It's Complicated: Lung Dendritic Cells in Chronic Obstructive Pulmonary Disease

Complex situations require timely transmission of accurate information between individuals with specific skillsets. For the immune system, much of this communication must occur "face-to-face" in secondary lymphoid organs, the lymph nodes, Peyer's patches, and the spleen. However, real crises require regional command centers closer to the threat. Accordingly, chronic immune stimulation induces tertiary lymphoid structures within organs that normally lack them. In distal lung parenchyma, tertiary lymphoid structures are better known as lung lymphoid follicles (LLFs). This phenomenon ("lymphoid neogenesis") appears to be central to chronic obstructive pulmonary disease (COPD) pathogenesis, although whether as a cause, a consequence, or both has been unclear (1).

Evidence is mounting that LLFs may contribute to lung damage in COPD. Emphysema is conclusively linked to their appearance both in pathological human tissues (2–6) and in murine models (4–8) and is strongly associated with lung expression of B cell–characteristic genes (9–11). LLFs in COPD contain germinal centers, where B cells undergo immunoglobulin class-switching and affinity maturation through interactions with a specialized $CD4^+$ T-cell subset, T follicular helper (T_{FH}) cells. Clearly, something important is happening in LLFs.

Thus, understanding LLF development could identify unique targets to arrest emphysema at earlier stages, potentially even in preclinical phases (12). That so many of these cited and related papers (13–18) on this topic were published by the *Journal* reflects a long-standing editorial appreciation of its clinical relevance and fundamental importance. Whether blocking development of LLFs (which some call inducible bronchus-associated lymphoid tissue) would be prudent in COPD or other chronic lung diseases depends on the degree to which LLFs are necessary to protect against lung pathogens. Because data are conflicting (19), this question remains an important research topic. Deciding how best to block LLFs requires a greater understanding of their genesis. At the level of cytokine networks, there appear to be two induction pathways that differentially depend on IL-17 (20). The most obvious suspect for an essential cell type is the lymphoid tissue inducer, an innate lymphocyte that is required for secondary lymphoid organ formation. Although lymphoid tissue inducers appeared to be dispensable in one landmark study of murine influenza infection (21), this point merits examination using other lung pathogens, especially bacteria. The question remains: What cell types drive LLF appearance in smoking-induced COPD?

The answer appears to be type II conventional dendritic cells (cDC2s), as shown by Naessens and colleagues (pp. 535–548) in this issue of the *Journal* (22). Dendritic cells (DCs) were known to contribute importantly to COPD pathogenesis (23), but the relative roles of type I conventional DCs (cDC1s) (found in airway mucosa and vascular walls and crucial to generating regulatory T cells, T-helper cell type 1 [Th1] immunity, and cytotoxic $CD8^+$ T cells) versus cDC2s (found in airway lamina propria and traditionally considered to activate Th2 and Th17 responses) had been uncertain. Naessens and colleagues started by single-cell RNA sequencing lung parenchymal leukocytes to distinguish among lung antigen-presenting cell types in an unbiased fashion. However, their primary approach used flow-sorted lineage-negative, HLA-DR^+ cells selected for expression of CD141 for cDC1s and CD1c plus CD172a (SIRP-1α) for cDC2s (to which we will return). They tested the ability of lung cDC subsets to induce differentiation of allogeneic-naive T cells into T_{FH}-like cells and performed *in situ* imaging using Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage IV lung tissue.

Results demonstrate multiple interesting findings. First, lung cDC2s were the most efficient type of lung antigen-presenting cells at inducing T-cell expression of ICOS, PD-1, and CXCR5 (characteristics of T_{FH}-like cells) and of OX40 (CD134/TNFRSF4), a receptor that fosters the survival of activated T cells. Second, lung cDC2s most effectively caused T cells to produce CXCL13, a chemokine that drives LLF development (5), and IL-21, which has complex effects in COPD (24). $ICOS^{hi}PD\text{-}1^{hi}$ T cells were the dominant producers of those two molecules. Congruently, the frequency of lung $ICOS^{hi}PD\text{-}1^{hi}$ T cells was greater in participants with GOLD stage II COPD than in nonobstructed individuals. Third, lung DCs of subjects with GOLD stage II COPD showed greater expression of the ligand for OX-40 (CD252/OX40L) and greater ability to induce T_{FH}-like cells. Blocking experiments showed that the OX40/OX40L interaction was crucial to induce T_{FH}-like cells. Fourth, regardless of airflow obstruction, lung cDC2s (relative to cDC1s) expressed more G-coupled protein receptor 183, an oxysterol receptor essential for B cells and DCs to localize properly within lymph nodes. Finally, in GOLD stage IV lungs, cDC2s were abundant in the follicular T-cell zone of LLFs. Thus, to the degree that it is possible to deduce longitudinal behavior from cross-sectional data, cDC2s appear to be doing all the right things to induce germinal center formation within LLFs during COPD progression.

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Given that wealth of novel data, why then do we still say, "It's complicated"? The answer is cDC2 heterogeneity. Unlike cDC1s, which derive exclusively from bone marrow precursors, recent single-cell RNA sequencing studies show that cDC2s are not a unitary population in humans or mice (25, 26). Some cDC2s arise from the same precursors as cDC1s (27), with local signals on arrival in target organs determining their fate. But even the cDC2s derived by this route contain two further subsets that differ in transcription factor expression but are difficult to distinguish by surface markers. Importantly, these two subsets mediate diametrically opposed functions of tolerance (cDC2A, $Tbet^{+}$) versus inflammation (cDC2B, $Tbet^{-}$, $ROR\gamma t^{+}$) (26). That only the cDC2B subset circulates in human blood (26) emphasizes the necessity to study cDCs in tissues, a strength of the study by Naessens and colleagues (22).

Further complexity arises because monocytes recruited during acute inflammation can differentiate into a phenotype indistinguishable from cDC2s. Selection of cDC2s based on CD1c expression, as used by Naessens and colleagues, would include both of the recently appreciated antiinflammatory and proinflammatory cDC2 subsets plus cDC2s derived from $CD14^{+}$ monocytes (25). The authors acknowledged these limitations, which highlight the near impossibility for studies that require pathological human lung tissue (and hence years to perform) to keep up with the rapidly evolving discoveries of basic science. Nevertheless, theirs is a significant contribution that will inform the design of future studies.

Hence, the next important research goals will be to define the exact phenotype and origin of the cDC2 subset driving LLF appearance in chronic lung diseases and to delineate the triggers for that destructive behavior. We predict that a key step will be determining which of the two $CD14^{+}$ monocyte lineages ($CD14^{+} + CD16^{-}$ "classical" vs. $CD14^{+} + CD16^{+}$ "intermediate") differentiates during inflammation into the cDC2 phenotype. ■

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Antibiotic Retreatment for Acute Exacerbations of Chronic Obstructive Pulmonary Disease

Resolution of exacerbations of chronic obstructive pulmonary disease (COPD) can be incomplete and, even after resolution, relapse after a short period of baseline symptoms is common. It has been shown that incomplete resolution of exacerbations is associated with persistent airway and systemic inflammation (1, 2). One possible explanation of this persistent inflammation is either incomplete resolution of airway bacterial infection or a bacterial superinfection in the airway following an initial viral infection (3). A course of antibiotics, often a repeat course, is commonly prescribed in this situation, with little evidence to support this practice.

In this issue of the *Journal*, the study by Ritchie and colleagues (pp. 549–557) admirably sets out to address this difficult clinical question in a randomized controlled trial (4). The investigators studied whether antibiotic retreatment of incompletely recovered COPD exacerbations with ciprofloxacin prevented subsequent exacerbations or prolonged the time to next exacerbation within a 90-day period. The study randomized 144 patients but was unable to show an effect of antibiotic retreatment on time to the next exacerbation or significant effects on lung function or quality of life.

Although the Ritchie and colleagues' study is very important because it is the first of its kind to examine the question of antibiotic retreatment in a randomized controlled trial, the study has some potential flaws (4). Importantly, before we abandon the use of additional antibiotics in nonresolving exacerbations of COPD, we need to carefully examine whether the patients enrolled in this study would have been the ones who would have been expected to benefit from additional antibiotics.

Reliable indicators of bacterial infection in COPD are sputum purulence and the presence of an Anthonisen type 1 exacerbation with dyspnea, increased sputum volume, and increased sputum purulence (5). Of the patients in this study, only a small minority (22/144; 15%) had a type 1 exacerbation, and sputum purulence was present in only 41/144 (28%) at the time of randomization. Furthermore, at the time of entry into the study, 17% of the study patients reported complete resolution of their symptoms but were randomized to retreatment with antibiotics because of an elevated serum CRP (C-reactive protein). In retrospect, it seems apparent from examination of the baseline characteristics of the patients enrolled in this study that most did not have clinical evidence of bacterial airway infection at the time of randomization, and thus they did not meet traditional clinical criteria for antibiotic treatment nor would they have been expected to benefit from additional antibiotics.

Studies that have examined the symptomatic resolution of exacerbations with validated patient-reported outcome tools have shown that the median time to symptomatic resolution ranges from 9 to 16.5 days (6). However, there is considerable variation around this time frame, and some patients who are destined to recover have not yet reached their baseline level of symptoms 14 days after the initial onset of their exacerbation. These slow resolvers appear to be a significant proportion of the patients included in this study, as evidenced by the fact that the median time to full resolution of symptoms following randomization to retreatment was only 3 days in those treated with antibiotics and 4 days in the group treated with placebo. If these patients were indeed experiencing a persistent or superimposed bacterial infection, spontaneous resolution in 3 to 4 days would be highly unusual.

Understanding the value of an antibiotic treatment is enhanced by corresponding bacteriological data. If exacerbations are suspected to be bacterial, sputum yields potential pathogens in about half the instances (7). If bacterial persistence or superinfection are

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