

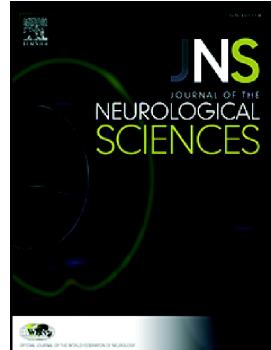


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Part I. SARS-CoV-2 Triggered ‘PANIC’* Attack in Severe COVID-19

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Abstract

The COVID-19 pandemic has produced a world-wide collapse of social and economic infrastructure, as well as constraining our freedom of movement. This respiratory tract infection is unprecedented in that it targets the most distal and highly vulnerable aspect of the human bronchopulmonary tree, specifically, the alveoli that are responsible for the loading of oxygen upon red cell hemoglobin for use by all of the body's tissues.

In most symptomatic individuals, the reaction is a productive and mild immune-mediated syndrome, with limited damage to the lung tissues. About 20% of those affected experience a virus capable of producing what can only be described as a cataclysmic set of immune activation responses that can culminate in the diffuse and irreversible obliteration of the distal alveoli, that then leads to a virtual collapse of the gas-exchange apparatus.

Here, in Part I of a duology on the characterization and potential treatment for COVID-19, we define severe COVID-19 as a consequence of the SARS-CoV-2 virus' ability to trigger what we now designate for the first time, as a 'Prolific Activation of a Network-Immune-Inflammatory Crisis', or 'PANIC Attack, in the alveolar tree. In Part II we describe an immunotherapeutic hypothesis worthy of the organization of a randomized clinical trial in order to ascertain whether a repurposed, generic, inexpensive, and widely available agent is capable of abolishing 'PANIC'; thereby preventing or mitigating severe COVID-19, with monumental ramifications for world health, and the global pandemic that continues to threaten it.

Never before has civilization been confronted with a more formidable enemy, one that is wholly invisible, often resulting in no identifiable symptoms, and yet, it is among the most transmissible of microbial agents known; the cause of the most rapidly disseminated viral pandemic in recorded history. The monumental penetrance of this microbe has culminated in the virtual collapse of nearly every endeavor which entails the close proximity of one human being to another. The only exception is the delivery of medical care to those requiring the most intensive management, and whereby such interventions cannot be achieved by our newly established and widely adopted diagnostic, interventional, and continuity of care method for non-emergent, non-surgical patient-care, TELEMEDICINE. The pace by which such methods were put into place, and have been rapidly refined with respect to ease of access, adherence to patient protections and privacy, along with consensual adoption on both sides of the provider-patient therapeutic alliance, has been nothing less than breathtaking.

The majority (~80%) of those infected by the severe acute respiratory syndrome (SARS) virus, the third member of the Coronavirus family, and second variant of SARS (SARS-CoV-2), will experience a mild to moderate flu-like respiratory tract illness, Coronavirus Disease 2019 (COVID-19), although it is conspicuously differentiated from just about every other pathogen targeting the human respiratory tree. Without equivocation, the SARS-CoV-2 virus is endowed with two fundamental characteristics, which when combined together, can foment incontrovertibly one of the most ominous semiologic courses of illness recognized in modern medicine.

Firstly, the SARS-CoV-2 virus targets its binding site, through which it mediates its viral tropism. Specifically, the SARS-CoV-2 surface glycoprotein, ‘spike’, binds to the angiotensin converting enzyme (ACE) 2 receptor, which is broadly distributed throughout the body. This

Coronavirus binds with particular predilection for the extreme terminus of our bronchopulmonary anatomy, especially the alveolar gas-exchange apparatus, which is responsible for the continuous loading and subsequent delivery of oxygen to all of the tissues of the body.

Secondly, while the majority of COVID-19 patients will mount an appropriate, coordinated, and highly regulated host-immune-response to the etiologic agent, approximately 20% of infected individuals shall instead be subjected to the consequences of the SARS-CoV-2 virus triggering of a ‘prolific activation of a network-immune-inflammatory crisis’, or ‘PANIC’ Attack. The latter host response involves the widely and indiscriminantly activated limbs of the entire immune response network, which results in a confluence of convergent immune effector elements [Table 1]. Targeted sites include the most delicate and vulnerable of our life-sustaining circuitries, resulting in the catastrophic obliteration of lung alveoli by exceeding a damage threshold for these indivisible and non-regenerating gas-exchange organelles.

Overwhelming the limiting threshold for damage to our 600 million alveoli has been associated with the abrupt collapse of circulatory oxygen saturation and delivery, which becomes refractory to any intervention, thereby presaging multiorgan hypoxic-ischemia and death. The designation of severe COVID-19 can be characterized by a number of risk factors and clinical manifestations that implicate the nervous system as either a target tissue of the disorder’s pathophysiologic underpinnings, or as playing a fundamental role in the compromise in the fidelity of centrally integrated regulatory mechanisms which both ‘sense’ and respond to alterations in respiratory metrics. To illustrate one such pathway, trace the partial pressures of oxygen and carbon dioxide as codified by the sensing apparatus in the carotid bodies, and transmitted via the nerves of Hering (small branch of the glossopharyngeal nerve), to the

solitary tract and nucleus localized to the caudal medullary tegementum. The integrity of the complex connectivity of this circuitry must be maintained in order to achieve its goal of regulating all functions of the respiratory system to maintain oxygen saturation and delivery to ensure continuous organ tissue viability.

A principal goal herein is to confirm both face and construct validity for the principle of this new definition for a poorly coordinated and dysregulated sequence of coincidentally activated limbs of the immune network (i.e. PANIC), and the consequences of such upon the primary target tissue for an infection as virulent and strategy ominous as the SARS-CoV-2 agent. Further, given the prolific immune activation associated with microbial-induced ‘PANIC’, in Part II of this publication, we advance the hypothesis that effective therapy should thereby provide a pleiotropic strategy commensurate with the range of the immune system’s diversity of activation by the SARS-CoV-2 virus.

The SARS-CoV-2 agent triggers PANIC which the authors find reminiscent of phenomenology which we have previously identified (and published in *the Journal of the Neurological Sciences*) as ‘monumentally severe central nervous system (CNS) inflammatory syndromes’ that were associated with multiple sclerosis (MS), neuromyelitis optica (NMO), and Sjogren’s syndrome myelitis; all of which were refractory to conventional, even intensive, immunotherapy. The successful rescue intervention reported utilized the application of high-dose methotrexate with leucovorin rescue (HDMTX-LR), an intensive and highly pleiotropic anti-inflammatory strategy [1]. Since then, we have treated a broadening diversity of other causes that we believe to be variants of ‘PANIC’, including other post-infectious encephalomyelitides (e.g. post-adenovirus) [Figure 1] and post-vaccinal (e.g. post-dTap) encephalomyelitides [Figure 2]. Though classically defined as nuances of acute disseminated

encephalomyelitis (ADEM), they were, however, recalcitrant to conventional immunotherapy, but were stereotypically abolished utilizing our HDMTX-LR treatment strategy.

COVID-19: It Began in China

In December 2019, in Wuhan, Hubei Province; China reported an outbreak of a highly communicable viral infection. Initially, it was reported to be a form of treatment resistant pneumonia. Subsequently, it was recognized that the etiologic agent was, in fact, a novel Coronavirus. Humans had never before been exposed to this infectious agent, and carried no immunity to prevent its spectrum of clinical manifestations, ranging from mild, even asymptomatic courses, to severe, even fatal disease, with a particular targeting of the respiratory tract [2]. SARS-CoV-2, the etiological agent of COVID-19, has now infected patients across 210 countries and territories, and has fomented a catastrophic global crisis, associated with medical, economic, and psychosocial ramifications of immense magnitude. The World Health Organization declared the outbreak of SARS-CoV-2 as a Public Health Emergency of International Concern on January 30, 2020 [3-8].

SARS-CoV-2 transmission is easily disseminated via aerosolization and droplets, with recent evidence suggesting that even non-amplified speech can effectively project oropharyngeal derived material containing virus to become airborne and capable of traveling distances typical for standard conversation [9]. Unlike the antecedent epidemics of SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), the SARS-CoV-2 (COVID-19) originated in Wuhan, Hubei Province, China and subsequently and rapidly disseminated globally confirming its designation as a *pandemic*. This ultimately led the world to ‘shelter in place’, because without an effective therapy, the best chance to quell the perpetual dissemination of infectivity, is to avoid transmissible contacts. Given the striking

transmissibility of SARS-CoV-2, in conjunction with the large proportion of both mildly affected and asymptomatic individuals harboring infection, both ‘shelter in place’, together with widespread availability of testing to confirm infection, as well as active immunity, represents our best strategy to prevent further dissemination and also to identify newly infected cases as early as possible.

COVID-19-Associated Clinical Manifestations

According to currently available data, at the time of submission of this article, approximately 80% of individuals who become infected with SARS-CoV-2 will experience a relatively mild, short duration syndrome; with many being wholly asymptomatic. The other 20% of SARS-CoV-2 infected patients will progress to exhibit a severe variant of this infection characterized by fever and a respiratory tract ‘flu like’ illness. They may suffer from fever, cough, pharyngitis, headache, dyspnea, myalgias, chest pain, nausea, vomiting, confusion, and interestingly, anosmia and dysgeusia in a small albeit conspicuous proportion of patients (~5%) [9-11]. This severe disease transitions to an accelerated course of predominant respiratory deterioration, requiring intensive multidisciplinary management, including respiratory support with pressure ventilation. Additionally, there is a high predilection for a diverse constellation multi-organ derangements, with the most severely affected individuals exhibiting multi-organ failure [2].

In the initial Wuhan cohort, characteristic chest CT findings were identified in 98% of 41 patients, with involvement of both lungs, and features consistent with the ‘so-called’ ground glass opacifications. Of those patients that deteriorated, most required intensive care unit (ICU) management, while paradoxically exhibiting a resolution of lung consolidations in comparison to those not requiring admission to the ICU [2]. The median duration (in days)

between the onset of symptoms and the passing of clinically relevant and prognostic milestones of the severely affected was also documented in the Wuhan patients. Specifically, admission to hospital (7 days), onset of dyspnea (8 days), diagnosis of ARDS (9 days), admission to ICU (10.5 days), and mechanical ventilation (10.5 days). The timing from hospital admission to diagnosis of ARDS was also reported to be as short as two days, at which point such patients had a mortality rate of 15% [2].

Risk factors associated with the designation of severe COVID-19 disease included male gender, advanced age, and pre-existing co-morbid conditions, especially hypertension, diabetes, morbid obesity, and smoking [2]. The clinical pathology for the most severely affected patients was most commonly comprised of interstitial pneumonia with rapid transition to ARDS, septic shock, and evidence of the liberation of acute phase reactants in conjunction with macrophage activation. Additionally, serum elevation of C-reactive protein (CRP) and D-dimer, were correlated with liver dysfunction and hyperferritinemia, laboratory findings which can be accompanied by the syndrome of disseminated intravascular coagulation (DIC) [2].

Neurological Manifestations of SARS-CoV-2 Induced COVID-19

In the severely ill COVID-19 patients, front-line healthcare workers have been largely preoccupied with providing adequate life-support, prioritizing the integrity of the circulatory system, and keeping blood oxygen saturation above the threshold where end organ damage ensues. In those with a rapid transition from minor symptoms to urgent admission to an ICU, the majority will require ventilator support, along with continuous observation and repeated assessments of each of the major body systems. Given the potential for any given COVID-19 patient to abruptly deteriorate, clinical management mandates a systematic sequence of intensive care measures and protocols principally aimed at avoiding a period of hypoxic

ischemia. It is for this reason, at least in part, that our understanding of the neurologic manifestations of COVID-19 lags behind our understanding of the more omnipresent and existential concerns that are associated with patients so severely affected by this infection.

The ICU priorities can hinder the ability of any subspecialist to carry out their assessments, as well as limit recommendations for those investigations that require the patient to leave the ICU setting (e.g. as with the performance of imaging studies including CT and MRI). Each departure for additional testing must be cautiously weighed against the risks and benefits of such investigations. Despite these limitations, we have now gained some insights into complications of the SARS-CoV-2 infection that target the CNS and peripheral nervous system (PNS) [12-19].

COVID-19 neurological manifestations are thought to result from indirect and direct actions of SARS-CoV-2 on the CNS and PNS. Indirect actions of the virus are related to injury to peripheral organs, such as the lungs, kidneys, liver, and heart. For example, myocardial and lung invasion by SARS-CoV-2 could result in cardiac failure and arrhythmias, which could in turn increase the secondary risk of stroke [20-23]. Alternatively, SARS-CoV-2 could directly damage the brain and spinal cord either via hematogenous spread or via neural propagation into the CNS [24].

Other coronaviruses, such as SARS-CoV1 and HCoV-OC43 have been shown to enter into the nasal passages and spread to the olfactory bulb, pyriform cortex, dorsal nucleus of the raphe in the brainstem, and spinal cord [25, 26]. Of note, ablation within the olfactory pathway can prevent the neural spread of MHV coronavirus in animals [27]. Cellular entry is thought to occur through the angiotensin-converting enzyme 2 receptor (ACE2R). Interestingly, ACE2R is

expressed widely in the CNS within neurons, motor cortex, hypothalamus, thalamus, and brainstem [28-33].

The physical distribution of ACE2R in the brain could account for some of the clinical symptoms observed in patients. For example, direct neuronal injury within the brainstem cardiorespiratory centers in the medulla, could explain the peak disease cardiovascular and respiratory complications in COVID-19 patients [25]. In addition, anosmia and ageusia in COVID-19 patients may be related to viral entry through ACE2R within the olfactory system or within the hypothalamus.

A recently published retrospective observational case series focused upon the neurologic manifestations of COVID-19 from Wuhan, China, and included 214 consecutive hospitalized patients with laboratory-confirmed diagnosis [10]. This analysis showed that approximately 60% of patients were designated as having mild disease, with the remainder characterized as having severe infection, defined with respect to their respiratory status. Approximately 36% of the cohort exhibited neurological manifestations, with those designated as having more severe disease being more likely to significantly harbor comorbid conditions with hypertension being most common, as well as being significantly less likely to present with the more typical respiratory symptoms (at least at presentation) of COVID-19, such as cough in the context of fever [10].

Patients most likely to present with neurologic symptoms tended to be significantly older, and also afflicted with severe infection, with the majority of neurologic features occurring in the CNS (e.g. stroke, potentially influenced by features of a thrombotic microangiopathy, with or without cardiac disease, impaired consciousness, headache, dizziness, anosmia, dysgeusia, etc.), versus the PNS. These same individuals were also significantly more likely to

present with elevated D-dimer levels (reminiscent of a consumptive coagulopathy, such as DIC), along with multiorgan derangements, like hepatic transaminitis, renal insufficiency signified by rising blood urea nitrogen (BUN) and creatinine levels, and serum elevation of muscle creatinine kinase levels. It has been established that inflammatory disease tips the coagulation cascade to a ‘pro-coagulant state’. This has been observed in neuroinflammatory disorders, including MS and its animal counterpart, experimental autoimmune encephalomyelitis (EAE) [34].

Some of the patients presented with neurologic features in the absence of typical COVID-19 symptoms, and tested negative by chest CT imaging and by COVID-19 blood testing. Days later, these individuals manifested the features of cough and sore throat, in conjunction with lymphopenia and the evolution of characteristic ‘ground-glass’ opacification lesions demonstrated on repeat chest CT. Indeed, their SARS-CoV-2 infections were later corroborated by nucleic acid testing [10]. Alternatively, those patients presenting with PNS features did not significantly correlate with any laboratory assessments. Dichotomizing patients categorically into severe versus non-severe COVID-19 did not significantly correspond to the presence or absence of PNS involvement.

Virology of the Severe Variant of SARS-CoV-2 Infection

SARS-CoV-2 represents the third and most-widespread Coronavirus zoonosis, preceded by SARS-CoV and MERS-CoV. All three of these zoonoses are believed to have originated in bats and transitioned to humans via intermediate hosts [35]. The Coronavirus’ namesake is derived from its structural ‘crown-like’ appearance. The virus is composed of a positive sense single stranded RNA physically associated with a nucleocapsid, within a phospholipid bilayer, with the envelope decorated with externally projecting spike glycoproteins

[36]. The viral spike interacts with its receptor, the angiotensin converting enzyme receptor-2 (ACE-2r), on susceptible target cells to facilitate entry and viral replication (i.e. viral tropism) [37].

With the identification of the ACE-2r receptor as the binding site for SARS-CoV-2 spike protein and its corresponding tropism, there was a groundswell of concern that ACE inhibitors and/or angiotensin receptor blockers would confer an increased risk upon infected patients. However, patients with COVID-19, who were treated with ACE inhibitors or angiotensin receptor blockers, continue to derive both cardiovascular and renal protection, and in fact, the discontinuation of these agents may in fact be harmful [38].

'Irrational Exuberance' of Immune-Mediated Inflammatory Networks

Evidence is rapidly mounting to suggest that the severe lung damage in COVID19 is the result of both the activation of diverse limbs of host immune networks, in conjunction with exaggerated activities of each of these responses to the SARS-CoV-2 virus [Figure 3]. To more accurately account for viral induction of the coincident confluence of converging inflammatory cascades, and to differentiate the characteristics of the immune-mediated responses in those designated to have mild versus severe COVID-19 disease, we have, for the first time to our knowledge, coined an acronym that reflects the severe magnitude and cataclysmic evolution of the severe variant of COVID-19: In essence; the PANIC Attack.

The Ominous Target for the SARS-CoV-2 'Triggered' PANIC Attack

The terminus of the broncho-pulmonary tree is the most important target of SARS-CoV-2, and is comprised of a duct system consisting of about 100 alveolar sacs, each composed of 20-30 alveoli. All totaled, there are some 600 million alveoli in the lungs [39]. Each individual

alveolar membrane is 1-cell-thick and necessitates an intimate and tight juxtaposition of the alveolar epithelium with that of the pulmonary capillary endothelium, also with the thickness of a single cell. The single-cell arrangement, and ultra-tight juxtaposition of the capillary anatomy with that of the alveolar epithelium, constitutes an ideal architecture for the principal goal of pulmonary physiology, the loading of oxygen onto red cell hemoglobin and the expulsion of CO₂ from the lungs into the ambient air [Figure 3].

The convergent inflammatory cascades focused upon this highly delicate and vulnerable bronchopulmonary gas-exchange scaffolding is what makes this particular member of the Coronavirus family so ominously dangerous [Table 1]. A further challenge relates to the *limited window of opportunity* to therapeutically intervene before the obliteration of a corpus of alveoli, from which survival is no longer feasible. Many who have succumbed to COVID-19, have done so while being carefully managed on pressure ventilation, with adequate respiratory rate (whether patient or ventilator triggered), and continuous delivery of oxygen, commensurate with optimization and persistency of O₂ saturation kinetics. The addition of positive end expiratory pressure (PEEP) ensures that the bronchopulmonary terminus remains patent to facilitate effective gas diffusion. Another dividend of pressure ventilation is that it contributes to the process of effective pulmonary toilet, now referred to as Pulmonary Hygiene, and involves the use of medications, devices, and/or maneuvers, which are principally aimed at optimizing mucus clearance mechanisms [40].

Without effective mucus clearance, there is a greater chance of organization of inspissated mucus combined with protein-rich fluid, produced secondary to localized inflammatory activities, material from the immune-mediated debris-field (including that from the exfoliation of destroyed alveoli epithelial cells), and trapped microbial material, which normally

enters the lung with ambient airflow. Despite the capabilities of state of the art pressure ventilation devices, many COVID-19 patients who fail to wean off ventilator support exhibit a precipitous deterioration, heralded by the abrupt onset of oxygen desaturation, despite full mechanical support and control over the delivery of high oxygen concentration in conjunction with maximized PEEP.

While the obliteration of a threshold level of alveoli may be responsible for the observation of catastrophic collapse of the gas-exchange apparatus, it has been suggested that the SARS-CoV-2 virus may disseminate into the caudal medullary tegmentum and inflict damage upon the CNS cardiorespiratory centers, such as the solitary tract and nucleus. This Center is endowed with the ability to sense and respond to alterations in the partial pressures of CO₂ and O₂, via information transmitted by the carotid bodies through the nerves of Hering, branches of the glossopharyngeal cranial nerve IX [41-43].

These observations suggest that there needs to be urgent intervention capable of uncoupling the unchecked inflammatory PANIC targeting the gas-exchange anatomy of the lung. Once a critical disease burden has destroyed a sufficient number of alveoli, further management is futile. Such patients can no longer saturate hemoglobin with the minimal threshold of oxygen in order to sustain the viability of the body's primary organ systems. For instance, protracted periods of hypoxic-ischemia will have nearly instantaneous adverse effects upon those systems requiring the highest supply to demand bioenergetic signatures, such as the brain, heart, kidneys and liver.

Other medical manifestations of the COVID-19 infection include a vasculitis with risk of progressing to gangrene [44-46]. Some patients have evidence of spleen destruction and diffuse atrophy of lymph nodes, and their associated regional chains [45]. Others can harbor

evidence of anti-phospholipid, anti-cardiolipin, and anti- β_2 -glycoprotein antibodies, each of which might signify the presence of a corresponding syndrome, with ramifications that only serve to intensify the demands upon patient management, and those responsible to deliver it [47].

The Pathophysiology of the SARS-CoV-2 Triggered PANIC-Attack

Role of Complement in the SARS-CoV-2-Triggered PANIC-Attack

The Coronavirus can enter cells within the lungs via the endosomal pathway, or by fusion mechanisms that also allow for the development of syncytia [Figure 3]. Destruction of alveolar cells expressing ACE-2r by SARS-CoV-2 could theoretically be orchestrated by post-viral replication cell bursting, or by antigen-antibody complex triggering antibody-dependent cellular cytotoxicity (ADCC) via activation of the complement pathway [48,49]. Activation of the complement pathway would result in the C3a and C5a fragments acting as anaphylatoxins and chemotaxins, serving to recruit neutrophils, monocytes, macrophages, and eosinophils into the target tissue. Upon arrival, these cells would release their immune effector mediators, including free radicals and reactive oxygen species (such as superoxide). Alternatively, distal activation of the complement pathways involving C5 convertase leads to the assembly of the C5b-C8 coordinated membrane attack complex (MAC) and the subsequent traversal of C9 into the MAC channel and across the cell membrane (i.e. the alveolar epithelium), leading to osmotic derangements culminating in cell death [Figure 3]. In order to ascertain the role played by the complement system in the pathobiology of COVID-19, a recently reported case series characterized the recovery of four ICU patients with severe COVID-19 associated pneumonia or ARDS, in response to eculizumab, an antibody against C5 convertase, which ultimately prevents the assembly of MAC, and thereby ADCC [50].

Eculizumab has been FDA approved for a number of conditions where pathogenic antibody is complement fixating, and upon engagement with its antigen, there occurs the initiation of the assembly sequence involving C5b-C8, that ultimately culminates in the formation of the MAC, through which C9 can then traverse and breach the integrity of the cell membrane sufficient to promote cell death. The conditions that have been shown to be effectively treated by eculizumab include hemolytic anemia syndrome (HUS), paroxysmal nocturnal hemoglobinuria (PNH), myasthenia gravis, and most recently AQP4⁺ neuromyelitis optica spectrum disorder (NMOSD) patients [51]. Administration of eculizumab early in the disease course may ultimately shed light on the role of complement-dependent injury pathways on the disease burden in the distal bronchonuillmonary circuit. The diverse spectrum of innate and adaptive immune activation, ignited by the SARS-CoV-2 agent, is compositionally part of our 'PANIC' Attack hypothesis.

We hypothesize that the early presence of both IgM and IgG directed to spike is germane to the activation of complement within the lung by the classical pathway, which is principally triggered by the recognition of antigen-antibody complexes. The latter of which must be of appropriate isotype in order to 'fix', and thereby activate, the classic complement cascade [Figure 3]. The alternate and lectin activated complement cascades are also likely ignited in the lung of severely affected COVID-19 patients. The former can be activated by either C3b or via contact with surface epitopes, such as those liberated by damaged cells, while the latter pathway is antibody-independent and becomes activated when mannose binding lectin (MBL) binds to glycosylated moieties upon the surface of pathogens.

Upon convergence with the serine esterase sequence activation of the complement cascade, all three paths ultimately lead to cleavage products, such as the anaphylatoxins and

chemotoxins, C3a, C4a, and C5a, which can increase vascular permeability in concert with promoting the redistribution of circulating neutrophils, eosinophils, monocytes, and macrophages into the site of the tissue localization where the complement cascades were activated [52,53]. Upon their arrival, these leukocytes can elaborate a number of highly potent and injurious immune effector elements (i.e. free radicals, superoxides, etc), which together further contribute to both the process of attempted neutralization of the immune challenge, which is SARS-CoV-2, as well as potentially fomenting a considerable amount of damage to the surrounding host tissue structure (i.e. bystander damage) [51-56]. The anaphylatoxins C3a and C5a further intensify and perpetuate organ tissue damage in COVID-19, where it is principally focalized to the peripheral bronchopulmonary anatomy via escalation of IL-1, IL-6, TNF α , as well as mast cell histamine degranulation [57-59].

Role of the Cytokine ‘Storm’ in SARS-CoV-2 Triggering of the PANIC-Attack

While IL-6 is elevated in about 35% of mild COVID-19 patients, 76% of severely affected patients exhibit elevation of this pro-inflammatory cytokine. In fact, COVID-19 patients characterized as having severe disease have corresponding escalations of IL-6, TNF- α , IL-2, MCP-1, MIP-1A, IL-10, IL-7, and G-CSF, especially in ICU patients [1]. Further, evidence of cytokine release syndrome (CRS) or ‘storm’ was confirmed by an escalation in IL-6 levels [60] in conjunction with inadequate levels of the negative regulatory suppressor of cytokine signaling 3 (SOC 3) [61].

Perhaps the laboratory finding of greatest conspicuity in those with confirmed COVID-19 is lymphopenia, posited to potentially represent the consequence of the broadening of SARS-CoV-2 distribution and apoptosis, the terminal phenomenon of viral infection [62,63]. An

alternative explanation is that the lymphopenia may be due to a cortisol burst from stress [10.1161/circ.98.15.1587/c]. The sequestration of lymphocytes into the lungs may also be a feasible hypothesis for the circulating lymphopenia (let's recall that only 3-5% of the body's mononuclear cells are in circulation at any given time). Despite the lymphopenia, widespread lymphocyte activation is a stereotypic observation in those with the disorder, particularly associated with the severe variant [64,65].

The pulmonary interstitium reveals a predominance of CD8⁺ T cells, a response considered to be crucial for SARS-CoV-2 clearance. However, the concomitant presence of elevated levels of IL-6, and IL-8, impair the ability of T cells to prime dendritic cells against the virus, and also limit macrophage clearance of the pathogen. Similar to MERS, also characterized by augmented circulating levels of IL-6, there is a reduced production and elaboration of anti-viral cytokines, such as the type I interferons (IFN alpha and beta) [66,67].

While the lung is the principal (and most crucial target of attention for those with severe COVID-19, the distribution of the ACE-2r is sufficiently wide that we are confronted with the controversy as to whether damage to the kidneys, GI tract, heart, skin, CNS, and PNS is a consequence of the reduced oxygen-carrying capacity of blood, secondary to the damage to the lung's gas exchange apparatus, or whether viral targeting of endothelium in other tissue beds can also foment the PANIC Attack, and the resultant injury mechanisms associated with such uncoordinated and poorly regulated immune activation.

The time is upon us to move urgently in order to identify therapeutic strategies which can bring to bear the necessary diversity of neutralization mechanisms commensurate with the wide spectrum of activated inflammatory mechanisms triggered by the SARS-CoV-2 virus, and which collectively represents the newly defined designation of PANIC Attack, extensively

characterized in this paper, and which we have proposed to be directly responsible for the pathobiological underpinnings of the severe variant of COVID-19.

In Part II of our 2-part series on the COVID-19 pandemic, and the corresponding global human crisis it has produced, we advance the innovative hypothesis that the repurposed application of high-dose methotrexate with leucovorin rescue, represents a particularly interesting candidate therapy, worthy of investigation within the context of a randomized, controlled clinical trial, in order to confirm or refute the contention that this WHO designated essential treatment can uncouple the SARS-CoV-2 triggered constellation of immune activities that compositionally represent the highly injurious PANIC Attack which leads to obliteration of the bronchopulmonary alveolar gas-exchange apparatus, predisposing the severe COVID-19 patient to disabling morbidity, and a significant risk of mortality.

Methotrexate with leucovorin rescue is an FDA approved and generic treatment regimen, that is inexpensive, widely available, and one with an extensive experiential track record of well-identified adverse events and toxicities, while also being associated with a corresponding and longstanding effective spectrum of risk mitigation strategies. Most importantly, high-dose intravenous methotrexate with leucovorin rescue (HDMTX-LR) represents a treatment strategy which is endowed with an impressively broad heterogeneity of anti-inflammatory properties spanning the human immune network, and which strikingly align with each of the currently identified components of the newly defined PANIC injury construct, fomented by the SARS-CoV-2 agent.

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Figure Legends

Figure 1: See Figure

Figure 2: See Figure

Figure 3: Here we present the principal target of the SARS-CoV-2 virus, the most distal extent of the bronchopulmonary tree; the alveoli and its complex architecture and tight juxtaposition with respect to the capillary terminals of the most distal pulmonary arteries and pulmonary veins. **A)** We present the component parts of the SARS-CoV-2 agent. Note that the virion has a nucleocapsid composed of a positive single strand RNA and phosphorylated nucleocapsid protein, which is then enclosed within a sphere delimited by membrane bilayers of phospholipid. Projecting out of the virion is the spike protein; the most important component part, given that the SARS-CoV-2 tropism is contingent upon the binding of spike to its counter-receptor, the angiotensin converting enzyme 2 receptor (ACE-2r). **B)** Demonstrates a pull-out in order to reveal the normal grape-like cluster arrangement of lung alveoli (on the left), in contrast to **C)** the irregular ‘deflated’ appearance of this terminal anatomic specialization that serves to uniquely package the sheer enormity of required surface area ($\sim 70m^2$) in order to provide a gas-exchange apparatus sufficient to meet the body’s demand for a continuous and uninterrupted supply of oxygen, while also serving as a portal for the expulsion of ‘bioenergetic exhaust’, in the form of carbon dioxide. **D)** represents the indivisible functional unit for gas-exchange, comprised of a single cell thick lining of which is comprised of a single cell thickness of epithelium. However, an added level of complexity relates to the differentiation of the cellular subtypes of the alveolar epithelial cells with the type I pneumocyte representing the principal cell responsible for gas exchange; the type II pneumocyte secretes surfactant, crucial for reducing alveolar surface tension which prevents alveolar collapse; and the type III pneumocyte or alveolar macrophage (aka ‘dust cells’) which are mobile and serve as a kind of

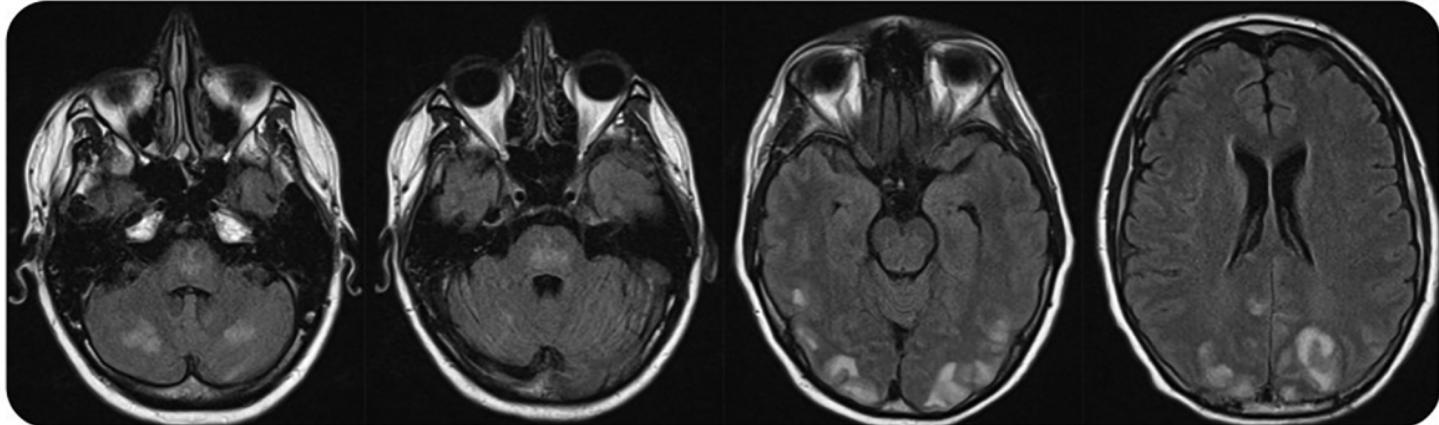
'vacuum cleaner' capable of removing a wide diversity of contaminants and microbial elements in order to minimize interference 'clean' gas-exchange. Inside the alveolus we illustrate the aerosolized entry of the SARS-CoV-2 virus, and some of the immune elements which have trafficked to this site, secondary to immune network activation by the virus. This includes the accumulation of macrophages, mast cells, polymorphonuclear neutrophils (PMNs), along with the cellular release of each cell's effector elements, such as cytokines, chemokines, free radicals, and reactive oxygen species. On the outer circular perimeter of the figure, we have magnified the circumferential organization of the alveolar epithelium in order to illustrate the distinctive mechanisms which collectively represent the SARS-CoV-2 triggered 'prolific activation of a network immune-mediated, inflammatory crisis ('PANIC'). **E)** We illustrate the sentinel step in viral tropism, vis a vis binding of the spike protein on the virion surface to the angiotensin converting enzyme type 2 receptor (ACE-2r) on the surface of the alveolar epithelium. Subsequent to this binding interaction is the entry of the virus into the epithelium via the endosomal pathway, culminating in the release of viral RNA in preparation for replication and eventual release of new virions, thereby perpetuating an amplification of the viral lifecycle and the corresponding acceleration in the destruction of lung alveoli, until a threshold burden of disease is established, beyond which the body's continuous demand for oxygen can no longer be achieved, at which point bioenergetic collapse occurs and heralds in the rapid demise and ultimately death of the patient. **F)** A distinctive facet of the PANIC Attack is illustrated where an activated immune cell has entered a terminal phase, programmed cell death or apoptosis. An inflammatory form of this cell-death sequence involves the massive release of cytokines and chemokines at the termination of the cell's viability, and is referred to as pyroptosis. **G)** We illustrate adaptive immune mechanisms triggered by the virus, and which

sets into motion the development of both humoral (antibody generation), as well as cellular (with phenotypes determined by the preferential synthesis and release of cytokine and chemokine elements, which we can dichotomize into a categorical scheme of pro-inflammatory vs anti-inflammatory or immunoregulatory profiles) immune activities. Such processes commence with the organization of the immunologic ‘synapse’, which includes processed antigen (e.g. viral epitope; such as the SARS-CoV-2 peptide) coupled to major histocompatibility complex II proteins in an antigen presenting cell (such as a macrophage, dendritic cell, or B cell), then shuttled to the cell surface where this complex interacts with a T cell receptor; and the subsequent activation of response priming mechanisms. Priming can result in T cell mediated activities, principally via cytokine and chemokine networks, whereas humoral or B cell networks involve a complex array of activities which commence with somatic hypermutations within the immunoglobulin gene complex, which promotes the development of affinity (a means by which an antibody can bind to its cognate antigen) and avidity (a measure of the binding strength between an antibody and the antigen) maturation, followed by class switching from the acute IgM subtype to the convalescent and memory IgG phenotype. **H)** We illustrate alveolar epithelial membrane, albeit with a segment of the SARS-CoV-2 membrane integrated at the cell surface in what is referred to as syncytia (a kind of chimeric fusion of membranes). In this case, we can observe that the integrated or syncytial membrane expresses spike protein. However, rather than interacting with the ACE-2r, the PANIC Attack process has generated antibodies which bind to spike protein giving rise to either macrophage Fc receptor binding to the Fc portion of the anti-spike antibody and clearance via the reticuloendothelial system (RES), or activation of the complement fixation site on the anti-spike antibody which sets into motion a series of immune activities, which can foment further

damage to the alveolar gas exchange apparatus. Activation of the lectin complement pathway, the alternative pathway (via contact with surfaces, including dead cellular debris), and the classical complement pathway all converge to activate a series of serine esterases, leading to the liberation of C3a and C5a, which serve as anaphylatoxins and chemotaxins promoting innate immune activities such as the migration of cellular elements (e.g. neutrophils, monocytes, macrophages, and eosinophils) into the alveoli, whereupon their arrival, their effector species (super oxides, free radicals, histamine, etc) can be released and damage the delicate architecture of the gas exchange apparatus. **I**) We illustrate how the complement system activities also involve the coordination between C5b-C8 in the assembly of the membrane attack complex (MAC), which contains a central channel, whereby C9 traverses the MAC and perforates the epithelial cell membrane culminating in death and ultimately exfoliation; a cataclysmic step in the destruction of the integrity of the alveoli, and failure of localized gas exchange.

Table 1

A)



B)

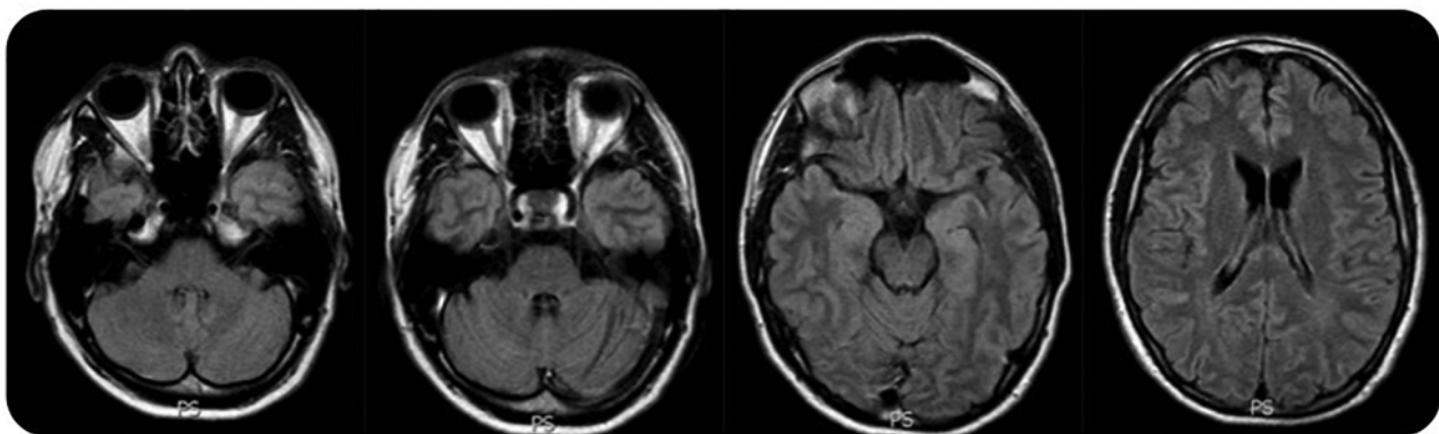
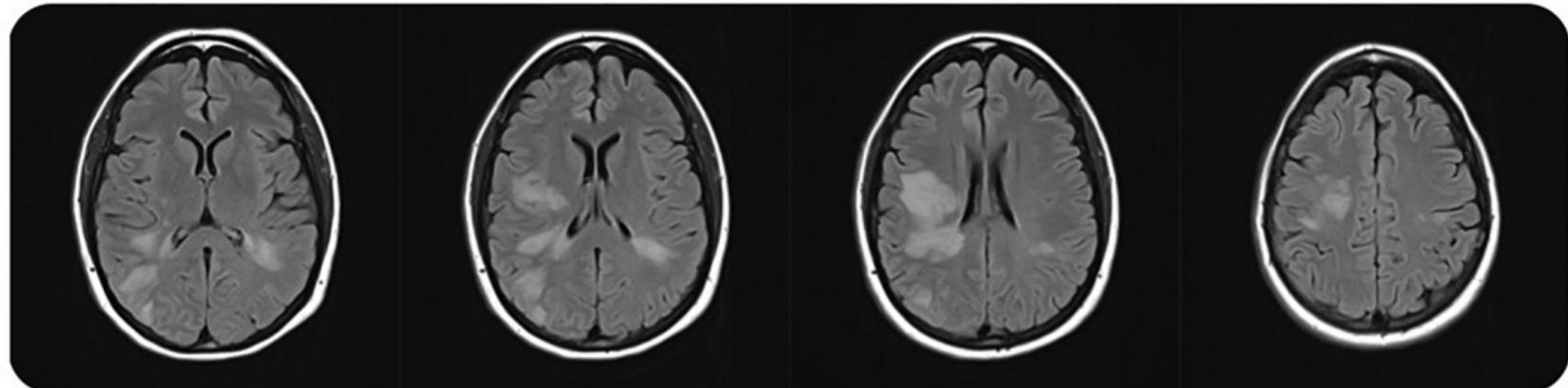


Figure 1

A)



B)

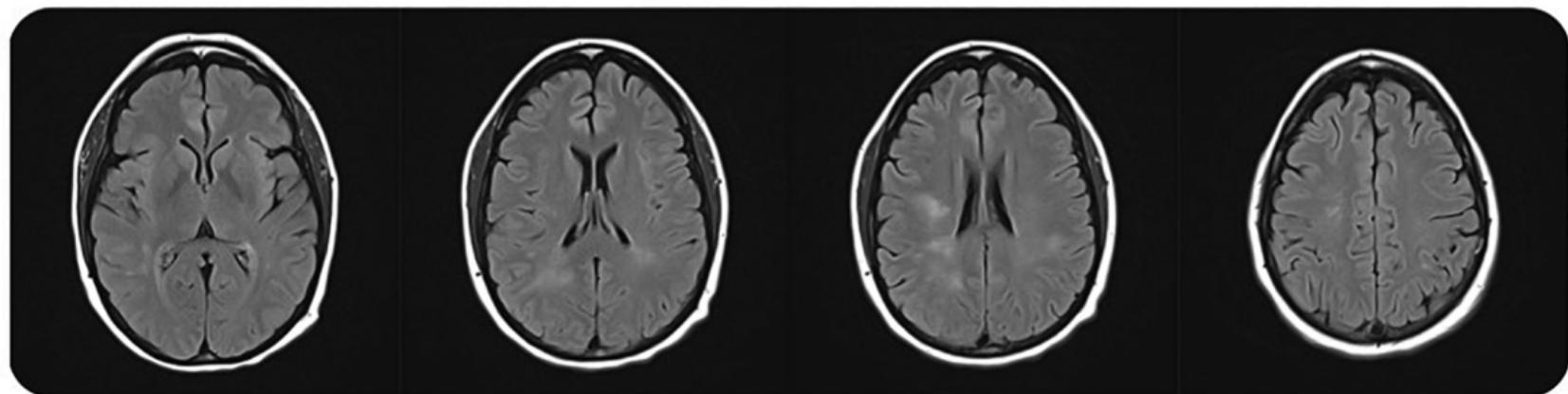


Figure 2

The Anatomy of a 'PANIC' Attack

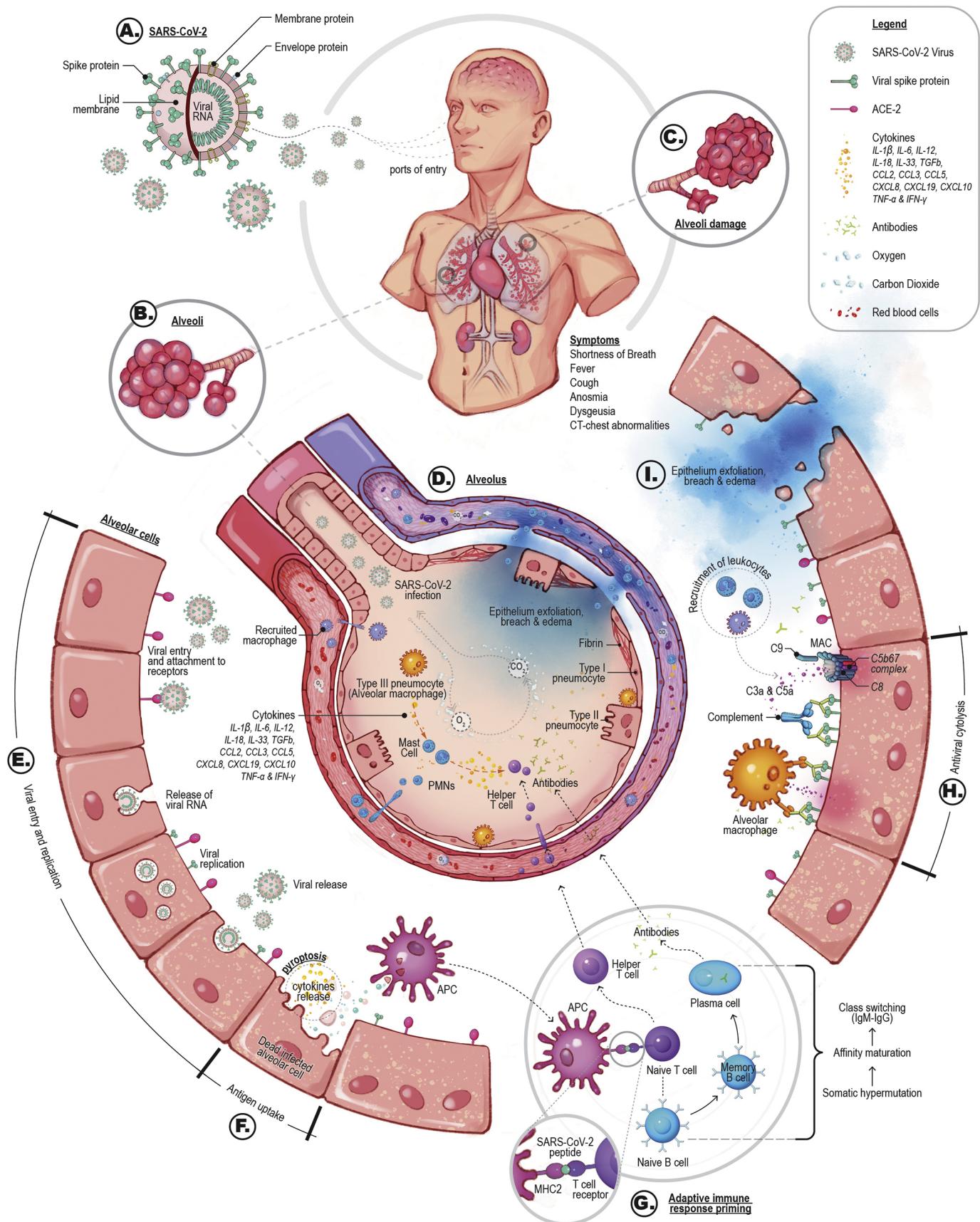


Figure 3