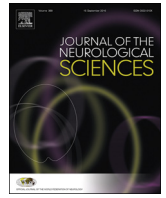




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Review Article

Part I. SARS-CoV-2 triggered 'PANIC'¹ attack in severe COVID-19

Elliot M. Frohman^{a,b,c,*}, Nicole R. Villemarette-Pittman^d, Esther Melamed^a,
Roberto Alejandro Cruz^a, Reid Longmuir^{e,f}, Thomas C. Varkey^{a,g}, Lawrence Steinman^h,
Scott S. Zamvilⁱ, Teresa C. Frohman^{a,b,c,*}



^a Department of Neurology, The Dell Medical School, The University of Texas at Austin, Austin, TX, United States of America

^b Department of Neurosurgery, The Dell Medical School, The University of Texas at Austin, Austin, TX, United States of America

^c Department of Ophthalmology, The Dell Medical School, The University of Texas at Austin, Austin, TX, United States of America

^d Department of Neurology, LSUHSC School of Medicine, New Orleans, LA, United States of America

^e Department of Ophthalmology and Visual Sciences, Vanderbilt University Medical Center, Nashville, TN, United States of America

^f Veterans Affairs Medical Center, Nashville, TN, United States of America

^g Colangelo College of Business, Grand Canyon University Phoenix, AZ, United States of America

^h Department of Neurology, Stanford University School of Medicine, Palo Alto, California, United States of America

ⁱ Department of Neurology and Program in Immunology, University of California San Francisco, San Francisco, California, United States of America

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic has produced a world-wide collapse of social and economic infrastructure, as well as constrained our freedom of movement. This respiratory tract infection is nefarious in how it targets the most distal and highly vulnerable aspect of the human bronchopulmonary tree, specifically, the delicate yet irreplaceable 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 disease is a mild immune-mediated syndrome, with limited damage to the lung tissues. About 20% of those affected experience a disease course characterized by a cataclysmic set of immune activation responses that can culminate in the diffuse and irreversible obliteration of the distal alveoli, leading 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 ability of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 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.

1. Introduction

Rarely before has civilization been confronted with such a formidable enemy, one that is wholly invisible, often resulting in no identifiable symptoms, and yet, it is highly transmissible and the cause of a rapidly disseminated viral pandemic. 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 majority (~80%) of those infected by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) experience a mild to moderate flu-like respiratory tract illness, Coronavirus Disease 2019 (COVID-19). The minority of those infected (~20%) experience a severe disease course that can result in death [1]. Without equivocation, SARS-CoV-2 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.

* Corresponding authors at: Neurology, Neurosurgery, & Ophthalmology, MS and Neuroimmunology Center, The Dell Medical School at The University of Texas at Austin, United States of America.

E-mail addresses: Elliot.frohman@austin.utexas.edu (E.M. Frohman), nville@lsuhsc.edu (N.R. Villemarette-Pittman), Esther.Melamed@austin.utexas.edu (E. Melamed), Roberto.CruzSaldana@austin.utexas.edu (R.A. Cruz), Steinman@stanford.edu (L. Steinman), zamvil@ucsf.neuroimmunol.org (S.S. Zamvil), Teresa.frohman@austin.utexas.edu (T.C. Frohman).

¹ Prolific Activation of a Network-Immune Inflammatory Crisis [PANIC].

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Table 1
Prolific Activation of a Network-Immune Inflammatory Crisis [PANIC]:A New Pathophysiologic Signature for Severe COVID-19.

Prolific Activation of a Network-Immune Inflammatory Crisis	
'PANIC' Principles	
1	SARS-CoV-2 entry <ul style="list-style-type: none"> ☞ Spike engages ACE-2. ☞ Entry via fusion or endocytosis.
2	Cytokine release syndrome <ul style="list-style-type: none"> ☞ Macrophages are triggered by exposure to virus to produce cytokines. ☞ Inflammatory cells recruited to lung.
3	Adaptive immune response <ul style="list-style-type: none"> ☞ APC uptake of viral components & migration to lymphoid tissue. ☞ Initiation of T-cell & B-cell responses. ☞ T-cells move to lung. ☞ Antibodies in blood & lung.
4	Complement fixation <ul style="list-style-type: none"> ☞ Anti-spike antibodies bind to infected cells & fix complement. ☞ C3a & C5a recruit polymorphonuclear leukocytes, eosinophils, monocytes, macrophages into alveolar space. ☞ Generation of further inflammatory mediators. ☞ Assembly (C5b-8) of the membrane attack complex (MAC); C9 penetrates alveolar cells killing them via cellular cytotoxicity.
5	Inflammatory tissue damage <ul style="list-style-type: none"> ☞ Alveoli epithelial exfoliation. ☞ Reduced gas exchange. ☞ Reduced surfactant.
6	Factors complicating management <ul style="list-style-type: none"> ☞ Poor mucus clearance. ☞ Pulmonary edema. ☞ Bronchial smooth muscle constriction. ☞ Net-positive hydration status.

Firstly, SARS-CoV-2 targets its entry receptor, through which it mediates its viral tropism. Specifically, the SARS-CoV-2 surface glycoprotein, 'spike', binds to the angiotensin converting enzyme (ACE-2-r) receptor [2], 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 ability of SARS-CoV-2 to trigger 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 cataclysmic obliteration of lung alveoli by exceeding a damage threshold for these indispensable and slow to repair gas-exchange structures – often exceeding the repair and replacement mechanism leading to ultimate end stage organ damage and potentially death.

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 tegumentum. 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 strategically ominous as SARS-CoV-2. 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 SARS-CoV-2.

SARS-CoV-2 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 [3]. Since then, we have treated a broadening diversity of other causes that we believe to be variants of 'PANIC', including other post-infectious (e.g. post-adenovirus) [Fig. 1] and post-vaccinal (e.g. post-dTap) encephalomyelitides [Fig. 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.

2. 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

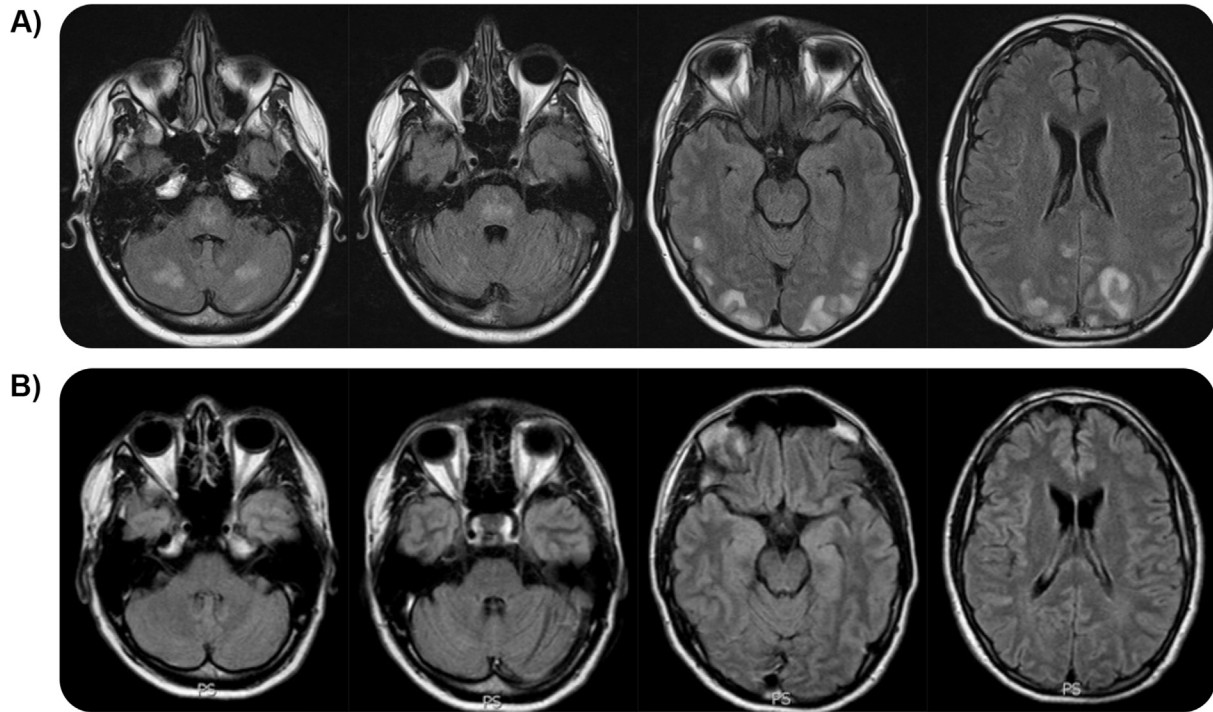


Fig. 1. A) Axial FLAIR-weighted MRI prior to HDMTX-LR, B) Axial FLAIR-weighted MRI after HDMTX-LR for adenovirus triggered PANIC.

severe, even fatal disease, with a particular targeting of the respiratory tract [4]. 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 [5–10].

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 [11,12]. Unlike the antecedent epidemics of SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 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

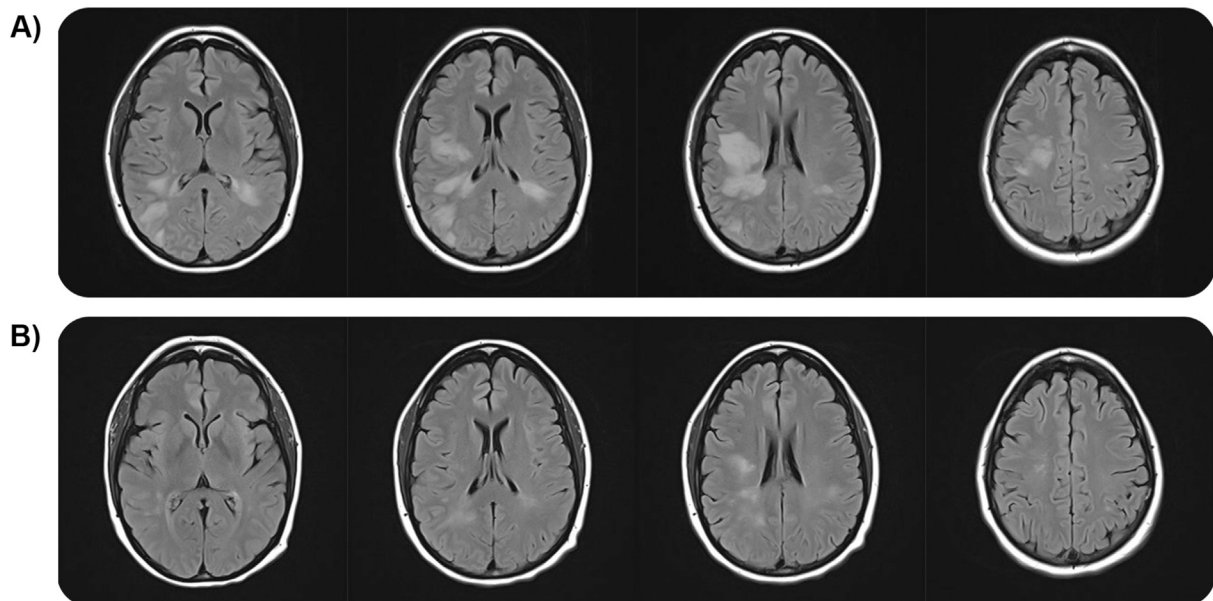


Fig. 2. A) Axial FLAIR-weighted MRI prior to HDMTX-LR, B) Axial FLAIR-weighted MRI after HDMTX-LR for a 3rd trimester vaccinal (dTap) triggered PANIC-associated encephalomyelitis.

active immunity, represents our best strategy to prevent further dissemination and also to identify newly infected cases as early as possible.

3. 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%) [11,13,14]. 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 of multi-organ derangements, with the most severely affected individuals exhibiting multi-organ failure [15].

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 [4]. 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 acute respiratory distress syndrome (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% [4].

Risk factors associated with the designation of severe COVID-19 included male gender, advanced age, and pre-existing co-morbid conditions, especially hypertension, diabetes, morbid obesity, and smoking [16]. The clinical semiology 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), as well as hypercoagulable derangements, which can lead to stasis, clot formation, and ultimately altered or abolished blood flow dynamics within the alveolar capillary network [4].

4. 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 respiratory and circulatory systems, 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 SARS-CoV-2 infection that target the CNS and peripheral nervous system (PNS) [14,17–23].

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 or pulmonary embolism as a result of stasis within the chambers of the heart [24–27]. Alternatively, SARS-CoV-2 could directly damage the brain and spinal cord either via hematogenous spread or via neural propagation into the CNS [28].

Other coronaviruses, such as SARS-CoV-1 and human coronavirus OC43 have been shown to enter into the nasal passages and spread to the olfactory bulb, pyriform cortex, dorsal nucleus of the rafe in the brainstem, and spinal cord [29,30]. Of note, ablation within the olfactory pathway can prevent the neural spread of MHV coronavirus in animals [31]. Cellular entry of SARS-CoV-2 occurs through the ACE-2-r. Interestingly, ACE-2-r is expressed widely in the CNS within neurons, motor cortex, hypothalamus, thalamus, and brainstem [32–37].

The physical distribution of ACE-2-r 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 [30]. In addition, anosmia and ageusia in COVID-19 patients may be related to viral entry through ACE-2-r 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 [14]. 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 [14].

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) [38].

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 [14]. 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.

5. Virology 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 [39]. 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 [40]. The viral spike interacts with its receptor, ACE-2-r, on susceptible target cells to facilitate entry and viral replication [41].

With the identification of the ACE-2-r 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 might be harmful [42].

6. ‘Irrational exuberance’ of immune-mediated inflammatory networks

Evidence is rapidly mounting to suggest that the severe lung damage in COVID-19 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 SARS-CoV-2 [Fig. 3]. To more accurately account for viral induction of the coincident confluence of converging inflammatory cascades, and for differentiation of 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.

7. 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 [43]. 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 [Fig. 3].

The convergent inflammatory cascades focused upon this highly delicate and vulnerable bronchopulmonary gas-exchange scaffolding is what makes this Coronavirus 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 [44].

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, these microbes include such species as MRSA and other pharyngeal and enteric species. 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 SARS-CoV-2 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 [13,45,46].

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.

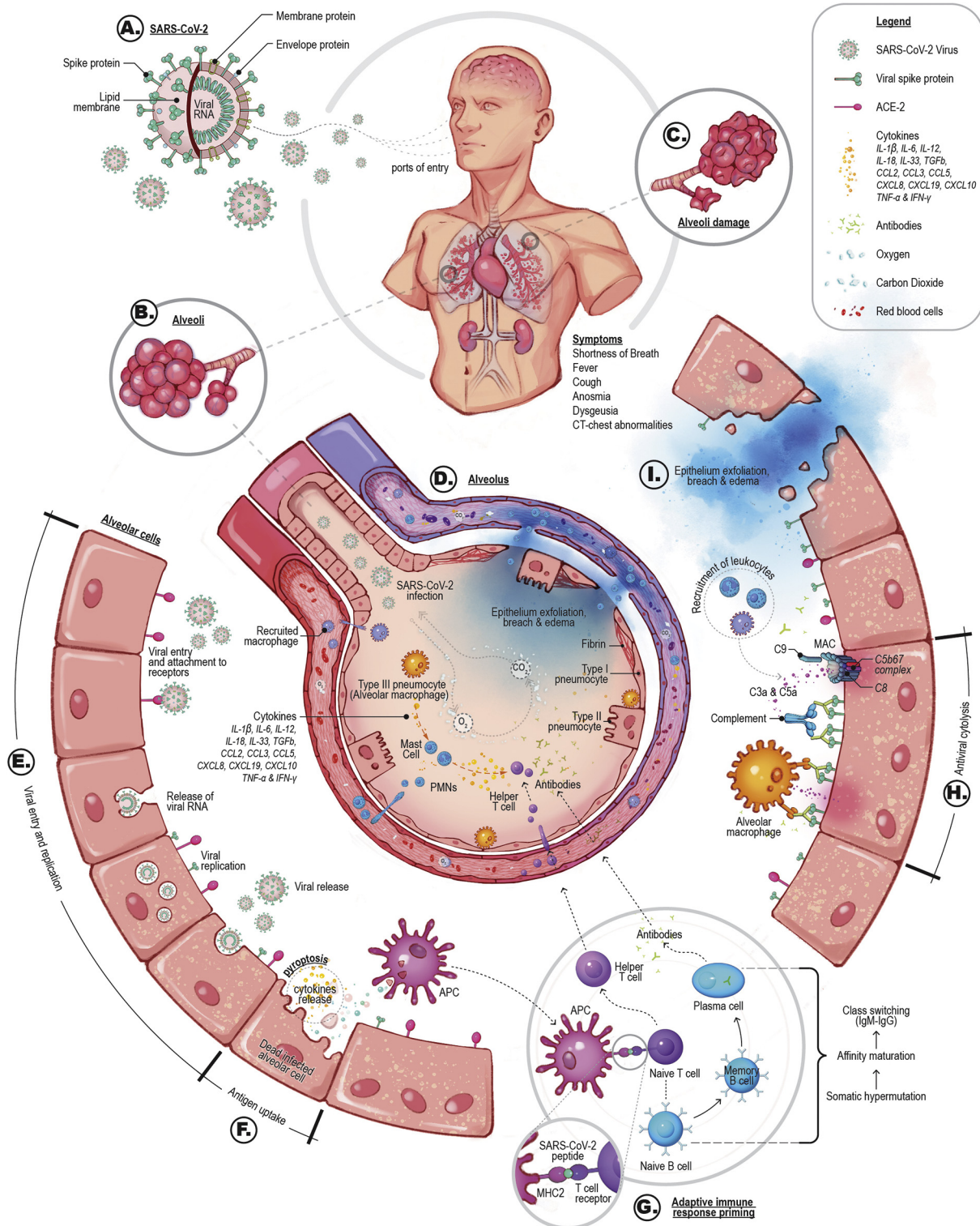
Other medical manifestations of the COVID-19 infection include a vasculitis with risk of progressing to gangrene [47–49]. Some patients have evidence of spleen destruction and diffuse atrophy of lymph nodes, and their associated regional chains [47]. Others can harbor evidence of anti-phospholipid, anti-cardiolipin, and anti-β₂-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 [50].

8. The pathophysiology of the SARS-CoV-2 triggered PANIC-attack

8.1. Role of complement in the SARS-CoV-2-triggered PANIC-attack

SARS-CoV-2 can enter cells within the lungs via the endosomal pathway, or conceivably by fusion mechanisms that might also allow for the development of syncytia [Fig. 3]. Destruction of alveolar cells expressing ACE-2-r by SARS-CoV-2 could theoretically be orchestrated by post-viral replication cell bursting, or by antigen-antibody complex triggering complement-dependent cytotoxicity (CDC) via activation of the complement pathway [51,52]. Activation of the complement

The Anatomy of a 'PANIC' Attack



(caption on next page)

pathway would result in the C3a and C5a fragments acting as anaphylatoxins, through increasing the leakage of the capillary beds, 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 [Fig. 3].

In order to ascertain the role played by the complement system in

Fig. 3. Here we present the principal target of SARS-CoV-2, 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 components of SARS-CoV-2. Note that the virion has a positive single strand RNA, which is enclosed within a sphere delimited by membrane bilayers of phospholipid. Projecting out of the virion is the spike protein; the most important component, given that SARS-CoV-2 tropism is contingent upon the binding of spike to its entry receptor, the angiotensin converting enzyme 2 receptor (ACE-2-r). 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 occurs in severe COVID-19 D) Represents the indivisible functional unit for gas-exchange, comprised of a single cell thick lining 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 with 'clean' gas-exchange. Inside the alveolus we illustrate the aerosolized entry of SARS-CoV-2, 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 'proliferative activation of a network immune-mediated, inflammatory crisis ('PANIC'). E) We illustrate the sentinel step in viral entry and replication, via a vis binding of the spike protein on the virion surface to ACE-2-r 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 infected cell has entered a terminal phase, apoptosis. 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, which set 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 this case, we can observe that the cell membrane expresses spike protein. However, rather than interacting with ACE-2-r, 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 complement system activation also involves 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 cell death and ultimately exfoliation; a cataclysmic step in the destruction of the integrity of the alveoli, and failure of localized gas exchange.

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 CDC [53].

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 uremic syndrome (HUS), paroxysmal nocturnal hemoglobinuria (PNH), myasthenia gravis, and most recently AQP4⁺ neuromyelitis optica spectrum disorder (NMOSD) patients [54]. 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 bronchopulmonary 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 [Fig. 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 chemotaxins, 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 [55,56]. 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) [57,58]. The anaphylatoxins C3a and C5a further intensify and perpetuate organ tissue damage in COVID-19 via escalation of IL-1, IL-6, TNF α , as well as mast cell histamine degranulation [59-61].

8.2. Role of the cytokine ‘Storm’ in SARS-CoV-2 triggering of the PANIC-attack

While IL-6 is elevated in about 33% 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 [4,62]. Further, evidence of cytokine release syndrome (CRS) or ‘storm’ was confirmed by an escalation in IL-6 levels [63] in conjunction with inadequate levels of the negative regulatory suppressor of cytokine signaling 3 (SOC 3) [64].

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 [65,66]. An alternative explanation is that the lymphopenia may be due to a cortisol burst from stress. 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 [48] (Preprint: Wan S et al. (2020) Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). medRxiv. DOI: <https://doi.org/10.1101/2020.02.10.20021832>).

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) [67,68].

9. Conclusion

While the lung is the principal and most crucial target of attention for those with severe COVID-19, the distribution of the ACE-2-r 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 counterbalancing mechanisms commensurate with the wide spectrum of activated inflammatory mechanisms triggered by SARS-CoV-2, 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 HDMTX-LR, represents a particularly interesting candidate therapy, worthy of investigation within the context of a randomized controlled clinical trial. This trial is needed 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, 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.

Author contributions

Elliot Frohman: conception, critical revision of manuscript for intellectual content.

Nicole Villemarette-Pittman: critical revision of the manuscript for intellectual content.

Esther Melamed: critical revision of manuscript for intellectual content.

Roberto Alejandro Cruz: critical revision of manuscript for intellectual content.

Reid Longmuir: conception: critical revision of manuscript for intellectual content.

Thomas Varkey: Critical revision of manuscript for intellectual content.

Lawrence Steinman: critical revision for intellectual content and accurate immunologic foundations.

Scott Zamvil: conception, critical revision of the manuscript for intellectual content.

Teresa C Frohman: conception critical revision of manuscript for intellectual content.

Author disclosures

Elliot Frohman: Has received speaker fees from Genzyme, Alexion, Novartis, and consulting fees from Biogen and Serono.

Nicole Villemarette-Pittman: Serves as Managing Editor for the *Journal of the Neurological Sciences*.

Esther Melamed: served as a consultant and received honoraria from EMD Serono and Genentech.

Roberto Alejandro Cruz: Has received speaker fees from Alexion.

Reid Longmuir: consultant for Horizon.

Thomas C. Varkey: Nothing to disclose.

Lawrence Steinman: Dr. Steinman is on the Editorial Boards of The Proceedings of the National Academy of Sciences, and the Journal of Neuroimmunology. He has served on the Editorial Board of the *The Journal of Immunology* and *International Immunology*. He has served as a member of grant review committees for the National Institutes of Health (NIH) and the National MS Society.

He has served, or serves, as a consultant and received honoraria from Atara Biotherapeutics, Atreca, Biogen-Idec, Celgene, Centocor, Coherus, EMD-Serono, Genzyme, Johnson and Johnson, Novartis, Roche/Genentech, Teva Pharmaceuticals, Inc., and TG Therapeutics. He has served on the Data Safety Monitoring Board for TG Therapeutics. He serves on the Board of Directors of Tolerion and Chairs the Scientific Advisory Board for Atreca.

Currently, Dr. Steinman receives research grant support from the NIH and Atara Biotherapeutics.

Scott Zamvil: Dr. Zamvil is Deputy Editor of *Neurology*, *Neuroimmunology and Neuroinflammation* and is an Associate Editor for *Frontiers in Immunology* and *Frontiers in Neurology*. He serves on the Advisory Committee for the American Congress on Treatment and Research in Multiple Sclerosis (ACTRIMS) and is a standing member of the research grant review committee for the National Multiple Sclerosis Society (NMSS). He has served on the Editorial Board of the *Journal of*

Clinical Investigation, *The Journal of Immunology* and *The Journal of Neurological Sciences*, and has been a charter member of the National Institutes of Health (NIH) Clinical Neuroimmunology and Brain Tumors (CNBT) grant review committee.

He has served, or serves, as a consultant and received honoraria from Alexion, Biogen-Idec, EMD-Serono, Genzyme, Novartis, Roche/Genentech, and Teva Pharmaceuticals, Inc., and has served on Data Safety Monitoring Boards for Lilly, BioMS, Teva and Opexa Therapeutics.

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