A Little Complement Goes a Long Way: A Perspective from the Pleural Space

Tuberculosis (TB) remains one of the leading causes of pleural effusion in developing countries (1–3). Tuberculous pleural effusion (TPE) is characterized by an excess accumulation of inflammatory cells and fluid in the pleural space. Although 5% of patients with TB develop TPE globally, the prevalence of this problem varies drastically (4, 5). In the United States, only 3% of patients with TB develop TPE, whereas in other countries (e.g., South Africa), up to 20% of patients with TB develop TPE (2, 4). Furthermore, immunocompromised patients with TB are reported to have higher percentages of TPE than immunocompetent patients (2, 5). Although the incidence of TB may be decreasing in countries like the United States, the incidence remains high in developing nations, justifying the need for continued research (5).

Although untreated TPE can resolve in 4–16 weeks, untreated patients can develop active TB (5). TPE, in most cases, is characterized by increased numbers of inflammatory cells (6). Although acute disease (<2 wk) is characterized by high numbers of polymorphonuclear cells or neutrophils, over time this population shifts to primarily lymphocytes. In one report, up to 90% of the inflammatory cell population consisted of lymphocytes (7). TPE is also characterized by the relative paucity of pleural mesothelial cells (2).

Monocytes are critical for TB progression (8, 9). In the lung, resident and recruited monocytes phagocytose foreign materials, including the invading *Mycobacterium tuberculosis* (*Mtb*). When this process is successful, these monocytes can "wall off" the infection and form granulomas, which may lead to the development of latent disease (10). However, in some cases the engulfed *Mtb* do not die and instead proliferate and eventually kill the monocytes. The dead but infected monocyte is then engulfed by other monocytes, supporting the *Mtb* proliferation cycle (8, 9). Although the role of monocytes in the proliferation and containment of TB is under active investigation, the mechanism of their recruitment to the pleural space and the role of pleural mesothelial cells in this process have been unclear.

In this issue of the *Journal*, Luo and colleagues (pp. 454–464) provide an in-depth analysis of the role of anaphylatoxins in nonclassical monocyte recruitment (11). Specifically, they investigated the role of anaphylatoxin expression by mesothelial cells in monocyte migration into the pleural space. Anaphylatoxins are derived from complement activation and can lead to anaphylactic shock (12–14). They are produced by cleavage of the complement proteins C3, C4, and C5 to form C3a, C4a, and C5a. The convertases responsible for this cleavage are generated by the classical, lectin, or alternative pathway. Anaphylatoxins can also be generated without complement activation via select proteases expressed by residential cells or pathogens. They can induce vasoconstriction and increase vascular permeability (13, 14). Anaphylatoxins can also induce both innate and adaptive immune responses (12–14). In this study, Luo and colleagues interrogated the ability of C3 and C5 to stimulate chemotaxis of inflammatory cells that express their cognate receptors, C3aR and C5aR. Because the TB protein Mpt64 potently induced C3a and C5a expression in pleural mesothelial cells, the authors investigated their potential role as the primary effector cells in initiating the recruitment of monocytes into the pleural space. They did not, however, determine whether Mpt64 exerted similar effects on monocytes or fibroblasts, which are also likely to be present in the pleural and subpleural mesothelium.

These investigators also found that monocyte percentages were increased in the pleural effusions of patients with TPE compared with patients with transudative effusions. Although this control population was predominantly comprised of classical monocytes, they found the nonclassical monocytes, $CD14^{+}CD16^{+}$, also accumulated in the pleural fluid of patients with TPE in comparison to the peripheral blood. These sentinel observations led them to seek to elucidate the mechanism for nonclassical monocyte accumulation in the pleural space of patients with TPE.

The authors next identified activated complement and other key factors involved in complement activation in patients with TB pleurisy. They found that the components for classical, lectin, and alternative complement activation pathways were elevated in the pleural tissues of patients with TB pleurisy. These components included C1q, Factor B, and mannose-binding lectin (MBL), among others. These proteins were also elevated in the pleural effusions of these patients. As expected, the activated complement proteins C3a and C5a were likewise elevated in these pleural effusions. Although pleural mesothelial cells were identified as the likely source of C3a and C5a, both mesothelial cells and nonclassical monocytes expressed C3aR and C5aR, and thus likely contributed to the C3a- and C5a-mediated proinflammatory effects. In contrast, classical monocytes expressed less C3a, C5a, C3aR, C5aR, and chemokines than the nonclassical monocytes. However, classical macrophage responses to anaphylatoxins were not determined. The finding that complement activation is promoted by pleural mesothelial cells is novel and introduces a new paradigm by which these cells can contribute to the pathogenesis of TPE.

Because chemokines (CCL2, CCL7, etc.) were increased in TPE compared with transudates, the authors next sought to determine whether anaphylatoxins could regulate chemokine production. As anticipated, C3a and C5a induced chemokine production by both pleural mesothelial cells and nonclassical monocytes. Furthermore, C3a and C5a directly induced inflammatory cytokine production by monocytes, as IL-1β, IL-17,

and IL-27 were increased in the presence of anaphylatoxins. However, in this study, the authors measured monocyte migration using nonstimulated mesothelial cells. Paradoxically, these cells induced monocyte migration in the absence of stimulation by anaphylatoxins. This finding limits the postulated importance of anaphylatoxin-induced cytokine production for monocyte migration as may occur in the context of TPE.

Although this study supports the hypothesis that anaphylatoxins play some role in increased cytokine production and nonclassical monocyte recruitment, several questions remain. Although monocytes are anticipated to play a role in the early stages of TPE development, their role in more established disease is unclear. Furthermore, are the effects demonstrated by anaphylatoxins limited to nonclassical monocytes? If similar or disparate results were demonstrated in classical monocytes or lymphocytes, the importance of this study would increase dramatically. Because active anaphylatoxins are relatively short-lived due to rapid cleavage and deactivation, the notion that they play a significant contributory role in maintenance of TPE seems unlikely. Although these questions remain to be resolved, the findings of this provocative study support the need for further investigation of the range of effects that anaphylatoxins have on pleural mesothelial cells in TPE and perhaps in other forms of pleural injury. ■

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

Torry A. Tucker, Ph.D.
Department of Cellular and Molecular Biology
The University of Texas Health Science Center at Tyler
Tyler, Texas

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