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An Emotional Molecular Pathway in Pulmonary Hypertension–Alternative Complement System

The article by Frid and colleagues (pp. 224–239) in this issue of the *Journal* shows the significance of the alternative complement system in pulmonary hypertension (PH) (1). The discovery of this antimicrobial and immune-surveillance system has an emotional history (2). The classical pathway of complement activation by antibody–antigen complexes was discovered in the 1890s. Roughly half a century later, a brilliant biologist and biochemist, Dr. Pillemer, and his team discovered that the complement pathway could be activated by properdin in the absence of antibody–antigen complexes (3). Their discovery soon became very controversial; however, the Pillemer lab did not have the technical means to experimentally dispel all criticisms, and perhaps as a result of this controversy compounding existing mental issues, Dr. Pillemer died of suicide (2). His original observations were corroborated in the ensuing decades, and the pathway that he discovered was termed the alternative pathway (2). The alternative pathway is the ancient part of the complement system, with critical components present in insects and echinoderms (4). The lectin pathway was discovered later and represents yet another way to activate complement (5). Researchers in basic science are currently discovering further links between these pathways, such as those between the lectin and alternative pathways (6). What makes the complement system so important (5)? In a cascade of proteolytic activation steps, the complement factors C5b and C6–C9 form the lytic complex, which is an essential defense mechanism that can destroy microbes or faulty cells. In addition, many of the intermediary complement fragments (e.g., C3a and C5a) have major immune-regulatory effects.

Frid and colleagues investigated the role of complement cascade activation in regulating proinflammatory and proproliferative processes during the initiation of experimental hypoxic PH and tested whether it can serve as a prognostic biomarker of outcome in human pulmonary arterial hypertension (PAH; Figure 1). The authors stained lung tissues from experimental PH models and patients with PAH, analyzed genetic murine models lacking specific complement components or circulating immunoglobulins, cultured human pulmonary adventitial fibroblasts, and performed a network medicine analysis of plasma from patients with PAH. Pulmonary perivascular–specific activation of the complement cascade was identified as a consistent critical determinant of PH/PAH in experimental animal models and humans. In experimental hypoxic PH, proinflammatory and proproliferative responses were complement (alternative pathway and C5) dependent, and immunoglobulins, particularly IgG, were critical for activation of the complement cascade in which Csf2/GM-CSF (granulocyte–macrophage colony–stimulating factor) was identified as a primary complement-dependent inflammatory mediator.

In their study, Frid and colleagues used omics to interrogate the association between the complement system and PAH. Omics studies are popular because of their potential for discovering new molecular disease mechanisms or new disease subgroups (for example, the pulmonary vascular omics network [7]). Here, the authors used omics to identify three classes of molecular deviations: *1*) disease-causing genes (e.g., hemophilia caused by defective factor VIII or factor IX genes), *2*) disease risk–increasing genes (e.g., increased risk of PAH in BMPR2 [bone morphogenic receptor 2] mutation carriers), and *3*) molecular pathways that can be critical for disease pathogenesis (e.g., the complement pathway). The filters used to identify these three distinct molecular deviations have a decreasing stringency, with the third, molecular pathways that can cause disease, having the least-stringent discovery filter.

It is to be remembered that not all PH is caused by aberrant complement activation. C5-deficient mice can develop the PH phenotype. The FVB mouse strain is a popular transgenic

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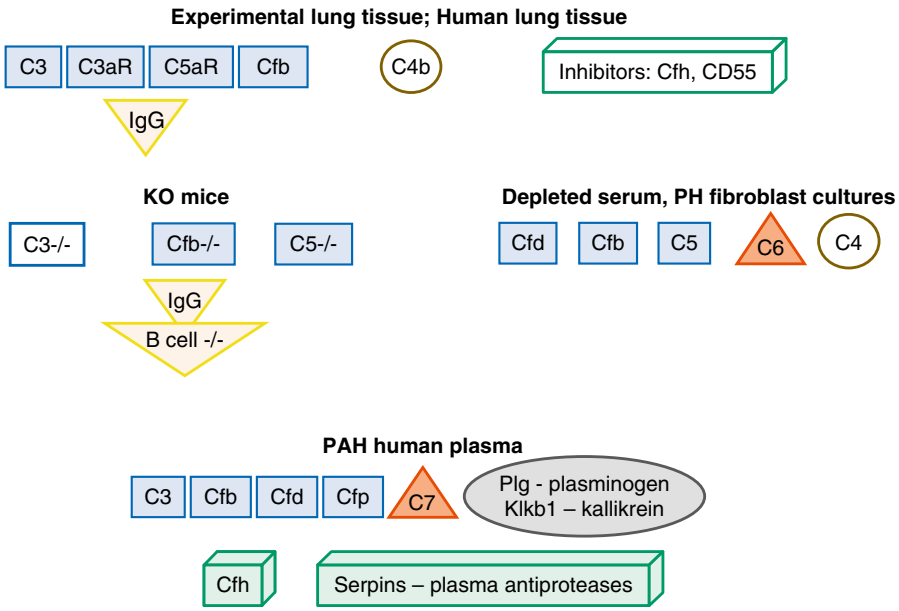

Figure 1. Schematic representation of the sequential experimental interrogation of the complement system presented in the article by Frid and colleagues (1). The complement components and interacting molecules are symbolized by colors and symbols. The shaded symbols denote factors that were found to be significantly different or have significant effects. The open symbols denote factors for which the study found no significance. The highly interactive complement network serves essential antimicrobial and immune surveillance functions by creating a membrane attack complex (red triangles) of C5b, C6, C7, C8, and C9 via a series of proteolytic reactions (5). The classical and lectin pathways (brown circles) lead to the generation of activated C4-C2 complex, which then activates C3, and in turn via activated C5 generates the membrane attack complex. The alternative pathway (blue squares) leads to activated C3 by using Cfb, Cfd, and Cfp, which then activates C5 and the membrane attack complex. C5 can also be activated in the alternative pathway via proteases of the coagulation system (kallikrein, plasmin [gray oval], and thrombin). By producing antibody (IgG; yellow triangles), B cells can activate the alternative pathway. Complement inhibitors (Cfh and CD55 [green boxes]) and serum protease inhibitors (serpins) are the endogenous blockers of the system. C = complement; CD = cluster of differentiation; KO = knockout; PAH = pulmonary arterial hypertension; PH = pulmonary hypertension.

strain that lacks C5, and FVB mice that carry a transgene of a mutated BMPR2 gene develop PH (8, 9). Complement factor mutations are rare (10), with the C2 mutation being the most common (1 in 20,000). These mutations can cause significant defects in antimicrobial defenses and autoimmunity, including systemic lupus erythematosus (SLE) (10). Compared with the general population, patients with SLE have a higher incidence of deficiencies in the classical/lectin pathway components C1, C2, and C4 (10), and PH (11); however, the functional relevance of the alternative complement pathway in SLE-associated PH is not known.

Frid and colleagues found that activation of the alternative complement pathway was antibody dependent. The significance of B cells and antibodies in PH/PAH has long been appreciated (11–14). However, the other implication of the significance of alternative complement pathway activation for PH/PAH is that this pathway can be activated without antibodies. Therefore, it is possible that new therapies aimed at removing antibodies by depleting B cells (15) may not be effective for every patient with PH, even those with a documented autoimmune-type history, because the alternative complement pathway could bypass the requirement of an antibody. The study by Frid and colleagues raises a question as to the yet unknown nature (specificity and isotype) of the antibody that induced experimental PH. The alternative complement activation may explain the mechanism underlying the previous observation that antibodies of an isotype class that does not activate the classical pathway can be critical initiators of some aspects of the PH phenotype (16). In the plasma complement–PAH network interrogated by Frid and colleagues, the involvement of antibody is not known, and it is not known whether interactions between the alternative, classical, and lectin pathways also contribute to the dysregulation of the complement system in PAH.

Frid and colleagues highlight the role of an essential molecular antimicrobial and immune surveillance pathway of antibodies and alternative complement activation in PH and PAH. This issue requires our attention and further investigation. ■

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