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Brief Communication

Activation of classical and alternative complement pathways in the pathogenesis of lung injury in COVID-19

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ABSTRACT

Lung inflammation and damage is prominent in people infected with SARS-Cov-2 and a major determinant of morbidity and mortality. We report the deposition of complement components in the lungs of people who succumbed to COVID-19 consistent with the activation of the classical and the alternative pathways. Our study provides strong rationale for the expansion of trials involving the use of complement inhibitors to treat patients with COVID-19.

1. Introduction

Acute respiratory syndrome following infection with coronavirus 2 (SARS-CoV-2) accounts for the catastrophic morbidity and mortality of the current corona virus-2-associated disease known as COVID-19 [1,2]. Numerous immune cell abnormalities have been reported along with the production of inflammatory cytokines including interleukin-6 (IL-6) and have been assigned pathogenic roles [3,4]. In parallel, a puzzling hypercoagulation has been reported in a significant portion of infected individuals with catastrophic sequelae [5]. Yet, the exact mechanisms of excessive inflammation and hypercoagulation in COVID-19 patients remain poorly understood. The endothelial damage, thrombophilia, thrombotic microangiopathy, and the aberrant inflammatory environment which accompany severe COVID-19 infection suggest excessive complement activation which can account for end-organ damage but the mechanisms of activation have not been addressed [6–8]. Entities with excessive complement activation such as atypical hemolytic uremic syndrome and antiphospholipid antibody syndrome, exhibit many similar histopathologic and clinical features with severe COVID-19 [9]. Although multiple studies have reported increased complement activation in patients with COVID-19 [7,8,10–12], the involved pathway(s) of activation have not been addressed.

In this brief report we explored the deposition of major components of the complement system in lung tissues of people who succumbed to COVID-19 in order to understand which pathway was primarily activated. We report that components of the classical (C1q, C4d) and alternative (Factor H, C3d) pathways along with C5b-9 and IgG and IgM were deposited in the lungs of people with COVID-19 indicating the activation of both pathways.

2. Materials and methods

Five consecutive patients, two male and three female with an age ranging from 58 to 91 years who expired between April 22 and May 6, 2020 at the Beth Israel Deaconess Medical Center (BIDMC), Boston from SARS-CoV-2 infection were included in this study. Minimally invasive ultrasound-guided autopsy was performed to collect lung tissue within 3 h of death to maintain tissue viability. SARS-CoV-2 nucleocapsid viral load varied from 10 to 21,407 copies/mg total RNA [13]. Histopathologic changes included patchy pulmonary edema, acute alveolar hemorrhage, interstitial lymphocytic infiltrates, abundant alveolar macrophages and type-II pneumocyte hyperplasia. Detailed description of pathology and molecular findings have been presented in a previous study [13]. Lung tissues were also collected from autopsy material of two subjects, one male and one female, 65 and 59 years of age who had expired in the pre-COVID-19 era (one who had suffered a middle cerebral artery syndrome stroke and another who had a diagnosis of Type I diabetes mellitus) to serve as a control. Family members were consented

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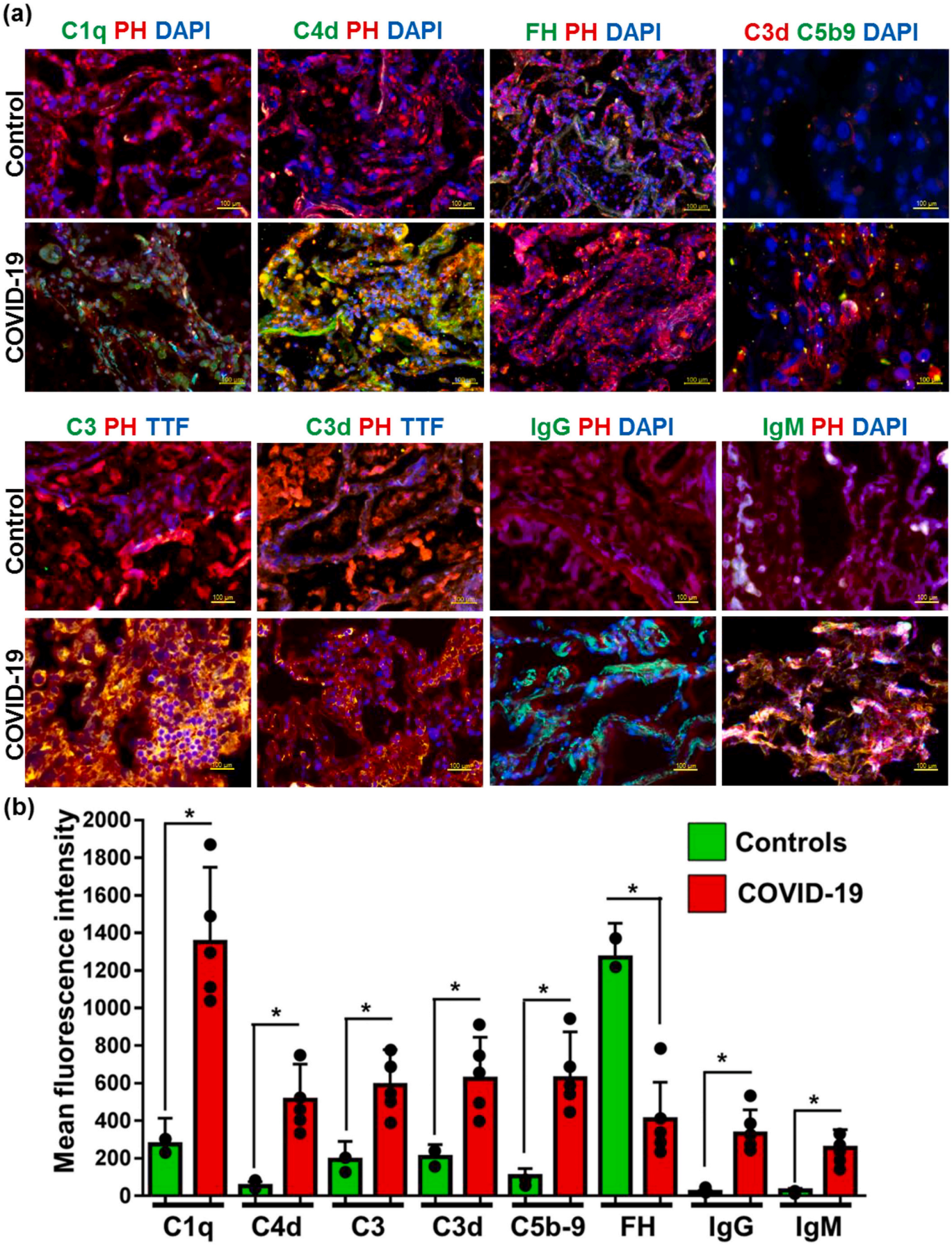

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Fig. 1. Deposition of complement components in the lungs of people with COVID-19 (a) Immunohistochemical fluorescence images obtained from formalin-fixed paraffin sections of lung tissues stained with complement molecules (C1q, C4d, FH, C3, C3d and C5b-9), immunoglobulins (IgG and IgM) and counter stained for cytoskeleton (PH - phalloidin), alveolar epithelial cells [thyroid transcription factor (TTF)-1] and nucleus (DAPI). (b) Mean fluorescence intensity analysis of fluorescence images confirmed the higher deposition of C1q, C4d, FH, C3 C5b-9, IgG and IgM and reduced deposition of FH in compare with controls (*Statistical significance was accepted at $p < 0.05$).

for limited autopsies by a pathologist during a witnessed phone call immediately after the death and after referral from the intensive care unit team. Research using autopsy tissue for this project was approved by institutional review board (IRB) of BIDMC. A Health Insurance Portability and Accountability Act (HIPAA) waiver was granted by the IRB for access to the patients' charts for each project using the tissue. Tissue was provided to research teams per previously IRB-approved research protocols.

Immunohistochemical staining and fluorescence intensity measurements were performed to evaluate the deposition of complement molecules and immunoglobulins. Immunohistochemical images obtained from formalin-fixed paraffin sections of lung tissues stained with complement molecules [C1q, C4d, FH, C3, C3d (all from Quidel) and C5b-9 (Abcam)], immunoglobulins [IgG and IgM (Santa Cruz Biotechnology)] and counter stained for cytoskeleton [phalloidin (Thermo Fisher), alveolar epithelial cells [thyroid transcription factor (TTF)-1 [14] (Santa Cruz Biotechnology)] and nucleus [DAPI (Thermo Fisher)] using standard protocols. Images were captured and quantitative fluorescence intensity measurements were performed with a Zeiss Axio Observer.A1 inverted fluorescence microscope (ZEISS, Germany).

Numerical data is expressed as mean ± SD. Analysis was performed using statistical software (Graphpad Prism, USA). Comparisons among groups were performed by Mann– Whitney *U* test or one-way ANOVA, followed by Bonferroni's multiple comparison test. Statistical significance was accepted at $p < 0.05$. Experiments were performed in triplicate or quadruplicate.

3. Results and discussion

In Fig. 1 we demonstrate that SARS-CoV-2 infection induced the deposition of complement molecules from classical pathway (C1q, C4d,) and the split product C3d reflecting activation of C3 which is shared by all activation pathways. We also found increased deposition of the membrane attack complexes (C5b-9). A reduced deposition of complement factor H (FH), a key inhibitor of the activation of the alternative pathway [15] was observed. Moreover, lung tissues from people with COVID-19 displayed significantly increased deposition of IgG and IgM (Fig. 1A). Quantitative image analysis further confirmed a significantly higher deposition ($P < 0.05$) of the complement molecules (C1q, C4d, C3, C3d and C5b-9) and immunoglobulins and decreased deposition ($P < 0.05$) of FH (Fig. 1B). Parallel C3, C3d and staining for the alveolar epithelial cell marker TTF-1 and phalloidin demonstrated that higher deposition of complement molecules occurred not only in alveolar epithelial cells but in all resident lung tissue cells. We did not appreciate any differences in the deposition levels of all tested molecules between specimens from men and women.

Complement activation which has been previously associated with ischemia, trauma [16–19], bacterial and viral pneumonia, acute respiratory distress syndrome and infections with corona viruses linked to severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS) [20,21] leads invariably to lung inflammation and respiratory failure [22,23]. The complement system, which plays a critical early role in pathogen clearance by the innate immune system, is also involved in major complications of COVID-19, including coagulopathy and multiorgan failure [8,24].

The deposition of C1q and C4d clearly implies the activation of the classical pathway of the complement and this is further supported by the observed deposition of IgG and IgM. It is unknown whether the deposited immunoglobulin represents antibody generated against SARS-CoV-2. Alternatively, it may represent naturally occurring antibodies present in all healthy individuals which have been shown to bind to ischemia or otherwise injured tissues and activate complement [25].

The deposition of C3d implied activation of C3, a common component of all complement activation pathways indicating the involvement of the alternative pathway. Our finding that the levels of the factor H, a well-established inhibitor of the alternative pathway, are decreased support the involvement of this pathway. It is unclear why the levels of factor H are decreased and it would be interesting to consider that people who do not fare well in response to infection with SARS-Cov-2 may have factor H variants that are not fully functional. In addition, there is *in vitro* evidence that the highly pathogenic coronavirus N protein aggravates lung injury by MASP-2-mediated complement overactivation of the lectin pathway [26] and therefore, the lectin pathway is also involved. C5b-9, which is apparently responsible for cell destruction was found here amply deposited in the lung tissues of people with COVID-19. Previously, it was reported that plasma levels of sC5b-9 were high in the moderate and severe COVID-19 patients who required mechanical ventilation [27].

Our findings further support the use of complement inhibitors to limit lung and probably other pathology in COVID-19 people. Indeed, inhibition of C3 activation with compstatin, a small peptide C3 inhibitor, in patients with COVID-19 has been used successfully to treat COVID-19 patients in whom a faster lymphocyte recovery, decrease in neutrophil numbers and significant reduction of neutrophil extracellular traps -driven thrombo-inflammation were noted [10–12]. Along the same line, eculizumab, a C5a neutralizing antibody, has shown advantageous effect in patients with COVID-19 infection [28]. Existing *in vitro* platforms [29] can also be utilized to study the biology of COVID-19 related cellular injury and drug toxicity.

In conclusion, we report the deposition of components of the complement in lung tissues of people who succumbed to COVID-19. The pattern of deposition bespeaks to the activation of both the classical and the alternative pathways and provides additional rationale for the expansion of clinical trials in which complement activation inhibitors are used.

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