



⊗ T-Follicular Helper-like Cells in Sarcoidosis: Lending a Helping Hand

T-follicular helper (Tfh) cells were first defined 12 years ago as a specialized subset of CD4⁺ T cells that expresses CXCR5, PD-1, and ICOS and the lineage-defining transcription factor, Bcl-6 (1–3). Tfh cells are strategically placed in lymphoid organs and are considered as nonmigratory compared with nonfollicular T-helper cells (4), which migrate from secondary lymphoid organs to nonlymphoid organs. In the past years, Tfh cells have gained attention as the major cell type regulating germinal center formation and B-cell antibody production and are involved in multiple immune disorders and infections (5). In this issue of the *Journal*, Bauer and colleagues (pp. 1403–1417) report the presence of Tfh-like cells in the BAL fluid of patients with sarcoidosis (6). These cells display features of Tfh cells while lacking CXCR5, which distinguishes Tfh cells from other T-cell subsets and is required for T-cell migration toward the T cell–B cell border of secondary lymphoid organs, and Bcl-6. *In vitro*, coculture of these Tfh-like cells with blood B cells induced B-cell proliferation and antibody production. This article thus adds an important role for Tfh-like cells in pulmonary sarcoidosis and potentially other immune-mediated lung disorders.

Sarcoidosis is a granulomatous lung disease that occurs in individuals of all ages, sexes, and ethnic backgrounds and is characterized by the accumulation of large numbers of activated CD4⁺ T cells and B cells in the lung (7). Although the cause of sarcoidosis remains unknown, a recent study identified a T-cell epitope derived from *Aspergillus nidulans* and suggested a potential role of this organism in driving Löfgren syndrome, an acute form of sarcoidosis (8). Multiple studies have delineated the phenotype of CD4⁺ T cells in the BAL fluid of patients with sarcoidosis, including the presence of CD4⁺ T cells expressing T-helper cell type 1 (Th1) and/or Th17 phenotypes as well as FoxP3-expressing regulatory T cells (9). Regulatory T cells have also been shown to be dysfunctional in sarcoidosis (10), and this dysfunctional state may contribute to the increased frequency of Th1- and/or Th17-polarized CD4⁺ T cells in the lung. A role for B cells also exists in sarcoidosis on the basis of the presence of increased IgA levels and the antivimentin antibodies (11). However, the subset of CD4⁺ T cells involved in helping B-cell differentiation and maintenance in sarcoidosis has not been defined.

Bauer and colleagues (6) sought to understand the function of pulmonary Tfh and germinal center-like lymphocytes in sarcoidosis and used flow cytometry to identify CXCR5, PD-1, ICOS, CD40L, and IL-21 expression on Tfh cells in the BAL fluid. Unfortunately, CD4⁺ T cells in the BAL fluid lacked expression of CXCR5 and Bcl-6,

the two signature molecules that define Tfh cells. However, these cells expressed CD40L and IL-21 that promote B-cell expansion and plasma-cell differentiation. On the basis of CD40L and IL-21 expression, the authors named these cells as Tfh-like cells. Future studies assessing the presence of additional transcription factors, such as TCF-1 (T-cell factor 1) and the lymphoid enhancer-binding factor LEF1, in these cells will be useful to further characterize their Tfh-like status. Cytokine analysis of *in vitro*-stimulated CD4⁺ T cells revealed the presence of multiple subsets of T cells displaying Th1 (IFN- γ -secreting), Th17 (IL-17-secreting), and presumably Tfh-like phenotypes (IL-21) in the sarcoidosis BAL fluid. Secretion of IL-2, which is considered as a negative regulator of Tfh-cell differentiation (12), was also present. Therefore, future studies assessing the presence of CD4⁺ T cells that express either IL-21 alone or both IL-2 and IL-21 will surely add information about their role in sarcoidosis. Analysis of memory markers, CXCR3 and CD69, revealed a tissue-resident phenotype of these CD4⁺ T cells that was confirmed by RNA sequencing. Among the various tissues, BAL-fluid CD4⁺ T cells showed a dominant memory phenotype as compared with tonsils or blood.

Transcriptome analysis of BAL-fluid T cells was not of great help in assessing the relationship between Tfh-like cells and classical Tfh cells because both the surface marker and the lineage marker for Tfh cells, CXCR5 and Bcl-6, were absent. However, *in vitro* T cell–B cell coculture assays confirmed the ability of Tfh-like T cells in BAL fluid to induce B-cell plasmablast formation, similar to classical Tfh cells in the tonsils (13). In addition, *in vitro* IL-21-blocking experiments confirmed the ability of IL-21-producing Tfh-like cells to induce antibody production by B cells, indicating that these cells share functional homology with classical Tfh cells (Figure 1). Tfh cells contact B cells in organized structures present in the secondary lymphoid organs. In a nonlymphoid organ such as the lung, both sterile and pathogenic inflammation induce the formation of aggregates called ectopic lymphoid aggregates or tertiary lymphoid structures, which are comprised of T cells, B cells, and follicular dendritic cells (14). In ectopic lymphoid aggregates or loosely arranged aggregates, T cells exist in close contact with B cells, allowing T cell–B cell cooperation and T cell–mediated help to B cells. To find such aggregates and confirm the cooperation of Tfh-like cells with B cells, Bauer and colleagues (6) documented the close contact between Tfh-like cells and B cells. In contrast to ectopic lymphoid aggregates, most of the aggregates in the lungs of subjects with sarcoidosis were nonectopic (i.e., lacking follicular dendritic cells). The important highlight of this current manuscript is the presence of IL-21-producing Tfh-like cells in the lung of patients with sarcoidosis that are functionally similar to but phenotypically distinct from classical Tfh cells and provide necessary help to B cells to undergo class switching and formation of plasmablasts. Tfh-like cells have also been identified in the murine lung, playing a role in T cell–B cell cooperation (15). Future studies involving lineage tracing and gene depletion in mouse models will allow a further understanding the mechanism(s) involved in Tfh-cell differentiation and function that will significantly advance the field of Tfh-like cell biology. This study

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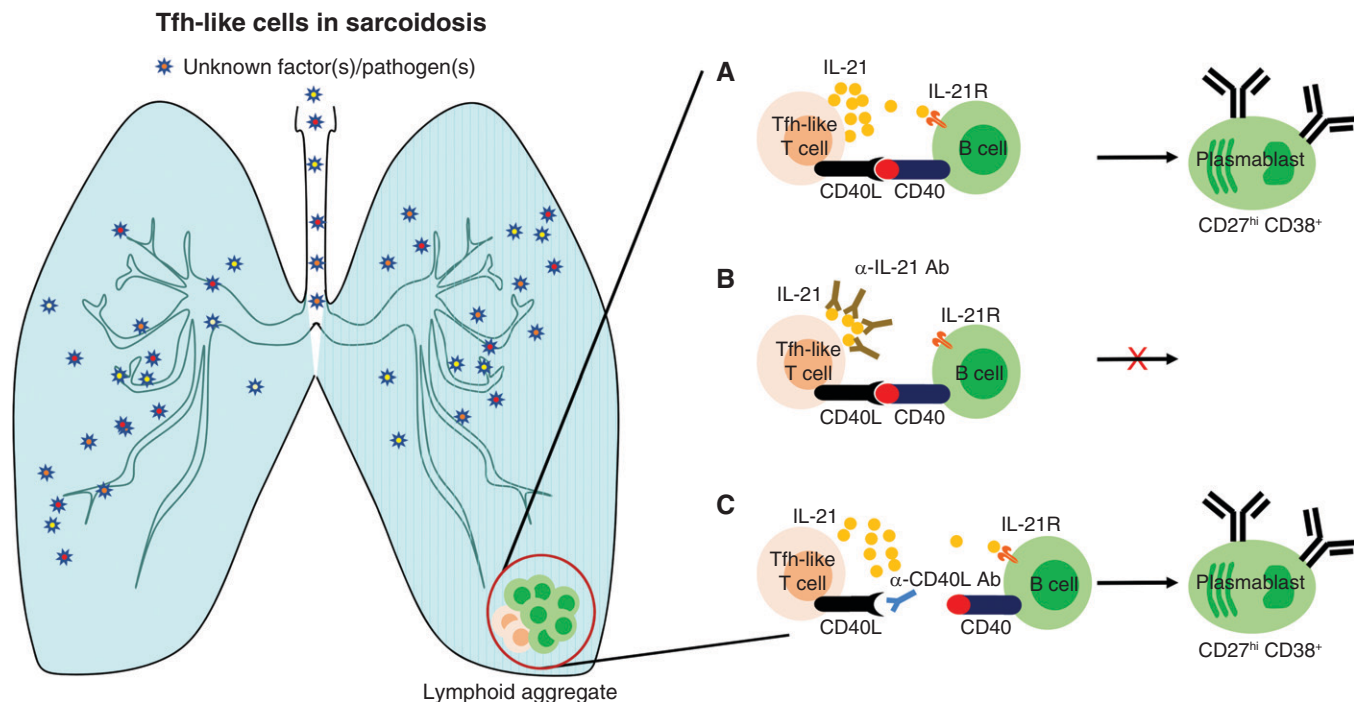


Figure 1. IL-21-producing T-follicular helper (Tfh)-like cells promote B-cell differentiation and antibody (Ab) production in the lungs. In sarcoidosis, an unknown factor causes the recruitment of leukocytes that forms cellular aggregates in the peribronchovascular region of the lungs. Within these aggregates, PD-1⁺ICOS⁺CXCR5⁺Bcl6⁺CD4⁺ T cells, named as Tfh-like cells by Bauer and colleagues (6), presumably interacted with B cells. IL-21 secretion from Tfh-like cells (A) induces B-cell differentiation into plasmablasts and (B) is inhibited in the presence of blocking IL-21 Ab. (C) B-cell differentiation and Ab production are independent of CD40-CD40L interaction.

also raises the possibility of new therapeutic strategies targeting Tfh-like cells in modulating disease activity and the use of Tfh-like cells in blood as a biomarker of disease activity. ■

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Cell Therapy with the Cell or without the Cell for Premature Infants? Time Will Tell

Bronchopulmonary dysplasia (BPD) remains one of the main complications in preterm infants born before 28 weeks' gestational age (GA) (1). Advances in perinatal care since the original description of BPD more than 50 years ago have allowed the survival of preterm infants as young as 22 weeks' GA. The corollary is that these infants are now born at the limit of biological viability because their lungs are still at the late canalicular stage when blood vessels and airways are just becoming juxtaposed. The task of protecting the ever more immature lung is becoming increasingly challenging. In a sense, neonatologists are victims of their own success. Not surprisingly, an increasing number of reports describe the long-term consequences of BPD in young adults. Pulmonary vascular disease, cardiac dysfunction, and emphysematous changes may result from early disruption of normal lung development, impaired repair processes, and early aging (2–4). Although incremental improvements in the use of our current therapies—such as less-invasive surfactant administration, for example (5)—can have an immediate positive impact on the incidence and severity of BPD, additional innovative treatments may be required to prevent and/or repair lung damage to substantially improve the respiratory outcome of micropremies.

Cell therapies for regenerative benefits represent such a promising approach. Mesenchymal stromal cells (MSCs) in particular have attracted attention in part because of their ease of isolation, culture, and expansion and because of their putative pleiotropic effects (6–8). Yet it is the immune-modulatory and reparative effects of MSCs that provided the biological plausibility for these cells to be tested in diseases with a strong inflammatory component such as the acute respiratory distress syndrome (9, 10) and BPD (11). Furthermore, MSCs do not engraft but rather act via a “hit-and-run” mechanism through cell-to-cell contact and the release of bioactive molecules contained in nano-sized particles termed exosomes or small extracellular vesicles (12, 13). These observations opened exciting prospects for cell therapies without the cell.

In this issue of the *Journal* (14), Willis and colleagues (pp. 1418–1432) follow up on their original findings (15) to explore more in detail the molecular mechanisms by which MSC-derived small extracellular vesicles (MEx) exhibit their lung-protective effects in a

well-established lung injury model in newborn mice exposed to hyperoxia. Biodistribution studies after intravenous injection revealed that MEx localize mostly to the liver and the lung. MEx interact with lung myeloid cells, restore the apportion of alveolar macrophages, and attenuate proinflammatory cytokine production. In a series of elegant experiments, the group demonstrates that MEx promote an immunosuppressive bone marrow–derived myeloid cell (BMDMy) phenotype: adoptive transfer of MEx-educated BMDMy, but not naive BMDMy, preserved alveolar architecture, blunted fibrosis and pulmonary vascular remodeling, and improved exercise capacity in this model. These findings provide further evidence for the antiinflammatory and reparative mechanisms of action of MSCs and their MEx.

Based on the above results, it is not surprising that MEx were found to accumulate mostly in the liver within 24 hours. Whether the liver could be the exclusive site of further macrophage/myeloid cell education or whether MEx migrate to the BM to directly interact with immune cells in this location deserves further exploration. Likewise, lineage tracing studies may answer the question whether educated cells subsequently migrate from the BM to the lungs or whether MEx only affect circulating immune cells. MEx administration early during the disease process was also able to blunt fibrosis, arguing in favor of early intervention and thus providing some clinical directions for these findings. Finally, it is uncertain whether identification of the MEx biological cargo will be critical for the clinical application of MEx therapy, although more understanding of the RNA and protein components that are most therapeutic might advance more focused therapies for preventing BPD in micropremies.

Although these observations demonstrate that much more needs to be learned about the biology of MSCs and their nanovesicles, the time is ripe for well-designed early-phase clinical trials to test the feasibility and safety of MSC-based therapies in preterm infants at risk of BPD. The results of the very first phase I trials suggest feasibility and short-term safety of a proprietary cord blood–derived MSC product administered as early as 10 days of life via the intratracheal route (16–18). Results of a phase II trial testing this same product in 66 preterm infants at 23–28 weeks' GA did not show a significant improvement in the primary outcome of death or moderate/severe BPD with MSCs compared with placebo (19). In that study, a subgroup analysis suggested an improvement in the secondary outcome of severe BPD (53% [8/15] to 19% [3/16]) with MSCs in the 23 to 24 gestational weeks group, but the study was underpowered, prompting a larger trial focused on these lower GA categories. Other cell products such as human amnion epithelial cells

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