Complement System in Lung Disease

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Abstract

In addition to its established contribution to innate immunity, recent studies have suggested novel roles for the complement system in the development of various lung diseases. Several studies have demonstrated that complement may serve as a key link between innate and adaptive immunity in a variety of pulmonary conditions. However, the specific contributions of complement to lung diseases based on innate and adaptive immunity are just beginning to emerge. Elucidating the role of complement-mediated immune regulation in these diseases will help to identify new targets for therapeutic interventions.

Keywords: immunology; fibrosis; transplantation; immune complexes

Clinical Relevance

Complement activation, an important immune response, can exacerbate lung injury. Understanding how complement proteins play a role in several of these lung diseases can help us gain insight into how to inhibit complement activation to attenuate further lung inflammation for future therapeutic interventions.

Lung diseases are increasing in prevalence and are expected to become the third leading cause of disease mortality and morbidity worldwide by 2020 (1). Therefore, understanding the biological processes involved may help elucidate mechanisms to ameliorate their harmful effects. Recent studies show that complement plays a key part in linking innate and adaptive immunity and that immunology can contribute to lung injury. Specifically, the role of immunity, including complement activation, is recognized increasingly in conditions such as ischemia reperfusion injury (IRI), acute lung injury (ALI), acute respiratory distress syndrome (ARDS), pneumonia, asthma, pulmonary arterial hypertension (PAH), idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), and obliterative bronchiolitis (OB) (2). This translational review highlights the key

components of the complement cascade and focuses on their role in these diseases.

Complement Proteins

The complement system has key roles in innate and adaptive immune responses. Three pathways may lead to complement activation (Figure 1): classical, lectin, and alternative (3). All three pathways lead to similar downstream enzymatic signaling, which results in cell lysis by pore formation, opsonization, and chemoattraction.

The classical pathway involves formation of immune complexes, with IgM or IgG antibodies binding to targets such as autoantigens (3), as well as teichoic acid expressed on gram-positive bacteria (4) or LPS expressed on gram-negative bacteria (5, 6). The complement C1 complex binds to the Fc portion of the antibody and initiates a series of enzymatic cascades to form complement convertases that produce

the anaphylatoxins C3a or C5a, which bind to their specific receptors and act as chemoattractants for phagocytes. Other complement by-products, such as C3b, can be involved in opsonization of pathogens to be destroyed by phagocytes. Complement activation may lead to cell death by formation of the membrane attack complex (C5–C9), which forms pores in the cell membrane, leading to cell lysis.

The lectin pathway involves mannose-binding lectin (MBL) binding to carbohydrates, such as D-mannose, fucose, and N-acetylglucosamine (7), on various microbial cell surfaces (8, 9), late apoptotic blebs, or necrotic cells (7). MBL is known to compete with C1q when binding to late apoptotic blebs because they bind to the same or adjacent epitopes on these apoptotic cells, as seen in Jurkat T cells (7). This activates mannose-associated serine proteases, which leads to downstream

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Figure 1. Complement pathways. Three pathways are known to activate the complement cascade. The classical pathway is initiated through binding of C1q protein to Fc receptor on the antibody–antigen complex. Activation of the lectin pathway is mediated through mannose-binding lectin that binds mannose (carbohydrates) on pathogens’ surface. Alternative pathway act as an amplification loop by spontaneously hydrolyzing C3 to initiate complement activation. All three pathways converge to form C3 convertase, which downstream leads to assembly of C5 convertase, thus forming membrane attack complex (MAC), which results in cell death. The complement pathways can be regulated at the level of C3 and C5 convertases or at MAC.

signaling similar to the classical pathway. MBL is a C-type lectin belonging to the collectin family and consists of a carbohydrate recognition domain that allows it to function as a soluble receptor binding to mannose-rich glycans on microbes that are also known as pathogen-associated molecular patterns. The lectin pathway also consists of ficolins, which bind to *N*-acetylglucosamine on microbes. Another way to activate lectin-mediated complement signaling involves polymeric IgA, which is important in mucosal immunity to defend against invading pathogens by binding to MBL to initiate complement cascade (7).

The alternative pathway is activated by a process known as “tickover” (10). This involves spontaneous hydrolysis of the thioester bond in complement protein C3 at a rate of approximately 1% of total C3 per hour (10). It activates a fluid-phase C3 convertase, which consists of Factor B binding to C3, thus allowing Factor D, a plasma protease, to cleave C3 into C3a and C3b. The alternative pathway is also known as the “amplification loop” because it can utilize the C3b from either the lectin or classical pathway and initiate the tickover process.

Role of Complement Proteins in Adaptive Immunity

The name “complement” refers to its ability to complement antibodies and other cells in eliminating pathogens from the host. The ability of complement to regulate humoral immunity on B cells has become a new area of focus. Complement receptor 2 (CR2), mainly present on B cells and follicular dendritic cells (FDCs), is part of the B cell coreceptor that includes CD19 and CD81. This coligation involving the B cell receptor and the CR2–CD19–CD81 complex augments B cell activation by decreasing the threshold for antigen dose in B cell activation, leading to clonal expansion (11). However, CR2 also binds to antigen-bound C3d, which is a subfragment of C3b. CR2 present on FDCs in germinal centers can interact with C3d–opsonized antigen, thus allowing FDCs to present this antigen to B cells, leading to humoral immunity (11). Although most diseases discussed in this review focus on systemic complement activation, complement proteins can be also be produced locally. It is important to highlight the effects of local complement production on lymphocytes, which play a role in adaptive immunity and in the systemic effects on inflammation and immunology.

Antigen-presenting cells are regulated by the complement system via C3a and C5a. For example, binding of each to their G-protein–coupled receptors up-regulates IL-12, MHCII, CD40, and CD80 on dendritic cells, which activates PI3K γ /AKT and inhibits cAMP/phosphokinase A, leading to Th1 cell activation and expansion (12). However, dendritic cell–induced T cell activation in the absence of C3a or C5a may result in down-regulation of costimulatory molecules and in initiation TGF- β secretion, which may lead to induction of Th17 cells or T regulatory cells (13). C3a- or C5a-induced activation of PI3K γ /AKT leads to increases in antiapoptotic genes, such as Bcl2, and down-regulation of apoptotic genes, such as FAS, thus enhancing T cell expansion, survival, and activation (13).

Complement Regulatory Proteins

Excess complement activation may lead to tissue injury and therefore is tightly regulated in part by membrane-bound complement regulatory proteins (CRPs) such as DAF/CD55, CD46 (or the murine homolog CRRY), CD59, and complement receptors CR1 and CR2. CRPs are expressed on many cell types, such as airway epithelial cells, fibroblasts, endothelial cells, and T and B lymphocytes. CD46 and complement receptor (CR1 or CR2) serve as cofactors that bind to C3b or C4b, allowing serine protease Factor I to inactivate them by proteolytic cleavage (14). Complement activation can also be regulated by preventing the assembly or dissociating reformed C3 convertase by decay acceleration factor (DAF/CD55), C4 binding proteins (C4BP), Factor H, or the rodent-specific complement receptor–related protein (CRRY) (14). Complement-mediated cell lysis can be prevented by inhibiting membrane attack complex–induced pore formation on cells (15).

Certain CRPs factor in regulating adaptive immune responses. CRPs such as CD46, CRRY, and DAF can participate in T cell activation. In mice, CRRY coligates with CD3 on T cells to induce IL-4 secretion (14). CD46 is a type 1 transmembrane protein that has two cytoplasmic tails, Cyt1 and Cyt2. Coligation of CD46 with CD3 may lead to T regulatory cell differentiation, depending on IL-2 concentrations present in the extracellular fluid (16, 17). The cytoplasmic tail Cyt1 is cleaved by presenilin/ γ -secretase after

CD46-mediated T cell receptor ligation, which can activate T cells, but cleavage of Cyt2 leads to T cell inhibition (17). DAF, a GPI-anchored protein, appears to suppress T cell responses in murine models *in vivo* by decreasing complement C3 activation and reducing levels of IFN- γ and IL-2 (18).

Complement Synthesis in Lung

Although most complement proteins are synthesized in the liver as inactive precursor enzymes known as “zymogens,” the lung may provide local source of complement proteins. In particular, pulmonary alveolar type II epithelial cells synthesize and secrete complement proteins C2, C3, C4, C5, and Factor B (19), whereas human bronchiolar epithelial cells can generate C3 (20). Local complement synthesis yields insights into the interaction between complement and lung disease. Inflammatory cytokines, such as IL-6, IL-1, TNF- α , IFN- γ , can initiate complement synthesis in cells such as resident polymorphonuclear leukocytes, epithelial cells, and fibroblasts (21).

Alveolar macrophages can synthesize complement proteins (Figure 2A). Macrophages from tissues other than the lung are also able to produce complement proteins under certain inflammatory conditions (Figure 2C) (22). *In vitro* experiments by Huber-Lang and colleagues demonstrated that alveolar macrophage-derived serine proteases cleave C5 produced by epithelial cells into C5a that, when bound to its receptor C5aR, initiated inflammatory signaling cascades (23). Activating alveolar epithelial cells with C5a, LPS, IL-6, or TNF- α can increase C5aR expression on alveolar epithelial cells but also can increase the affinity of C5a binding to C5aR (24). Immune complexes may induce local injury in the lung via complement activation. In this setting, the Fc portion of the immune complex binds C1q, leading to classical pathway activation (Figure 2B). Lung inflammation is further exacerbated by complement proteins acting as chemoattractants for neutrophils (Figure 2D). In addition, C5a can induce the release of proteolytic enzymes from neutrophils (Figure 2D).

IRI

Although it may occur in mechanical ventilation, pneumonia, hypotension, and aspiration (25), the most devastating effects of IRI are observed after lung

transplantation (26). Complement activation after reperfusion can lead to significant tissue injury due to production of C3a and C5a, which attract and activate neutrophils (26–29). Bosmann and colleagues have also shown a role for C5a in activating neutrophils by binding to its corresponding receptors (c5aR and C5L2) (30). This binding leads to formation of neutrophil extracellular traps that can lead to the appearance of extracellular histones, which can exacerbate conditions such as ALI (30). In a swine model, the administration of soluble CR1, a C3 and C5 convertase inhibitor, reduced lung edema, decreased neutrophil accumulation, and improved oxygenation of the transplanted lung (31, 32). Keshavjee and colleagues reported that CR-1 inhibition administered to human lung transplant recipients decreased the duration of mechanical ventilation and improved gas exchange (33). Combining the therapeutic effect of blocking complement and leukocyte adhesion molecules resulted in reduced reperfusion injury and improved oxygenation in an orthotopic allogeneic single left lung transplantation model in rats (29).

ALI

Using cobra venom factor, which is a functional analog of C3, to activate complement, Till and Ward (34, 35) reported formation of complement protein C5a, which has chemoattractant property to sequester neutrophils. This leads to oxygen radical-dependent lysis of endothelial cells and increased lung vascular permeability characteristic of ALI (34). C5a also up-regulated expression of adhesion molecules, such as P-selectin, on pulmonary endothelial cells, leading to neutrophil adherence to pulmonary vascular endothelium.

ARDS

Complement activation has been well documented in clinical and experimental ARDS. Zilow and colleagues observed that the ratio of plasma C3a to C3 was increased, whereas C5a was not detectable, in multiple trauma patients who later developed ARDS (36). In contrast, Hammerschmidt and colleagues detected elevated C5a levels in ARDS plasma from patients with ARDS (37), whereas Schein and colleagues confirmed that C3, C4, and Factor B complement proteins were not indicative of ARDS in the assessment of 59 patients with

septic shock (38). Duchateau and colleagues reported complement C5a-like activity in patients with ARDS and in patients without ARDS (39). Thus, the specific roles of complement proteins in ARDS remain undefined.

Pneumonia

Biochemical properties of many viruses, bacteria, and fungi can activate complement proteins and initiate the innate immune response for host protection. However, for the purpose of this review only a few selected examples are highlighted.

Classical and alternative complement pathways play a role in opsonization of pneumococci due to their various serotypes (40). The alternative pathway is activated by teichoic acid on the pneumococcal cell wall, and the classical pathway is activated by pneumolysin, a cytolytic toxin produced by *Streptococcus pneumoniae* (41). C3 has a protective role in the lungs in the early stage of infection with *S. pneumoniae* (42). Complement regulation is also critical in response to gram-negative bacteria. The morbidity of *Pseudomonas aeruginosa*, a common cause of hospital-acquired pneumonia (43), is exacerbated in mice by deficiencies of C3 (43).

The pathogenesis of respiratory syncytial virus (RSV), a cause of bronchiolitis and pneumonia, is in part mediated by the formation of RSV-specific immune complexes. Murine RSV is attenuated in mice deficient in C3 and B cells (44). Wells and colleagues indicated a role for complement proteins in antibody-mediated protection against *Pneumocystis carinii* pneumonia (45).

PAH

Increased pulmonary arterial pressure, pulmonary vascular resistance, pulmonary vascular remodeling, and pulmonary vasoconstriction characterize the pathogenesis of PAH (46). Increased plasma levels of C3 and C4a have been reported in PAH (47). Bauer and colleagues were the first to report the role of complement activation in PAH pathogenesis by showing that inhibition of C3 by use of C3-/- mice attenuated the right ventricular systolic pressure and right ventricular hypertrophy, pulmonary vascular remodeling, and prothrombotic effects of hypoxia (46).

IPF

Although the etiology of IPF remains elusive, certain factors, such as smoking and

Figure 2. Model of immune complex-mediated lung injury. (A) Normal airways. (B) Injury to the lung can be mediated by immune complex-mediated injury in the lung epithelium. Antigen inhaled or present in the lung is bound by its antibody. C1q binds to the Fc portion of the antibody and activates complement cascade. (C) Lung injury can also initiate inflammation by complement activation through local synthesis of complement proteins from alveolar macrophages or airway epithelium. (D) Lung inflammation is exacerbated by complement proteins that act as chemoattractants for neutrophils.

genetic predisposition, have been linked to this disorder (48). Very early studies revealed evidence of complement activation in IPF as shown by detection of complement-activating immune complexes and fragments of activated complement proteins in serum and bronchoalveolar lavage fluid of patients with IPF (49–52). Preclinical models of IPF pathogenesis showed that complement depletion attenuated bleomycin-induced lung fibrosis and suggests a possible association between complement activation and lung fibrosis (53). Addis-Lieser and colleagues examined the role of C5 in bleomycin-induced pulmonary fibrosis by demonstrating that C5 promoted fibrosis through TGF- β 1 and MMP-3 and that blocking C5 attenuated fibrosis (49). Schein and colleagues recently reported the presence of the autoantibody, anti-HSP-70 in patients with IPF (38). These data provide indirect evidence of complement activation in IPF pathogenesis, considering that HSP-70 antigen and anti-HSP-70 immune complexes were associated with worse lung function (54).

Asthma

Airway inflammation is a hallmark of allergic lung diseases such as asthma (55, 56). Although allergic asthma is characterized, in part, by production of the Th2 cytokines IL-17 and IgE, recent studies indicate a role for complement activation (57–60). For example, Nakano and colleagues (59) and Krug and colleagues (60) showed that C3a and C5a levels increased in bronchoalveolar lavage fluid in response to allergen challenge in patients with asthma. Ovalbumin-induced airway hyperresponsiveness (AHR) resulted in decreased bronchiolar expression of CD55 and CRRY in mice with locally increased C3a and C5a (J. Lott and D.S. Wilkes, unpublished observations). Lajoie and colleagues observed reciprocal roles of C3a and C5a in the regulation of experimental allergic asthma using a similar model in which they demonstrated a role for IL-17A mediating ovalbumin-induced allergic AHR through C5a deficiency, but C3a deficiency led to less AHR severity (61). Although the mechanisms by which C5 is protective and C3 is inflammatory in allergic asthma have not been fully elucidated, Kohl and colleagues have suggested that repression of Th-2-specific chemokines (CCL17 and CCL22) production by myeloid dendritic cells results in less homing of Th2 cells and

their immune response (62). To support the findings of the protective effects of C5 in allergic asthma, Haslam and colleagues (51) and Lewkowich and colleagues (63) suggested that plasmacytoid dendritic cells, which drive T regulatory cell-induced tolerance, may be regulated by C5-activating plasmacytoid dendritic cells, resulting in resistance to allergen-driven AHR. However, other studies suggest that C3a may regulate recruitment and activation of Th2 cells, contributing to the severity of allergic asthma (51, 64).

COPD

Recent studies reveal elevated levels of circulating C3a and C5a in patients with COPD, which provide indirect evidence that complement proteins may contribute to disease pathogenesis (65–68). C5a levels were significantly up-regulated in COPD as compared with healthy nonsmoking individuals (65), with further local up-regulation during COPD exacerbations (69). Exposure to cigarette smoke, the major risk factor for COPD, has been reported to activate alternative complement pathways (70). Specifically, tobacco glycoprotein is able to bind C1q, leading to production of C5a, which can enhance neutrophil recruitment (71, 72). Cigarette smoke and C5a can act in unison to induce the expression of adhesion molecules on epithelial cells to facilitate recruitment of

inflammatory cells to the site of injury, as reported by Floreani and colleagues (71). Examination of the lung proteome by Grumelli and colleagues (73) revealed that a reduction in CD46 expression correlated with a loss of lung function in COPD, which could help to explain complement activation reported in these patients.

OB

OB is a form of chronic lung allograft dysfunction that limits patient survival after lung transplant (74). In a murine orthotopic lung transplant model of OB, Suzuki and colleagues reported that airway epithelial expression of CD55 and CRRY was down-regulated and associated with up-regulated local levels of C3a and C5a (74). CD46 and CD55 were down-regulated with increased local levels of C3a and C5a in human lung transplant-associated OB (74). Neutralizing C5 abrogated OB, indicating that OB was in part complement dependent in mice (74). Interplay between IL-17-related pathways and complement was shown by demonstrating that neutralizing IL-17 reduced local C3a production, whereas CD55 and CRRY/CD46 expression was restored in murine lung allografts. Therefore, complement activation and IL-17 in OB were linked in this model. In contrast, Takenaka and colleagues reported that complement activation was not required for anti-MHC Class I

antibody-induced lymphocytic bronchiolitis, which is considered a precursor of OB, but lung pathology was IL-17 dependent (75). The differences between the studies reported by Suzuki and colleagues (74) and Takenaka and colleagues (75) are likely due to their animal models. The former used an orthotopic model of lung transplantation, resulting in down-regulation of airway CD55 and CRRY. The latter used an intact murine lung, with intact expression of CD55 and CRRY, in which antigen-specific antibodies were instilled.

Future Directions and Conclusions

Several studies suggest a role for activated complement proteins in the biological processes involved in lung diseases such as COPD, IRI, ALI, ARDS, pneumonia, PAH, IPF, allergic asthma, COPD, and OB. Future studies involving complement receptor inhibition and their downstream signaling cascade may lead to a better understanding of the mechanism of complement-mediated injury in these diseases. To reduce complement activation in lung diseases, it may also be beneficial to understand and elucidate how proteins, such as CRPs, contribute to these lung diseases. ■

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

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