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Sarcoidosis and T-Helper Cells
Th1, Th17, or Th17.1?

It is widely accepted that sarcoidosis is a noninfectious disorder of the immune system characterized by an abnormal antigen-mediated adaptive immune response culminating in the formation of granulomas in affected tissues. The lungs are most commonly affected, incriminating inhaled environmental antigens as initiators of the disease process. There are clear gene-by-environment interactions that predispose to sarcoidosis, with several genetic association studies implicating $CD4^+$ T-cell immune response genes in disease pathogenesis (1). The prototypical adaptive immune response in sarcoidosis is characterized by the presence of IFN-γ–producing $CD4^+$ cells in inflamed tissues (2), which, taken together with the essential role played by IFN-γ during granuloma formation in animal models (3), supports the idea that sarcoidosis is a type 1 T-helper cell (Th1) disease.

Despite the Th1 bias in sarcoidosis, however, we now realize that the immunopathology of sarcoidosis is complex, with evidence for activation of the innate immune system, dysfunction of regulatory T cells, and expansion of IL-17–producing cells including $CD4^+$ Th17 cells (4–7). Th17 cells are prevalent in epithelial surfaces such as the lungs, skin, and gut, where they contribute to host immune responses against bacterial, fungal, and mycobacterial pathogens largely by orchestrating the recruitment of inflammatory cells (8). Interestingly, Th17 cells have been implicated in the development of Crohn's disease, another disease characterized by noninfectious granuloma formation (8). Several lines of evidence point to a role for Th17 cells in sarcoidosis. First, the frequency of IL-17–producing T cells is increased in peripheral blood and lungs of subjects with sarcoidosis compared with controls (9). Second, IL-17A was shown to be essential for mature granuloma formation in response to mycobacterial infections in mice (10). Third, a recent large case-control study confirmed an association between genetic variants near the IL-23 receptor (which promotes Th17 responses) in different cohorts of subjects with sarcoidosis (11).

In this issue of the *Journal*, Ramstein and colleagues (pp. 1281–1291) used multiparameter flow cytometry to analyze bronchoalveolar lavage (BAL) T cells and report that a subset of Th17 cells that coexpress IFN-γ is particularly enriched in the lung in sarcoidosis (12). IFN-γ–producing Th17 cells (termed Th17.1 or Th17/Th1 cells) were previously detected in mouse and man, including in human subjects with sarcoidosis or Crohn's disease (13–15). Ramstein and colleagues identified Th17.1 cells by their coexpression of the chemokine receptors CCR6 and CXCR3, which had been previously linked with Th17 and Th1 cells, respectively (12). IFN-γ production by these $CCR6^+CXCR3^+$ Th17.1 cells rivaled that of canonical $CCR6^-CXCR3^+$ Th1 cells, suggesting that Th17.1 cells are major producers of IFN-γ in the sarcoid lung. Interestingly, whereas traditional Th17 numbers were elevated in the blood of patients with sarcoidosis, Th17.1 cells were specifically elevated in BAL (and not blood). One possibility is that IFN-γ^- Th17 cells are recruited from the circulation into the lung, and differentiate into IFN-γ^+ Th17.1 cells under the influence of local inflammatory signals. This mechanism is supported by the differential expression of CXCR3 on Th17.1 cells in BAL (and not in blood), a chemokine receptor expressed on effector T cells that plays a role in cell trafficking and inflammation. The factors that control the development of Th17.1 cells *in vivo* are not known, but several cytokines have been implicated in this process, including IL-1β, IL-12, and IL-23 (16). Engagement of Th17 cells with IL-12 and IL-23 promotes the activation of transcription factor Tbet (Tbx21, T-box expressed in T cells), which in turn regulates the expression of *IFNγ* and related chemokine genes (*CXCL9*, *CXCL10*, and *CXCL11*), leading to the Th17.1 phenotype (17). Interestingly, human IFN-γ^+Th17.1 cells were preferentially induced *in vitro* by exposure to *Candida albicans* in an IL-1β–dependent manner (18). It remains to be seen what controls the preferential expansion of Th17.1 cells in the lung in sarcoidosis, and it will be informative in future studies to dissect the contributions of pathogen-encoded signals and inflammatory cytokines to this process. Future studies investigating how cellular metabolism and redox balance influence Th17 subset development in sarcoidosis also seem worthwhile, as these processes are likely perturbed in granulomatous inflammation.

The study by Ramstein and colleagues builds on growing evidence that "not all Th17 cells are created equal" (12). Major insights into Th17 subset differentiation have come from mouse

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models using adoptive transfer and lineage tracing, approaches that are not feasible in human subjects. The idea is amply demonstrated in mouse models of experimental autoimmune encephalitis, where Th17 cells differentiated in the presence of IL-23 promote brain inflammation *in vivo*, whereas their counterparts differentiated in the absence of IL-23 are not (19). In these and other models, pathogenic Th17 cells are often characterized by coexpression of IFN-γ. Furthermore, fate mapping studies showed different outcomes for IL-17A$^+$ cells in the settings of experimental autoimmune encephalitis versus infection with *Candida*, even to the point of turning off the Il17 gene completely (20). Taken together, it appears that distinct Th17 subsets differentiate *in vivo*, depending on the disease state and/or pathogens encountered, and the challenge now is to relate different subsets to clinically meaningful outcomes.

The study by Ramstein and colleagues has several strengths, including the use of sophisticated immunophenotyping of rare blood and lung cell types from two different, well-characterized human cohorts (12). This approach provided a semiquantitative assessment of Th1, Th17, and Th17.1 cell populations in lung and blood compartments, and related *ex vivo* experiments confirmed the likely contribution of each cell type to IFN-γ–mediated inflammation in sarcoidosis. There are also some limitations in the present report, including that the authors relied on surface chemokine receptor expression to identify Th17.1 cells and did not confirm their findings with expression of lineage-defining transcription factors (e.g., RORγt$^+$ for Th17 cells and Tbx21 for Th1 cells). Although CCR6 is usually expressed by RORγt$^+$ Th17 cells, a recent report by Kara and colleagues demonstrated a shift from CCR6 expression to CCR2 expression in effector Th17 cells in an experimental autoimmune encephalitis model (21), suggesting CCR6-negative Th17 cells can develop *in vivo* in some settings.

What are the implications of this study? First, Ramstein and colleagues' findings suggest that classifying CD4$^+$Th cells into canonical Th1 or Th17 subsets is not sufficient to explain this complex human disease, and that if replicated in other studies, sarcoidosis might be better considered a "Th17.1 disease" (12). Second, if Th17.1 cells are pathogenic in sarcoidosis, then combined antagonism of Th1/Th17 pathways may be needed to achieve therapeutic efficacy in humans. This might explain the apparent failure of single-pathway antagonists in clinical trials. Given the important role for IL-1β in inducing Th17.1 cells, strategies targeting the IL-1 pathway may also be worth exploring in some patients. Finally, Th17 and Th17.1 cells are relatively resistant to the antiinflammatory effects of glucocorticoids, and it will be interesting in future studies to determine whether these cells are expanded in the subset of subjects with severe, steroid-resistant sarcoidosis. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Don't Let (D_L)CO Be Misunderstood

Why are patients with systolic heart failure and reduced ejection fraction short of breath when they exercise? It is surely a simple question, and one might be inclined to answer, "well, of course it's the cardiac output," but the answer is perhaps more complex. In this issue of the *Journal*, Kee and colleagues (pp. 1292–1300) provide some promising steps toward a better understanding of the mechanism, or at least one of the mechanisms (1). Their cohort of patients with severe congestive heart failure awaiting heart transplantation is a most interesting group to examine and represents the severe end of the spectrum in which histopathological evidence of pulmonary vasculopathy could be predicted to exist; specifically, basement membrane thickening, basal pulmonary hemosiderosis, and sometimes even endothelial cell proliferation resembling pulmonary capillary hemangiomatosis, according to prior studies, albeit in valvular heart disease and congenital heart disease (2–4). Whether these changes can and do regress with therapy, and to what degree, is a moot point (5). Improvements in diffusing capacity of the lung for carbon monoxide (D_{LCO}) have been reported with the use of angiotensin-converting enzyme inhibitors, spironolactone, exercise training, and acutely with a single dose of sildenafil (6–9), but not by ultrafiltration (10). Conversely, acute intravascular volume expansion with saline impaired alveolar-capillary membrane function and increased airflow obstruction in patients with left ventricular dysfunction, raising the possibility that abnormalities of pulmonary diffusion in heart failure might have a variable component that could be amenable to therapeutic intervention (11). However, even successful heart transplantation is not invariably associated with physiological evidence of improvement over time (12).

Systolic heart failure with reduced cardiac output and elevated filling pressures is but one cause of dyspnea in this group. Many heart transplant recipients have concomitant respiratory dysfunction resulting from cigarette smoking, and the real degree of functional limitation related to lung disease *per se* is often not fully appreciated until successful heart transplantation unmasks the lung disease, especially during exercise (13). In fact, many patients after heart transplant still report dyspnea on exertion despite ostensibly normal left ventricular ejection fractions. Deconditioning and frailty, specifically sarcopenia, are cogent confounding factors in this regard. It was once hoped that pretransplant cardiopulmonary exercise testing would be a critical tool to help determine the balance between heart and lung dysfunction, but in practice, the ability to fully test the pulmonary component may be limited by the severity of the heart failure, causing reduced alveolar-capillary membrane diffusing capacity (14).

The central hypothesis of this intuitive piece of work is that heart failure causes short-term, and possibly longer-term, changes in the pulmonary microvasculature, giving rise to a reduced D_{LCO}, which alters ventilation and perfusion matching, particularly during exercise, when cardiac output rises, and thereby increases total dead space ventilation. Dead space ventilation, of course, is inefficient and is associated with an elevated work of breathing. The authors argue that anatomical dead space should remained fixed, which suggests that the increase in measured dead space by the end of exercise in patients with higher maximum dead space is a result of an inability to increase pulmonary perfusion at the same rate as ventilation. Some consideration should also be given to the possibility that anatomical dead space may paradoxically decrease as a result of increased water volume in the sustentacular space, so that an increase in total dead space may actually signal a very considerable increase in alveolar dead space indeed.

In essence, the work is logically sound, but how good is the evidence? The study group of 87 consecutive heart transplantation referrals was heterogeneous, comprising a broad range of cardiac pathologies including ischemic (33%), idiopathic dilated cardiomyopathy (47%), hypertrophic obstructive cardiomyopathy (9%), congenital (5%), valvular (4%), and other (2%), but to counter this potential criticism, the focus was on the subgroup with the highest maximum dead space. As the authors clearly state, all patients in this study had severe systolic heart failure (heart failure with reduced ejection fraction) and were being assessed for transplantation. Hence, the findings might not apply to patients with diastolic dysfunction (heart failure with preserved ejection fraction), or indeed patients with systolic heart failure or patients with less severe disease. Notwithstanding these potential limitations, the study group, with the exception perhaps of its severity (mean New York Heart Association class, 2.9), could be encountered in any advanced heart failure program, so the message is likely of clinical relevance. The control group represents the other end of the spectrum and is drawn from an atypical population of fit younger nonsmoking subjects (100% male) with a mean peak work of 161% predicted. That aside, the emphasis is in the relevance of the D_{LCO}, which was undeniably normal in the controls (113% predicted).

Although somewhat beyond the scope of the study, it is intriguing to note that after review of pulmonary radiology reports of all patients, including chest computed tomography in 87%, no patient had reported evidence of emphysema or gas trapping, despite a mean smoking history of 18 pack-years in the 46% of ex-smokers in the study group. Details about computed tomography