supports the early transition of antigen-specific effector CD8 T cells to memory cells (Mortier et al., 2009). However, the expression of IL-15R on DCs selectively maintains central memory CD8 T cells, whereas the expression of the IL-15R on macrophages supports both central and effector memory CD8 T cells (Mortier et al., 2009). Other studies also suggest that DCs are important for the maintenance of memory CD8 T cells, but indicate that DCs are not the only cell type involved in IL-15 trans-presentation (Stonier et al., 2008). Importantly, McGill et al. (2010) go beyond just differentiating between DCs and macrophages; they find that both plasmacytoid DCs (pDCs) and CD8α$^+$ (CD8α DCs) pulmonary DCs make IL-15, as do alveolar macrophages. Moreover, these same populations express similar levels of IL-15R. However, pDCs and CD8α DCs, but not macrophages, were able to rescue the CD8 T cell response upon adoptive transfer into mice depleted of pulmonary DCs (McGill et al., 2008). Thus, the authors conclude that IL-15 is produced and trans-presented by both pDCs and CD8α DCs in the lung during influenza infection, that these same DCs present antigen, and

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that both functions are required for robust effector CD8 T cell responses in the lung.

A question not addressed by McGill et al. (2010) is where pulmonary DCs present antigen and IL-15 to effector CD8 T cells. One possibility is that pulmonary DCs present antigen and IL-15 in the airways (Fig. 1). However, CD8 T cells in the airways lose expression of IL-15R and do not respond to IL-15, suggesting that DCs do not promote the survival of CD8 T cells in the airways (Shen et al., 2008). Another possibility is that pulmonary DCs continue to migrate to the draining LN, where they present antigen and IL-15 throughout the course of infection and maintain the production of CD8 effector T cells. Although some work suggests that the initial presentation of influenza antigens occurs in the LN during the first 48 h after infection and that antigen presentation shifts exclusively to the lung after that (Legge and Braciale, 2003), more recent work suggests that DCs continue to migrate to the LN from the lung for many days after infection and that peak migration occurs around day 5 (Ballesteros-Tato et al., 2010). Moreover, pulmonary DCs adoptively transferred to the lungs of influenza-infected mice 5 d after infection migrate to the LN within 24 h. These migratory DCs include $CD103^{+}CD11b^{lo}$ epithelial DCs, as well as $CD103^{-}CD11b^{hi}$ parenchymal DCs (Ballesteros-Tato et al., 2010), both of which express low levels of CD8α and may correspond to the IL-15–presenting CD8α DCs described above.

A third possibility, and the one favored by McGill et al. (2010), is that DCs present antigen in situ in the lung, presumably in the parenchyma. This possibility is supported by previous data showing that antigen presentation to CD4 and CD8 T cells can occur in the lung in the absence of conventional lymphoid tissues (Constant et al., 2002; Moyron-Quiroz et al., 2004, 2006). If DCs can interact with T cells directly in the lung, where do these interactions take place? McGill et al. previously speculated that local antigen presentation in the lung may occur in local lymphoid tissues called inducible bronchus-associated lymphoid tissue (iBALT) that develop in the lungs after infection (Moyron-Quiroz et al., 2004; McGill et al., 2008).

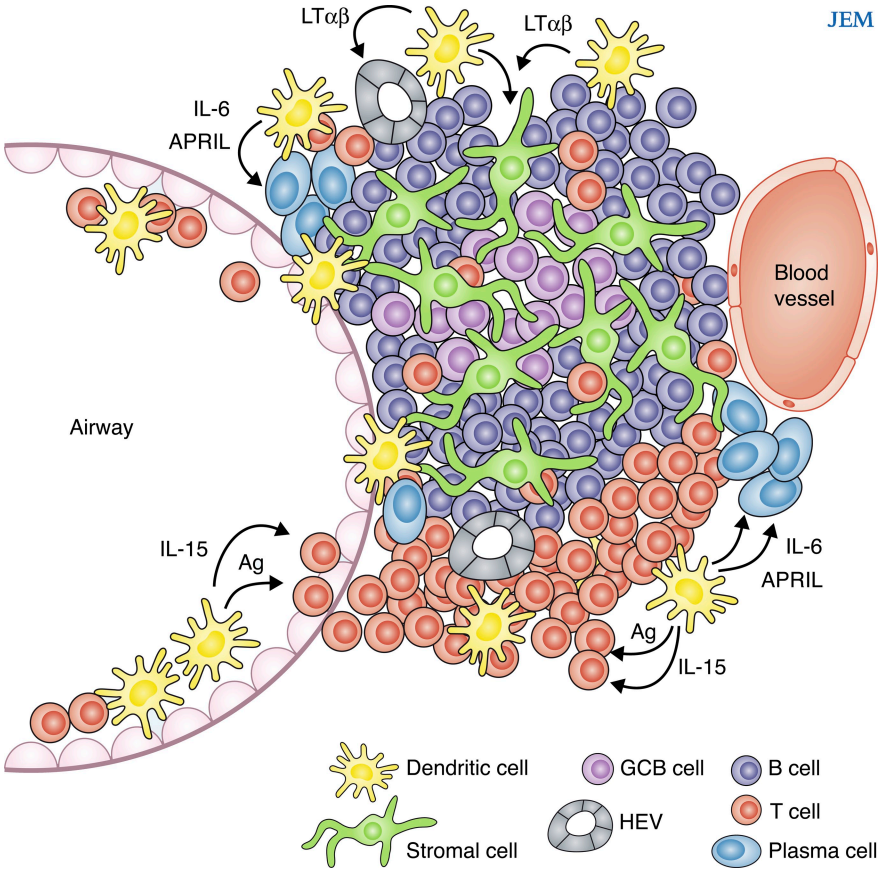

Figure 1. DCs facilitate lymphocyte recruitment, organization, and activation in the lung via multiple mechanisms. DCs may promote the survival and expansion of antigen-specific effector CD8 T cells in the lung by producing IL-15 and presenting antigen. This could occur either in the airways or in lung parenchyma, particularly in areas of iBALT. DCs may also promote the expansion or survival of plasma cells in the lung by producing IL-6 and APRIL. DCs may also indirectly affect lymphocyte activities by triggering differentiation of high endothelial venules (HEVs) or stromal cells via the production of LTαβ. In turn, the HEVs recruit lymphocytes into iBALT, whereas the stromal cells produce cytokines and chemokines that support lymphocyte survival and organization, including the formation of germinal centers.

Two recent studies (GeurtsvanKessel et al., 2009; Halle et al., 2009) indicate that pulmonary DCs are important for the maintenance and function of iBALT. GeurtsvanKessel et al. show that $CD11b^{hi}MHCII^{hi}$ DCs accumulate in the lungs after influenza infection and continue to increase in numbers, even after infection is cleared (GeurtsvanKessel et al., 2009). This increase correlates with the formation of iBALT areas, which contain central B cell follicles surrounded by DCs and some T cells (Fig. 1). Importantly, iBALT areas disappear upon depletion of pulmonary DCs, suggesting that DCs are somehow responsible for the maintenance of iBALT (GeurtsvanKessel et al., 2009). Similarly, Halle et al. find that modified vaccinia Ankara infection of the lungs leads to the progressive development of iBALT (Halle et al., 2009). Again, the maintenance of iBALT is dependent on $CD11c^{+}$ cells, as diptheria toxin (DT)–mediated depletion of $CD11c^{+}$ cells from the lungs of infected mice leads to the rapid dissolution of iBALT. Without intervention, iBALT areas are maintained for

weeks after infection is resolved, which is consistent with previous studies using mice lacking conventional secondary lymphoid tissues (Moyron-Quiroz et al., 2006).

Strikingly, Halle et al. show that pulmonary DCs transferred to the airways rapidly accumulate in iBALT (Halle et al., 2009), where they interact with T cells, present antigen, and, given the results of McGill et al. (2010), possibly trans-present IL-15. Ex vivo imaging of explanted lung tissues suggests that DCs can either migrate across the epithelium into iBALT or can enter afferent lymphatics that drain the airways and lead to iBALT (Halle et al., 2009). In either case, these data are an exciting departure from the standard model of mucosal lymphoid tissues, in which antigens are obtained by specialized epithelial M cells that obtain antigen from the lumen of the airways and then transfer it to DCs that lie in wait just below the epithelium (Kiyono and Fukuyama, 2004).

In addition to DCs, iBALT recruits adoptively transferred naive T cells, which can then respond to antigen-bearing DCs in situ (Halle et al., 2009; Fig. 1). In experiments using ex vivo lung tissue explants, Halle et al. (2009) also show that T cell dynamics in the iBALT areas are similar to those in conventional lymphoid tissues, with long-lasting stable contacts between transferred T cells and antigen-bearing DCs. These data are consistent with previous results showing naive and memory T cell responses to viral and protein antigens in the lungs of mice lacking conventional lymphoid organs (Moyron-Quiroz et al., 2006, 2004) and clearly demonstrate that iBALT is not simply a collection of inflammatory cells, but a fully functional local lymphoid tissue.

The B cell response is also altered by the presence of iBALT. GeurtsvanKessel et al. (2009) find influenza nucleoprotein-specific plasma cells and germinal centers are found in iBALT after influenza infection (Fig. 1). Germinal centers are reduced in size when $CD11c^+$ cells are depleted after a primary response, and IgA-secreting, but not IgM-secreting, plasma cells are reduced in number. Importantly, the number of long-lived nucleoprotein-specific plasma cells in the bone marrow is also reduced by the elimination of iBALT via DC depletion in the lung (GeurtsvanKessel et al., 2009), suggesting that many of the long-lived plasma cells in the bone marrow are derived from precursors in the lungs rather than the LN. Moreover, the dissolution of iBALT by DC depletion after primary infection is cleared results in reduced IgA responses in the lung and lower hemagglutinin-inhibiting activity in the serum after challenge infection (GeurtsvanKessel et al., 2009). Although these changes in the B cell response may well be attributable to the loss of iBALT, they may also reflect the loss of DC-derived plasma cell survival factors, such as IL-6 and APRIL (Mohr et al., 2009; Fig. 1). Thus, the depletion of DCs may lead to reductions in local plasma cell numbers via impairment of the plasma cell niche, which may or may not be dependent on iBALT. Nevertheless, other studies show that by inducing iBALT using intranasally delivered nanoparticles, subsequent antibody responses are accelerated upon infection with a variety of viruses (Wiley et al., 2009). Moreover, mice with iBALT exhibit reduced morbidity and mortality upon viral infection (Moyron-Quiroz et al., 2004; Wiley et al., 2009), suggesting that iBALT fundamentally alters the immune response in ways other than just the location of T and B cell priming.

Although pulmonary DCs are clearly important for the activation of T cells and for the survival of plasma cells in iBALT, it is not entirely clear how they maintain iBALT as an organized lymphoid tissue. GeurtsvanKessel et al. (2009) address this issue by showing that DCs in iBALT express homeostatic chemokines, including CXCL12 and CXCL13, as well as the cytokine lymphotoxin-β (LTβ), all of which are implicated in lymphoid organogenesis (Carragher et al., 2008). Although a potential role for the chemokines was not addressed in this study (GeurtsvanKessel et al., 2009), previous work shows that CXCL13 is not required for the formation of iBALT, per se, even though it does promote the formation of a proper B cell follicle (Rangel-Moreno et al., 2007). However, LTβR signaling is required for the maintenance of iBALT, as the administration of LTβR-Ig reduces the number of germinal center B cells and the number of iBALT areas in the lungs (GeurtsvanKessel et al., 2009). Although this experiment shows that LTβR signaling is important for the maintenance of iBALT, just as it is important for the maintenance of other ectopic lymphoid tissues (Gatumu et al., 2009), it does not indicate what cells might be the important source of LT. Given that the depletion of pulmonary DCs only modestly reduces LTβ expression and has no effect on either CXCL13 or CCL21 expression (GeurtsvanKessel et al., 2009), it seems that DCs are likely involved in the maintenance of iBALT via some other mechanism, such as the presentation of IL-15 to CD8 T cells (McGill et al., 2010). Because CD8 T cells are essential for the maintenance of germinal centers in ectopic follicles in other locations (Wagner et al., 1998), they may also be playing a role in iBALT formation and maintenance. Regardless, further experiments are needed to clarify the mechanisms by which DCs induce and maintain iBALT.

Collectively, the papers highlighted here demonstrate the importance of pulmonary DCs acting at the local level to modify the pulmonary environment in ways that facilitate local immune responses. Although these data directly apply to the activities of DCs in the lung, similar events are likely to occur in other peripheral, nonlymphoid organs and to impact local immune responses in those organs.

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