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REVIEW

# The role of C5a in acute lung injury induced by highly pathogenic viral infections

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**The complement system, an important part of innate immunity, plays a critical role in pathogen clearance. Unregulated complement activation is likely to play a crucial role in the pathogenesis of acute lung injury (ALI) induced by highly pathogenic virus including influenza A viruses H5N1, H7N9, and severe acute respiratory syndrome (SARS) coronavirus. In highly pathogenic virus-induced acute lung diseases, high levels of chemotactic and anaphylatoxic C5a were produced as a result of excessive complement activaiton. Overproduced C5a displays powerful biological activities in activation of phagocytic cells, generation of oxidants, and inflammatory sequelae named ''cytokine storm'', and so on. Blockade of C5a signaling have been implicated in the treatment of ALI induced by highly pathogenic virus. Herein, we review the literature that links C5a and ALI, and review our understanding of the mechanisms by which C5a affects ALI during highly pathogenic viral infection. In particular, we discuss the potential of the blockade of C5a signaling to treat ALI induced by highly pathogenic viruses.**

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## INTRODUCTION

The epithelium of the lung is vulnerable to damage caused by inhaled microorganisms and other noxious particles. Many studies suggested the presence of complement components at the alveolar epithelium, where inhaled airborne particles and microorganisms are deposited.[1-3] In addition, the complement system has been implicated in the development of acute lung diseases induced by highly pathogenic viruses including influenza A virus H1N1,[4] H5N1,[5] H7N9,[6] severe acute respiratory syndrome coronavirus (SARS-Cov),[7] Middle East respiratory syndrome coronavirus (MERS-Cov).[8] 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 identify new targets for therapeutic interventions.[9]

Complement activation leads to the formation of bioactive molecules, including the anaphylatoxins, C3a and C5a, and the lytic membrane attack complex (C5b-9).[10] The complement-activated product C5a is a strong chemoattractant and is involved in the recruitment of inflammatory cells such as neutrophils, eosinophils, monocytes, and T lymphocytes, in activation of phagocytic cells and release of granule-based enzymes and generation of oxidants.[10] C5a also displays other powerful biological activities including inducing ''cytokine storm.'' On the other hand, blockade of C5a signaling has demonstrated potential benefits in the treatment of acute lung injury (ALI) induced by highly pathogenic viruses. In this article, we summarize recent developments in our understanding of the role of C5a in mediating aute lung injury induced by highly pathogenic viruses.

## ACUTE LUNG INJURY INDUCED BY HIGHLY PATHOGENIC VIRAL INFECTIONS

### Highly pathogenic virus

Due to high mutation rates of viruses, every several years to decades a highly pathogenic virus emerges. Especially in the recent decades, there were more than five highly pathogenic viruses such as SARS coronavirus in 2002, avian influenza A/H5N1 virus in 1997, H1N1 virus in 2009, H7N9 virus in 2013, and MERS coronavirus in 2012. As exemplified by coronaviruses and influenza viruses, bats and birds are natural reservoirs for providing viral genes during evolution of new virus species and viruses for interspecies transmission.[11,12] This is the primary cause of an outbreak by jumping directly from bird to human.

The novel influenza A virus (IAV) pandemic poses a serious threat to public health. The data provided by the World Health Organization demonstrated that the 2009 H1N1 influenza pandemic caused over 18 138 deaths from outbreak to May 30, 2010; highly pathogenic H5N1 resulted in the deaths of 385 people from 2003 to Feb 27, 2015; the avian-originating H7N9 has resulted in over 560 human infections, leading to 135 deaths since emerging in 2013 to Feb 27, 2015.

Except for influenza A virus, coronaviruses such as SARS-CoV and MERS-CoV represent another serious threat to public health. Between November 2002 and July 2003, an outbreak of SARS caused an 8096 cases and 774 deaths according to World Health Organization. MERS-CoV was a novel human coronavirus that caused outbreaks of a SARS-like illness in the Middle East in March of 2014.[13] In two months, 536 laboratory-confirmed cases and 145 deaths have been reported globally.[14]

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There is an H5N1 vaccine for human use, but there is currently no H7N9, SARS or MERS vaccine available. Current vaccination strategies are still inadequate at providing protection against epidemic outbreaks. Thus, it is urgent to explore the mechanism by which highly pathogenic viruses induce diseases.

**Acute lung injury induced by highly pathogenic viral infections**

Although highly pathogenic virus infections have the different epidemiology, there is a similar rapid progression to acute respiratory distress syndrome (ARDS).[15] For example, histopathological changes in the lung from patients infected with H5N1 are highly similar to those of patients with SARS.[16] Except for influenza A H5N1 virus, avian influenza A H7N9 virus in 2013 also caused severe pneumonia.[17] Postmortem biopsy of 3 patients infected with H7N9 in 2013 showed acute diffuse alveolar damage: patient 1, who died 8 days after symptom onset, had intra-alveolar hemorrhage, whereas patients 2 and 3, who died 11 days after symptom onset, had pulmonary fibro proliferative changes.[18]

Patients infected with H5N1 develop rapidly progressive pneumonia, further resulting in ALI or ARDS.[19,20] ALI may be a critical cause of death in patients with H5N1 infection.[19,21] Like H5N1 infection, H7N9 also causes serious lung pathology. In addition, SARS-CoV infection caused ALI that may progress to life-threatening ARDS. MERS-CoV infection resulted in a more severe pneumonia than SARS-CoV infection.[22]

Respiratory distress is the most common cause of death in patients infected with highly pathogenic virus. In terms of therapy, lung protective ventilation is the cornerstone of supportive care.[23] Extracorporeal membrane oxygenation is routinely used in many centers for the treatment of severe respiratory tract infections. However, due to few effective treatment options, ALI is often fatal for patients infected with highly pathogenic viruses. This suggests that serious lung pathology should be of particular concern.

## COMPLEMENT AND C5a ACTIVATION IN ACUTE LUNG INJURY INDUCED BY HIGHLY PATHOGENIC VIRAL INFECTIONS

After a microorganism infection begins, the host quickly activates the complement system to clear infected pathogens.[24] During the complement activation, the high levels of products such as C5a are commonly involved in exacerbated inflammatory reactions that can cause direct harm to the host following infections.[25-27]

IAV belongs to the *Orthomyxoviridae* family with single-stranded negative-sense RNA virus,[28] and has the capacity to activate the complement system.[29] In addition, the avian influenza hemagglutinins typically bind alpha 2-3 sialic acid receptors, whereas human influenza hemagglutinins bind alpha 2-6 sialic acid receptors.[30] Thus, H5N1 replicates in the lower respiratory tract, then causes complement activation.[31] This suggests that upon influenza infection, the high levels of C3 and C5 including fragments C3a and C5a are produced.

Complement activation possibly contributes to the observed tissue damage in severe viral infection.[32] Studies demonstrated that ALI in H5N1-infected mice was caused by excessive complement activation such as release of C5a.[5] Thus, complement activation plays a critical role in the pathogenesis of virus-induced acute lung injury.

Among the complement activation products, the anaphylatoxin C5a is one of the most potent inflammatory peptides.[33] Increased levels of C5a were found in bronchoalveolar lavage fluid (BALF) and serum from patients infected with fatally H1N1 pandemic virus.[4,34] C5a had also been found to increase in BALF of mice infected with highly pathogenic avian influenza H5N1 but not following seasonal IAV infection.[35] On the other hand, BALF from recovered patients with ARDS demonstrated significantly reduced C5a-dependent chemotactic activity.[36] Thus, C5a might play a critical role in the pathogenesis of virus-induced acute lung injury.

## THE MECHANISMS UNDERLYING C5a-MEDIATED ACUTE LUNG INJURY INDUCED BY HIGHLY PATHOGENIC VIRAL INFECTIONS

**C5a-mediated inflammatory cells migrate into lung tissue**

Compared to normal controls, SARS patients had increased cellularity of BALF with increased alveolar macrophages.[37] Thus, mononuclear cell infiltration might have an important role in the pathogenesis of ALI induced by highly pathogenic viruses like SARS.

Anaphylatoxin C5a has been implicated in the pathogenesis of ARDS by mediating neutrophil attraction, aggregation, activation, and subsequent pulmonary endothelial damage.[38-41] Reversely, C5a-dependent chemotactic activity is significantly decreased in recovered patients with ARDS.[36] These suggest that C5a-mediated mobilization and activation of immune cells might be the central events to tissue injury caused by highly pathogenic viral infections.

Two chemoattractants C5a and interleukin 8 (IL-8) can be synthesized by cells in the lung (e.g., macrophages, epithelial cells, endothelial cells, smooth muscle cells and neutrophils).[33] IL-8 levels have also been found to correlate with neutrophil numbers and the degree of lung dysfunction.[42] C5a could strongly amplify IL-8 expression from human whole blood cells induced by lipopolysaccharides and other types of toll-like receptors agonists via extracellular-signal-regulated kinases 1/2 and p38, but not c-Jun N-terminal kinase.[43] The data suggest that C5a might be a critical effector molecule to mediate lymphocyte attraction by itself or indirectly by enhancing the production of IL-8.

Altogether, C5a-mediated lymphocyte attraction plays a critical role in the pathogenesis of ALI induced by highly pathogenic viruses.

**C5a-mediated neutrophil extracellular traps**

Neutrophil extracellular traps (NETs) are primarily composed of DNA from neutrophils, which bind pathogens with antimicrobial proteins. NETs are beneficial in antimicrobial defense and can help fight against invading pathogens. However, an excess of NETs contributes to the pathology of a number of diseases including those of the lung.[44] NETs are found in infection-related ALI models of influenza virus.[45,46]

*In vitro* studies demonstrated that C5a, in association with granulocyte-macrophage colony-stimulating factor, is able to induce the release of NETs.[47] C5a is also able to activate macrophages and endothelial cells and to promote vascular leakage and the release of NETs.[10] Thus, NETs are induced by C5a during IAV infection and are associated with alveolar damage in IAV-induced pneumonitis.[45]

The excess of NET components are potent factors in lung injury. NET increases the permeability of the alveolar-capillary barrier by cleaving endothelial actin cytoskeleton, E-cadherin and VE-cadherin.[48] The antimicrobial peptide LL-37 in NET structures presents cytotoxic and proapoptotic properties towards endothelial and epithelial cells.[49] NET also induces the release of proinflammatory cytokines.[48] The data suggest that C5a-mediated neutrophil extracellular traps aggravate ALI in patients infected with highly pathogenic virus.

**C5a-mediated release of reactive oxygen species**

C5a is a strong chemoattractant for neutrophils and monocytes; it then activates these cells to generate oxidative burst with release of reactive oxygen species (ROS), especially $O_2$ and $H_2O_2$.[10] A study demonstrated that ROS are primary pathogenic molecules in pneumonia from mice infected with influenza virus.[50] The amount and duration of exposure of generated ROS, released from respiratory, immune, and inflammatory cells, determined the extent of lung damage.[50] In lung fibroses, inflammatory cells produce a significantly greater amount of ROS. Critically, antioxidant treatment significantly reduces lung damage and mortality in influenza-infected mice.[51] These studies demonstrated a critical role of reactive oxygen intermediates (ROIs) in virus-induced epithelial damage.

C5a-C5aR interaction plays a critical role in oxidative burst.[52] Interception of C5a/C5aR signaling with a C5aR antagonist significantly inhibited oxidative burst in neutrophils induced with *E. coli*. Similarly, anti-C5a blocked the oxidative burst in whole blood induced with *Neisseria meningitides*.[53] Phosphorylation of $p47^{phox}$ is essential for assembly of NADPH oxidase and the subsequent production of $O_2$ and $H_2O_2$.[10] C5a is a strong activator of mitogen-activated protein kinase (including p42/p44), which is an important kinase for $p47^{phox}$ phosphorylation.

Except for directly affecting tissue damage, oxidant production might also be involved in signal transduction pathways. IL-8 expression is enhanced by the oxidant sensitive transcription factor nuclear factor-κB[54] activated in the lungs of influenza-infected mice.[55] This means that oxygen-derived free radicals might exert much greater effects on the pathogenesis of the disease by indirectly inducing other proinflammatory mediators. Thus, C5a-mediated oxygen-derived free radicals are thought to be important events in the pathogenesis of the disease.

**C5a-mediated release of histones**

Histones are essential regulators of genome function in eukaryotic cells. The NS1 protein of influenza A H3N2 subtype possesses a histone-like sequence (histone mimic), and could target the human RNA polymerase-associated factor 1 transcription elongation complex which has a crucial role in the antiviral response.[56] Thus, the virus used NS1 histone mimic to suppress human RNA polymerase-associated factor 1 transcription elongation complex-mediated antiviral response.

Diversely modified histone regulates gene replication, repair and transcription. After activation with influenza, H3K4me3 reduced association of interferon I (IFN-I) and IFN-III promoters in dendritic cells (DCs) to suppress antiviral gene expression.[57] In contrast to IFNs, the association of tumor necrosis factor-α (TNF-α) promoter was not disturbed.[57]

Histone can be excreted into cells to reduce intracellular histone to suppress antiviral gene expression. In the setting of ALI both in humans and in mice, histone presence has been found in BALF.[58] In addition, when polymorphonuclear leukocytes are incubated *in vitro* or *in vivo* with C5a, neutrophil extracellular histones-contained extracellular traps (NETs) develop.[59] These results suggest that engagement of C5a with its receptors led to the appearance of extracellular histones in BALF.

Extracellular histones significantly enhance inflammatory response by inducing nucleotide-binding domain and leucine-rich repeat containing family, pyrin domain containing 3 (NLRP3) inflammasome.[58] Furthermore, airway instillation of histones resulted in intense lung injury and inflammation, together with fibrin clots in pulmonary veins.[60] C5a-mediated release of histones has an important contribution to the pathogenesis of ALI.

**C5a-mediated the upregulation of adhesion molecules**

The process of leukocyte adhesion to endothelial cells is the first critical step in neutrophil migration into an area of inflammation. Adhesion molecules on the surface of endothelial cells have an important role in inflammatory cell migration. In fact, C5a can regulate the expression of adhesion molecules.[61] C5a directly activates endothelial cells to upregulate adhesion molecules such as P-selectin. In addition, C5a and TNF-α cooperate to enhance upregulation of intercellular adhesion molecule 1 and E-selectin.[62] Thus, C5a is an effective mediator in the first step in inflammatory cell migration into the lung.

Adhesion molecules on the surface of inflammatory cells also have an important role in inflammatory cell migration. *In vitro* studies demonstrated upregulation of CD1lb/CD18 expression on neutrophils induced by C5a.[10] In addition, C5a also induced the expression of β1 and β2 integrin on blood neutrophils.[63,64] Thus, enhanced adhesive interactions of neutrophils to endothelial cells promote inflammatory cell migration into inflammatory sites.

The adhesion molecules effectively enhanced pro-inflammatory cytokines such as TNF-α production by pulmonary macrophages, which, in turn, promotes the inflammatory response.[62] Blockade of CDllb, CD18, intercellular adhesion molecule 1, or P-selectin significantly reduced ALI damage by neutrophil content of the lungs.[65] Anti-C5a might protect tissue injury in various organs by limiting neutrophil sequestration through downregulating the expression of adhesion molecules.[10] These studies suggest that C5a-mediated upregulation of adhesion molecules promotes the inflammatory response.

**C5a-mediated adaptive immune response**

C5a induces innate immune cells including mast cells, neutrophils, and macrophages to release cytokines such as IL-12, TNF-α and macrophage inflammatory proteins-1α.[66] IL-12 is a strong activator of $CD8^+$ T cells, whereas TNF-α promotes transendothelial migration of T cells by up-regulating vascular adhesion molecules and induces IFN-γ expression in T cells.[66] These data demonstrate that C5a indirectly induces adaptive immune response by activating innate immune cells.

Apart from innate immune cells, human DCs[67,68] and T cells[69] also express the C5a receptor (C5aR, CD88). Thus, C5a is also a potent chemoattractant for human T cells,[69,70] B cells,[71] and DCs.[67,68,72,73] In addition, during the early inflammatory stage of a pathogen infection, DCs used C5a as a homing signal to take up Ag, and then were primed for helping T-cell function.[74] Thus, C5a induces adaptive immune response by recruiting for DCs.

CD28 and CD40L on T cells are important signaling for T-cell proliferation and differentiation induced by interaction of locally-produced C5a with C5aR on antigen-presenting cells (APCs). Accordingly, C5a could not activate $Cd80^{-/-}$ $Cd86^{-/-}$ and $Cd40^{-/-}$ APCs to induce T cell activation.[75] The data suggest that the local interaction of C5a and C5aR on APCs is critical to $CD4^+$ T cell proliferation and differentiation.

The binding of the C5a to the C5aR also plays an important role in $CD8^+$ T cell responses.[74] $CD8^+$ T cell activation during influenza infection requires C5a, which acts as a chemoattractant for T lymphocytes.[69,76] Thus, it is conceivable that C5a might elicit $CD8^+$ T cell response upon the input stimuli. Accordingly, C5aR antagonist reduced the frequency and absolute numbers of flu-specific $CD8^+$ T cells.

**C5a-mediated cytokines storm**

In patients infected with influenza A virus like H5N1, Inflammatory cytokines such as IL-1β, IL-8, and IL-6 play a major role in mediating and amplifying ALI and ARDS by stimulating by chemotaxis C5a.[77] C5a induces innate immune cells including mast cells, neutrophils,



Emerging Microbes and Infections EMI201528.3d 27/4/15 12:40:24

and monocytes/macrophages to release proinflammatory cytokines such as IL-12, TNF-α and macrophage inflammatory proteins-1α.[64] In addition, C5a also stimulates adaptive immune cells such as T and B cells to release cytokines such as TNF-α, IL-1β, IL-6, and IL-8.[78,79]

Many cytokines, triggered by highly pathogenic viruses like H5N1, has been called a ''cytokine storm''.[80] Cytokines were rapidly induced at 24h post infection with H5N1.[81] The pro-inflammatory cytokines including IL-1β and TNF-α might contribute to the severity of disease by promoting maximal lung inflammation caused by H5N1 viral infection.[82] Compared to healthy volunteers, H7N9-infected patients have significantly higher levels of cytokines such as IL-6, IFN-γ-inducible protein 10, IL-10, IFN-γ, and TNF-α.[83] A dangerous cytokine storm also occurs in SARS. The representative SARS-CoV ssRNAs had powerful immunostimulatory activities in inducing pro-inflammatory cytokines TNF-α, IL-6 and IL-12.[84] Elevated levels of some pro-inflammatory cytokines including moncyte chemoattractant protein-1, transforming growth factor-beta1, TNF-α, IL-1, and IL-6, produced by cells infected by SARS-CoV, might cause ALI.[85] In addition, a cytokine could induce other cytokines to further enhance the pro-inflammatory response. Take for example, elevated levels of TNF-α induced other cytokines like IL-6.[86] Thus, cytokine storm plays an important role in ALI.

Anti-TNF-α (etanercept) significantly reduced the damage of ALI.[87] The inhibition of macrophage migration inhibitory factor alleviated H5N1 influenza virus pneumonia in murine model by causing a significant reduction in pulmonary inflammatory cytokines IL-1β, IL-6 and TNF-α and IFN-γ-inducible protein 10[88] A widely used antiviral agent Arbidol hydrochloride efficiently inhibits both H1N1 strains and diminishes both viral replication and acute inflammation through suppression of inflammatory cytokines such as IL-1β, IL-6, IL-12, and TNF-α.[89] These studies indicate that blockade of cytokine storm is effective in treatment of infections with highly pathogenic virus.

### C5a-mediated immune paralysis

The severe H7N9 patients were in a state of immune paralysis with general leukopenia, low antigen-presenting capacity and impaired T cell response.[90] Those suffering fatal infections with H7N9 have particularly low proportions of peripheral blood T lymphocyte subgroups.[91] Previous studies have demonstrated that C5a induces thymocyte apoptosis, which in turn results in decreased number of T cells in circulation and attendant immunosuppression.[10,92] This suggests that in a striking contrast to neutrophils, thymocytes apparently receive pro-apoptotic signals from C5a.

During SARS-CoV infection, IL-6 and IL-8 induced by C5a inhibits the T-cell-priming ability of DCs.[93] Compared to significant up-regulation of inflammatory chemokines, the SARS-CoV-infected DCs showed low expression of antiviral cytokines (IFN-α, IFN-β, IFN-γ, and IL-12p40).[94] These studies are in accordance with the conclusion that the N-protein of SARS-CoV induced ALI by resulting in imbalance of pro-inflammatory and anti-inflammatory cytokines.[95] Many inflammatory and anti-viral genes were differentially expressed in SARS patients. Plenty of pro-inflammatory cytokines such as IL-1, TNF-α, and IL-8 significantly increased, whereas a number of IFN-stimulated genes like double-stranded RNA-dependent protein kinase, interferon-induced guanylate-binding protein-1 and 2, C-X-C motif chemokine 10 decreased in the acute severe case.[96] Like SARS-CoV, MERS-CoV viruses were unable to significantly stimulate the expression of antiviral cytokines (IFN-α and IFN-β) but induced comparable levels of TNF-α and IL-6.[8] C5a-C5aR interaction might potentiate the mitochondrial apoptotic pathway and/or enhance the expression of proapoptotic factors, such as TNF-α, which has been linked to thymocyte apoptosis, in turn reducing the expression of antiviral cytokines. This suggests that C5a-mediated immune paralysis plays a critical role in mediating pathogenic damage in severe patients infected with highly pathogenic virus like H7N9.

## THE EFFECT OF BLOCKING C5a ON ACUTE LUNG INJURY INDUCED BY HIGHLY PATHOGENIC VIRAL INFECTIONS

To evaluate the effect of C5a blockade, OmCI, a potent arthropod-derived inhibitor of C5 activation that binds to C5 and prevents release of C5a by complement activation, was used to treat mice infected with H1N1 pandemic virus. OmCI significantly inhibited neutrophil and macrophage infiltration in the airways, NETs formation, death of leukocytes, lung epithelial injury and overall lung damage.[4] The study suggests that targeting C5a could be a promising approach to reduce excessive inflammatory reactions associated with the severe forms of IAV infections.

C5aR was found to be expressed on upper (bronchial) and lower (alveolar) airway epithelial cells. An adenovirus construct (siRNA) was used to silence mRNA for C5aR in the lung and resulted in buildup of polymorphonuclear leukocytes, and lower levels of proinflammatory mediators in bronchoalveolar lavage fluid.[97] Antagonism of C5a receptors also significantly inhibited the development of ARDS induced by intravenous infusion of cobra venom factor, including neutrophil migration and bronchoalveolar vascular leakage, blood pressure alterations, pro-inflammatory cytokines including TNF-α levels in bronchoalveolar lavage fluid.[98] The study indicates that C5a signaling greatly contributes to inflammation and injury in the lung and was targeted to treat highly pathogenic virus infection. In addition, interception of C5a signaling has recently shown promising beneficial effects in small animal models of ALI/ARDS by reducing pro-inflammatory cytokines.[99]

Polyclonal anti-C5a antibody led to significantly reduced inflammation in lungs, alleviating ALI in H5N1-infected mice.[5] The study indicates that inhibition of C5a might be an effective clinical intervention for H5N1-induced ALI. However, studies in knockout mice demonstrated that C3 was required for protection from influenza infection, proper viral clearance, and associated with changes in cellular infiltration.[35] The data are in accordance with the fact that complement C5a is the leading mediator of the over-inflammatory response which induced ALI, whereas the lytic membrane attack complex (C5b-9) provide a protective role in controlling viral infection. Thus, we developed a neutralized humanized anti-human C5a antibody which only blocked C5a effects but did not affect the formation of C5b-9 membrane attack complex.

*In vitro* experiments demonstrated that a novel, neutralizing, humanized anti-human C5a antibody blocked the ability of C5a to induce granulocytes to express CD11b while not affecting the ability of C5b to form the membrane attack complex. African green monkeys were inoculated with H7N9 virus and then treated intravenously with anti-human C5a antibody. Anti-C5a treatment in H7N9-infected monkeys substantially attenuated ALI by reducing the lung infiltration of macrophages and neutrophils, and the levels of inflammatory mediators.[6] The data suggest that humanized anti-human C5a antibody might provide a potential therapeutic reagent for H7N9-infected patients.[100]

The role of C5a in the different viral infections and the effect of C5a blockade on acute lung injury were described in Table 1. Neutralizing, humanized anti-human C5a antibodies are being tested on H5N1-induced ALI in African green monkeys. It is reasonable to speculate



Emerging Microbes and Infections EMI201528.3d 27/4/15 12:40:24

**Table 1 The role of C5a in the different viral infections**

| Test | Result | Reference |
|---|---|---|
| 2009 H1N1 influenza patients | The C5a concentrations in 2009 H1N1 influenza patients both with and without severe complications were significantly higher than those in healthy individuals | 34 |
| H1N1-infected murine model | Blockade of C5a inhibited neutrophil and macrophage infiltration in the airways, NETs formation, death of leukocytes, lung epithelial injury and overall lung damage | 4 |
| HPAI H5N1 virus infected murine model | Anti-C5a Ab treatment also reduced lung injury and neutrophil infiltration especially on Day 5 after H5N1 virus infection. Also, anti-C5a Ab treatment increased survival rate, with 50% mortality in the C5a Ab group compared with 100% mortality in the control group on day 9 after H5N1 virus challenge. | 5 |
| HPAI H5N1 virus infected murine model | H5N1 influenza virus infected mice had increased levels of C5a activation byproducts as compared to mice infected with either seasonal or pandemic 2009 H1N1 influenza viruses. | 5, 35 |
| H7N9-infected monkey model | Anti-C5a treatment in H7N9-infected monkeys substantially attenuated ALI: it markedly reduced the lung histopathological injury and decreased the lung infiltration of macrophages and neutrophils. Moreover, the treatment decreased the intensity of SIRS; the body temperature changes were minimal and the plasma levels of inflammatory mediators were markedly reduced. The treatments also significantly decreased the virus titers in the infected lungs. | 6 |

Ab, antibody; HPAI, highly pathogenic avian influenza; SIRS, systemic inflammatory response syndrome

that the neutralized humanized anti-human C5a antibody would be a potential therapeutic option for H5N1-infected patients.[100]

## CONCLUDING REMARKS

The complement system, a part of innate immunity, plays a critical role in host defense against pathogens. Unregulated complement activation is likely to play a crucial role in the pathogenesis of lung diseases. The complement-activated product C5a displays powerful biological activities in the activation of phagocytic cells, generation of oxidants, release of histones and cytokine storm, and so on.[10] In particular, cytokine storm is believed to be responsible for many of the deaths during the 1918 influenza pandemic,[101] during the SARS epidemic in 2003,[7] MERS-Cov in 2014,[8] and the human deaths from H1N1,[4] H5N1[102] and H7N9.[6] There is growing awareness that there are key similarities in the contribution to the cytokine storm and the manifestation of lung pathology among the chronic respiratory diseases,[103] and the cause of death such as bleeding from Ebola virus.[104] C5a, as a key trigger to induce cytokine storm, could be an ideal target for many lung inflammatory diseases, and it would be important to assess the therapeutic potentials of C5a blockade in human clinical trials. We have evidence that humanized anti-C5a antibody greatly reduced lung histopathologic injury, as well as decreased lung infiltration of macrophages and neutrophils and the levels of pro-inflammatory cytokines including TNF-$\alpha$ in a monkey model of ALI induced by H7N9[6] and herbicide, paraquat (Shihui Sun *et al*, unpublished data). Thus, it is reasonable to speculate that blockade of C5a with a humanized anti-human C5a antibody would be a potential therapeutic target for highly pathogenic viral infection-induced acute lung injury.

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Emerging Microbes and Infections EMI201528.3d 27/4/15 12:40:25

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