At the Root of 3 "Long" Diseases: Persistent Antigens Inflicting Chronic Damage on the Brain and Other Organs in Gulf War Illness, Long-COVID-19, and Chronic Fatigue Syndrome

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ABSTRACT: Several foreign antigens such as those derived from viruses and bacteria have been linked to long-term deleterious effects on the brain and other organs; yet, health outcomes subsequent to foreign antigen exposure vary depending in large part on the host's immune system, in general, and on human leukocyte antigen (HLA) composition, in particular. Here we first provide a brief description of 3 conditions characterized by persistent long-term symptoms, namely long-COVID-19, myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), and Gulf War Illness (GWI), followed by a brief overview of the role of HLA in the immune response to foreign antigens. We then discuss our Persistent Antigen (PA) hypothesis and highlight associations between antigen persistence due to HLA-antigen incongruence and chronic health conditions in general and the 3 "long" diseases above in particular. This review is not intended to cover the breadth and depth of symptomatology of those diseases but is specifically focused on the hypothesis that the presence of persistent antigens underlies their pathogenesis.

KEYWORDS: Human leukocyte antigen, Gulf War illness, long-COVID, myalgic encephalomyelitis/chronic fatigue syndrome, persistent antigen

Introduction

Long-COVID-19, myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and Gulf War Illness (GWI) all share the following attributes. (a) They present with chronic symptomatology arising from involvement of various organ systems, including the brain; (b) they appear following an "event": long-COVID-19 after a COVID-19 infection with the SARS-Cov-2 virus, ME/CFS after an infection with any of various viruses ("postviral syndrome"), and GWI after exposure to potentially toxic chemicals and biologics (vaccine antigens) while serving in the first Gulf War of 1990-91; and (c) in all 3 conditions, women are more adversely affected than men (in long-COVID-19, ME/CFS, and GWI - below). *We attribute the development of long-COVID-19, ME/CFS and GWI to the presence of persistent antigen(s) to which patients were exposed and which could not be eliminated.* We further attribute the varied symptomatology to (a) continuous direct tissue damage by the offending antigen, (b) chronic inflammation accompanying tissue damage, (c) diverse immune reactions depending on the antigen itself, the organs affected, and the sex of the patients, and (d) potential autoimmunity stemming from chronic breakdown of tissue proteins and/or antigen fragments. All of the above are the result of the persistence of offending antigen(s);

hence we called this idea the "Persistent Antigen" (PA) hypothesis.[1] We attribute this antigen persistence (in otherwise immunocompetent people) to ineffective elimination of the offending antigen due primarily to a mismatch between the antigen and the patient's Human Leukocyte Antigen (HLA) genetic capacity.

The 3 "Long" Diseases

Long-COVID-19

Over 483 million people worldwide are estimated to have been infected with SARS-CoV-2, the virus responsible for the ongoing coronavirus-19 (COVID-19) pandemic, and over 6 million people have died as a result of infection.[2] Although the cases of SARS-CoV-2 have decreased rapidly over the last several months, the long-term effects of COVID infection are just beginning to take shape. Studies have documented that nearly 80% of patients develop one or more long-term symptoms,[3] commonly referred to as long-COVID or Post-Acute Sequelae of SARS-CoV-2 (PASC), and as many continue to experience symptoms 12 months after acute infection.[3,4] An even higher percentage of patients hospitalized with COVID experience persistent symptoms,[5] yet sizable numbers of individuals with mild or even asymptomatic cases experience

long-COVID[6-8] as well as vaccinated individuals following breakthrough COVID-19 infection.[9]

Several systems are affected by long-COVID including respiratory, cardiac, cutaneous, and CNS,[10] with the most common persistent symptoms (for the initial strain) including fatigue and post-exertional malaise, headaches, dyspnea, joint and chest pain, brain fog and memory impairment.[3,5,7,11] These effects have resulted in sustained impairment in daily functioning including inability to return to work months after infection.[11]

While the lungs are the primary site of acute COVID infection, several other systemic effects have been associated with long-COVID. Long-term effects of COVID infection, even among those with mild COVID cases, include "COVID-heart" which refers to dysrhythmias, cerebrovascular disorders, heart disease, myocarditis, heart failure, and thromboembolic disease well after acute infection, even among those with mild cases of COVID [12]; new onset Type 2 diabetes after recovery [13]; neurological events including Bell's Palsy, encephalomyelitis, Guillain-Barre syndrome[14]; autoimmune diseases[15,16]; and significant multi-organ impairment.[17] In addition, long-COVID is associated with brain gray matter loss especially in olfactory areas, whole brain atrophy, and cognitive disruptions associated with cerebellar atrophy.[18] Furthermore, patients with pneumonia associated SARS-CoV-2 infection have 30% higher odds of new onset dementia compared to other pneumonias.[19] Indeed, the effects on the brain are so substantial that some have referred to the commonly reported brain fog, difficulty concentrating, dizziness, and fatigue characteristic of long-COVID as "neuro-COVID."[20]

ME/CFS

ME/CFS is an umbrella term used to characterize a condition of uncertain etiology defined by extreme fatigue, neurocognitive problems, and autonomic dysfunction.[21] Similarities between long-COVID/PASC and ME/CFS have been widely recognized[11,22,23] as has overlap between GWI and ME/CFS.[24,25] Like GWI and long-COVID, ME/CFS lacks a definitive case definition though generally involves debilitating chronic systemic symptoms including fatigue, post-exertional malaise, unrefreshing sleep, cognitive impairment, orthostatic intolerance, and musculoskeletal pain.[21] Approximately 1% of the population suffers from ME/CFS with more women affected than men though the prevalence varies widely depending on case definition.[26] Among those affected, ME/CFS results in significant impacts on quality of life including significant social and occupational impairment and disability.[21,27,28]

GWI

GWI appeared in a large number of otherwise healthy veterans after the 1990-91 Persian Gulf War. Of the 693 826 veterans enlisted then,[29] about 1/3 (25%-32%, 173 456-222 021 veterans) reported symptoms from multiple organs, which have since been[24,30] and are even currently a major health problem,[29] especially in women veterans.[31,32] This Gulf War Illness (GWI) is a condition characterized by chronic and diffuse symptoms including muscle and joint pain, fatigue, neurological/cognitive/mood impairment, skin rashes, respiratory complaints, and gastrointestinal problems.[33,34] GWI symptoms prominently involve the brain, and numerous structural and functional brain anomalies have been associated with GWI,[35-40] yet symptoms span several organ systems suggesting broad systemic effects. No definitive objective indicator of GWI exists; rather, symptoms are evaluated via self-report and case status is determined according to 2 primary case definitions recommended by the Institute of Medicine.[41] There is currently no cure for GWI and treatments are aimed at alleviating symptoms piecemeal. 30 years after the Gulf War, veterans with GWI have continued to experience deteriorating health resulting in significant impairment.[24,42] In addition to GWI, GW veterans are at higher risk for several chronic health conditions including numerous infectious diseases as well as irritable bowel syndrome, chronic fatigue syndrome, and amyotrophic lateral sclerosis.[43]

The etiology of GWI is uncertain although numerous biological and chemical exposures have been implicated. In theater exposures such as burning pits, smoke from oil well fires, pesticides, organophosphates, sarin/cyclosarin nerve agents, pyridostigmine bromide and others have been associated with GWI.[44] However, GWI symptoms have also been reported among Gulf War era veterans who did not deploy (albeit to a lesser extent)[35] suggesting involvement of exposures not tied to service in theater. Whereas early evaluations documented significantly more GWI symptoms among deployed compared to non-deployed veterans, recent research demonstrated that as the Gulf War era veteran population ages, up to 80% of non-deployed veterans meet criteria for at least 1 case definition of GWI,[42] suggesting that Gulf War veterans, regardless of deployment status, are at high risk of poor health outcomes for reasons that are not entirely certain but appear to extend beyond deployment theater.

In addition to deployment-related exposures, several investigators have considered the role of vaccines as contributing to GWI. To limit effects of potential biological warfare agents, Gulf War veterans were administered the anthrax, botulinum toxin, and plague vaccines in addition to a spate of routine and geographic-specific immunobiologics including cholera, measles, meningitis, rabies, rubella, tetanus, yellow fever, and typhoid vaccines.[44] Altogether, up to 17 antigens were administered via vaccination in the first 2 weeks of training with additional vaccinations to follow.[45] Since the Gulf War, questions have been raised about the role of various vaccines in GWI; however, vaccination records for Gulf War veterans are limited rendering direct links between vaccine administration and GWI challenging to establish.[46] Nonetheless, there appears to

be evidence linking vaccines administered to Gulf War veterans with GWI, either due to the direct effects of the vaccine antigen(s)[47-52] or to vaccine adjuvants such as squalene.[53,54]

Effects of sex on prevalence and severity of the 3 "long" diseases

Sex has significant effects on the prevalence and/or severity of all 3 "long" diseases above. More specifically, (a) women are more likely to develop long-COVID-19 and are more adversely affected than men,[55-57] (b) women are more frequently and more severely affected with ME/CFS than men,[58] and (c) women veterans of the Gulf War are in worse health than men.[31,32]

Immune Response to Foreign Antigens

The human immune system has evolved to provide a range of defenses starting with mechanical and chemical barriers to protect against foreign antigens such as viruses and bacteria.[59] If those barriers are breached, several the innate immune system[60] pathways are promptly triggered to inhibit infection, protect cells, and eliminate infected cells. The initial line of defense involves the mobilization at the site of infection of macrophages and polymorphonuclear phagocytes (neutrophils, basophils, and eosinophils) to contain the infection. The involvement of adaptive immune mechanisms follows soon thereafter.

Adaptive immunity: HLA

Adaptive immune mechanisms are slower, starting about 4 to 10 days following infection, and include T-cell mediated immunity to resolve infection and B-cell mediated production of antibodies to clear lingering pathogens and protect against reinfection. If the infection is not controlled due to immune system disruption or evasion, antigen persistence can occur, the outcome of which can range from persistent infection with low viral load (eg, cytomegalovirus [CMV] and Epstein-Barr virus [EBV]) to latency with periods of reactivation (eg, herpes simplex viruses) to persistent viremia (eg, human immunodeficiency virus [HIV], hepatitis B virus, hepatitis C virus).[61-63] A central component of human immune defenses involves HLA. HLA genes, located on chromosome 6, code for cell-surface glycoproteins that play an essential role in host protection from foreign antigens (eg, viruses, bacteria, cancer neoantigens) by facilitating immune surveillance and initiating an immune response to eliminate foreign antigens.[64] There are 2 primary classes of HLA that work in concert via distinct mechanisms.

HLA Class I: Cytotoxic immunity.

HLA Class I molecules (HLA-A, B, C), which are expressed on nucleated cells, export small peptides (8-10 amino acid residues) from proteolytically degraded cytosolic viruses, bacteria, and tumors to the cell surface for presentation to CD8 + cytotoxic T cells to signal cell destruction.

HLA Class II: Humoral immunity.

Class II HLA molecules (HLA-DPB1, DQB1, DRB1), which are expressed on lymphocytes and professional antigen presenting cells (APC; eg, macrophages, dendritic cells, and monocytes), present larger peptides (12-22 amino acid residues) derived from endocytosed exogenous antigens such as viruses and bacteria to CD4 + T cells, facilitating antibody production and adaptive immunity. The success of antigen elimination requires, as an initial step, a match between epitopes derived from foreign antigens and the HLA receptor binding groove. Even a single amino acid difference can alter the binding groove,[65] thereby changing the landscape of antigens that a given HLA molecule can bind for presentation to T cells. HLA is the most highly polymorphic region of the human genome; each individual carries 12 HLA alleles (6 from Class I and 6 from Class II) inherited in a Mendelian fashion, determining the antigens that each individual can successfully bind to and eliminate. To that end, HLA variation is known to contribute to variation in disease susceptibility.[66]

Relations between sex and immunity

There are well established differences between men and women with respect to native, cellular and humoral immune response to pathogens and vaccines, as reviewed succinctly recently.[67,68] Briefly, women mount stronger immune responses to viruses and vaccines than men but also suffer more serious illness when infected and show more serious adverse effects to vaccination. These differences are attributed to a combination of hormonal and genetic factors, namely the differential effects of sex hormones (estrogen, progesterone, testosterone) on the immune response and to the genetic coding of several immune-related proteins on the X chromosome. It is thought that the more rigorous immune response protects women better than men from viral infections but, at the same time, renders them more susceptible to potential autoimmunity.

The Persistent Antigen Hypothesis

Since a match between antigen epitopes and proteins of the HLA receptor binding groove is the critical initial step in antigen presentation and elimination, and since each individual has a limited repertoire of HLA molecules, what then happens in the event of an HLA-antigen mismatch? This is the crux of the persistent antigen hypothesis[1] which posits that, in the absence of HLA with sufficient affinity and immunogenicity, the antigens persist, contributing to downstream deleterious effects. Such effects comprise 4 major categories: (a) a persistent antigen continues to cause direct tissue damage, (b) this damage induces chronic inflammation produced by a persistent immune response, including cytokine production, (c) ongoing breakdown of proteins can lead to autoimmunity, and (d) antigen persistence can alter differentiation of pathogen-specific CD8 + T cells resulting in functional exhaustion of effector T cells and defective immunological memory.[69,70] At the root of

this lies a HLA-antigen mismatch in that HLA-antigen binding is critically involved in antigen presentation to T cells: a HLA-antigen mismatch leads to the breakdown of antigen elimination pathways and antigen persistence. Several common viruses have been associated with antigen persistence including HIV, hepatitis B virus, hepatitis C virus, influenza, several human herpes viruses including EBV and CMV[3] and, more recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[22,71-75] Persistent antigens stimulate immune pathways aimed at their elimination, the downsides of which include chronic inflammation and tissue damage which may impact multiple organ systems.

Antigen persistence: Systemic effects

Many foreign antigens ultimately impact brain function; however, other organs are often the primary target. For example, hepatitis B and C viruses affect primarily the liver but systemic effects involving the eye, gut, kidney, thyroid, cardiovascular system, and central nervous system (CNS) have been associated with infection.[76] Influenza primarily affects respiratory epithelial cells, yet is also associated with neurological complications and CNS disease due to cross-reactivity with human brain tissue.[77,78] Similar systemic effects are seen in relation to other infections such as dengue fever for which viral antigens have been documented in the liver, spleen, lymph node, thymus, kidney, lung, skin, and in monocytes and lymphocytes,[79] and similarly for bacterial infections.[80]

Although the brain is protected by the blood-brain barrier (BBB), there is substantial evidence that the defenses conferred by the BBB are readily breached by foreign antigens either via peripheral nerves or via the blood supply through several mechanisms including infection of the endothelial cells lining the BBB permitting direct access to the brain, the "Trojan Horse" approach, or access through the choroid plexus which is relatively unprotected.[81,82] Inflammation, a common immune response to foreign antigens, has been found to increase BBB permeability,[83] permitting entry of foreign antigens into the brain. Indeed, evidence of persistence in brain tissue and associated neurocognitive disruptions have been documented for several neurotropic viruses including hepatitis C,[84] HIV,[85] herpes simplex virus,[86] influenza,[87] and zika virus,[88] among others.

Antigen persistence and chronic conditions

Numerous chronic conditions have been associated with viral or bacterial persistence. EBV infection, for instance, has been associated with systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, multiple sclerosis, and other diseases.[89,90] Herpes simplex virus and hepatitis B have been linked to Alzheimer's disease.[86,91] Hepatitis C virus has been linked with insulin resistance and type 2 diabetes as well as rheumatic diseases,[76] and several human herpes viruses (eg,

CMV, EBV, HHV6) have been associated with brain tumors.[92] Bacterial infections have also been linked to long-term sequelae.[93] For example, *helicobacter pylori* (*H. pylori*) infections are thought to cause severe gastroduodenal disorders,[94] bacterial infections have been implicated in chronic obstructive pulmonary disease,[95] and oral bacterial infections are associated with several non-oral systemic conditions including cardiovascular disease, diabetes mellitus, stroke, inflammatory bowel disease, and others.[96,97] These varied insults result in remarkably similar systemic effects indicative of equifinality related to antigen persistence.

In summary, the host immune system has evolved to maximize host health via elimination of pathogens; however, pathogen elimination in an otherwise immunocompetent host relies on congruence between an individual's HLA composition and foreign antigen epitopes, thereby constraining the pathogens one can successfully eliminate. In the absence of HLA-antigen congruence with sufficient binding affinity and immunogenicity, the antigen may persist to various degrees resulting in long-term sequelae including chronic low-grade inflammation, autoimmunity, and chronic disease. In the next section we provide an overview of 3 chronic conditions—Gulf War Illness, Long-COVID, and chronic fatigue syndrome—and highlight evidence suggesting a contributory role of antigen persistence.

Antigen persistence in long-COVID-19. Of the 3 diseases reviewed above, long-COVID is by definition a postviral syndrome, ME/CFS is possibly a postviral syndrome,[98] but GWI has not been associated with any infection. Accordingly, SARS-CoV-2 RNA or protein have been found to persist in the olfactory mucosa for at least 6 months[71] and have been found in the brainstem,[99,100] presumably having entered the brain retrogradely via olfactory sensory neurons.[71] SARS-CoV-2 RNA was found to persist in various organs up to 230 days,[72] and SARS-CoV-2 nucleic acids and immunoreactivity in the small bowel individuals 4 months after the onset of symptoms.[73] Finally, with respect to long-COVID-19, the offending antigen has been found in the cerebrospinal fluid (CSF),[101] and has been shown to persist[102] and be associated with adverse clinical outcomes[101,102]

Antigen persistence in ME/CFS. Similarly to GWI and long-COVID, ME/CFS is associated with immune system disruption[103] including systemic inflammation[104,105] and neuroinflammation.[106-108] Numerous infectious agents including several human herpes viruses (eg, EBV, HHV6, CMV) have long been suspected in the pathogenesis of ME/CFS [109-113]; indeed, CFS was historically known as "chronic Epstein-Barr virus syndrome."[114] Several lines of research suggest that viral reactivation or viral persistence underlies ME/CFS,[98,112] including evidence of HHV-6 antigen in peripheral blood mononuclear cells in patients with CFS,[115] evidence of active and latent HHV-6/HHV-7 infection in plasma samples of CFS patients,[116] and deficient EBV-specific B- and T-cell

memory response indicative of impaired ability to control early steps involved in EBV reactivation in CFS patients.[117] ME/CFS is also associated with autoimmunity,[118,119] and recent evidence suggest that molecular mimicry between viral and human proteins may contribute to the condition.[120] Finally, both Class I and Class II HLA have been implicated in ME/CFS, linking immune-mediated pathways targeting foreign pathogens in the pathogenesis of the chronic, debilitating condition.[121]

Although several brain anomalies are associated with ME/CFS,[122] brainstem abnormalities have been the most consistently documented.[108,122-124] The brainstem, a central hub in inflammation neurocircuitry,[125] is implicated in sickness behaviors such as malaise, lassitude, fatigue, numbness, coldness, muscle and joint aches, and reduced appetite.[126] Like ME/CFS, both long-COVID/PASC[22,127] and GWI[37,128] have been linked to brainstem anomalies. Since sickness behavior is triggered by proinflammatory cytokines resulting from activation of the innate immune system in response to pathogens,[126] it is likely that the overlapping sickness behavior symptoms associated with ME/CFS, GWI, and long-COVID may be similarly driven by exposure to pathogens; although notable differences between the conditions[129,130] suggest potential involvement of distinct pathogens.

Antigen persistence in GWI. Unlike long-COVID and ME/CFS where viral components (nucleic acids and/or proteins) have been identified or implicated as persistent antigens, no such connection exists for GWI. However, research during the past 5 years has produced direct evidence for the presence of persistent neurotoxic antigens in the blood of GWI patients.[50-52] Using an in vitro system of testing the effect of serum from GWI patients on the spreading and apoptosis of cells in N2A neuroblastoma cultures, it was found (a) that GWI serum has deleterious effects on the culture by reducing cell spreading and inducing cell death (apoptosis),[131] and (b) that these negative effects are prevented by the concomitant addition of serum from heathy GW veterans[131] and, partly, by the addition of pooled human IgG.[132] These findings documented the presence of harmful substances in GWI serum which can be neutralized by healthy GW serum and partly by IgG. A plausible hypothesis to explain these findings would be to attribute the adverse effects on circulating persistent antigens. Since every enlisted GW veteran was administered at least 17 vaccines, including vaccines against anthrax, botulinum toxin and plague, and since GWI appeared in all GW veterans, we entertained the hypothesis that such harmful persistent antigens in GWI serum are components of antigens contained in the administered vaccines. We then tested this hypothesis by evaluating the effect of specific antibodies against the various GW vaccine antigens and found that, indeed, such antibodies substantially reduced or even reversed the detrimental effect of GWI serum on N2A cells.[50-52] The most notable beneficial effect was that of the antibody against the anthrax antigen contained in the

vaccine, "protective antigen 63" (PA63),[50,51] as illustrated in Figure 1 for 15 GWI patients, as follows. The serum of each patient increased substantially cell apoptosis compared to heathy GW serum (Figure 1A), an effect that was very ameliorated by the addition of anti-anthrax PA63 antibody (Figure 1B). However, the apoptosis was not fully reduced to control level (Figure 1C), due to fact that other vaccine antigens also induced increased apoptosis.[52] These findings suggest that (a) vaccine-related neurotoxic antigens circulate in the blood of veterans with GWI, (b) the presence of these antigens may contribute to systemic GWI symptoms affecting multiple organs, and (c) deleterious effects can be neutralized by serum from healthy GW veterans suggesting promising therapeutics aimed at elimination of the persistent harmful antigens. Interestingly, the connection of GWI with vaccine antigens and the fact that GW women veterans are in poorer health overall than GW male veterans[31,32] are in keeping with the fact that women have more serious and prolonged side effects to vaccines.[67,68]

Finally, it should be noted that our persistent antigen hypothesis (and evidence for it) is not incompatible with an adverse effect of stress, inflammation and toxic exposures in GWI. In fact, as we argued in detail elsewhere,[37] those factors have been shown in various studies[133-141] to break and/or enhance permeability of the blood brain barrier, thus effectively allowing entrance to the brain of harmful persistent antigens. Such BBB breakdown has been specifically described in an animal model of GWI.[133] In that view, the combined effect of toxic exposures, stress and inflammation (of diverse origin) is to make the brain vulnerable by breaking the blood brain barrier and thus enable and/or potentiate the brain damage inflicted by otherwise circulating persistent antigens (eg, anthrax PA63 and other vaccine-related antigens) by allowing their entry to the brain. It should be noted that that mechanism is fairly general and does not apply only to GWI but to other disorders as well, including ME/CFS and long-COVID.

Antigen persistence and HLA

As mentioned above, HLA is essential for eliminating offending antigens shortly after infection (through CD8+ *T* cell cytotoxicity mediated by HLA Class I molecules) and preventing future infection (through CD4+ *T* cell initiation of antibody production by B cells). Assuming immunocompetence along the T cell immune response pathways, the first and crucial step in eliminating an offending antigen is a good match between epitopes of the antigen and HLA molecules. A mismatch with HLA Class I molecules will result in defective cytotoxicity of infected cells mediated by CD8+ *T* cells, whereas a mismatch with HLA Class II molecules will result in defective antibody production initiated by CD4+ *T* cells. Absence of both HLA Class I and II molecules (primary immunodeficiency disorder) is fatal due to overwhelming, mostly viral, infections.[142] (A similarly serious outcome comes from loss of innate immunity, as seen, eg, in agranulocytosis.)
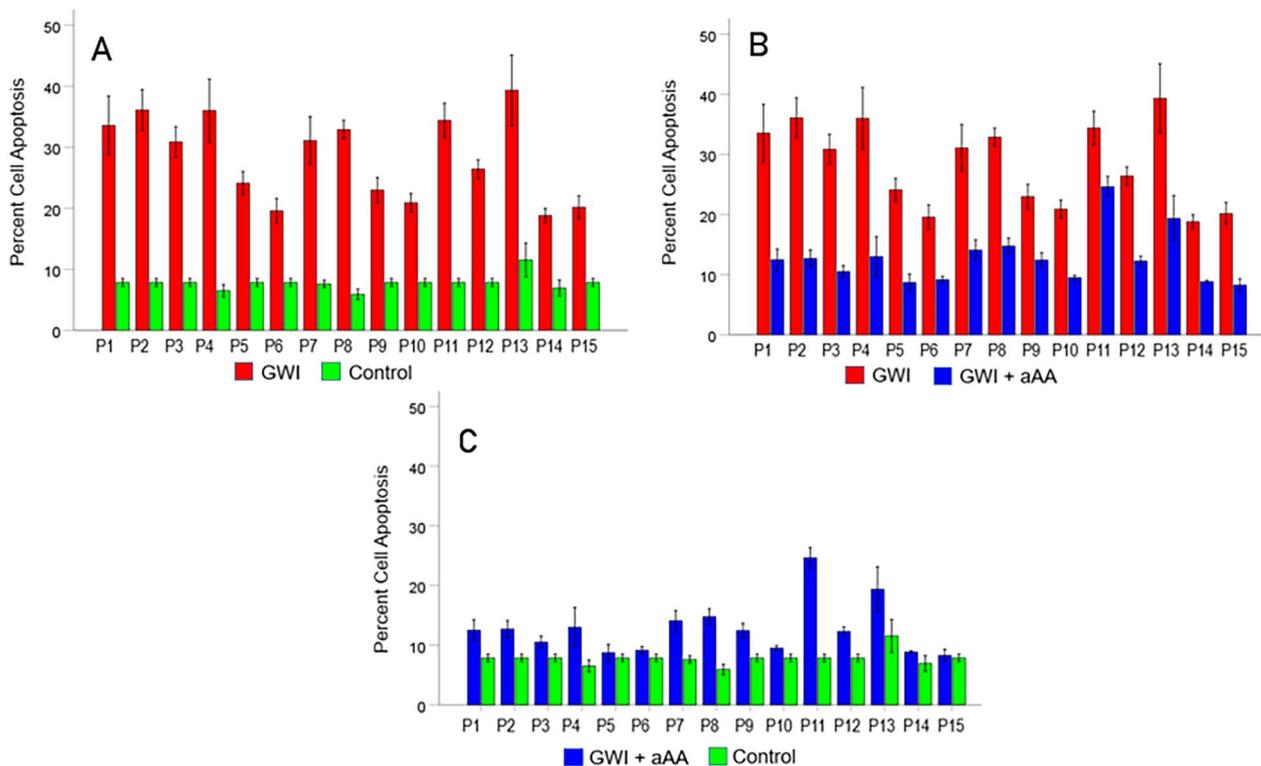
Figure 1. Evidence for the presence of anthrax protective antigen (PA63) in the serum of veterans with GWI. Data are from 15 GWI patients (P1-P15) compared to a healthy GW veteran control. Ordinate is percent apoptosis (cell death) in neuroblastoma N2A cultures following incubation with the stated treatment. (A) much higher percent apoptosis in incubation with each GWI patient serum vs. control. (B) systematic beneficial effect (reduction in apoptosis) in each patient when antibody against anthrax PA63 was added to the patient's serum. (C) apoptosis in GWI + anti anthrax antibody vs. apoptosis in control; the apoptosis in GWI + anti anthrax antibody is still higher than in the control presumably due to the presence of other persistent antigens in GWI.[59] The figure is a composite of figures 12, 13 and 14 in Bai et al.[57]

Long-COVID-19 and HLA

Studies have documented that sex, age, and pre-existing conditions are associated with increased risk of long-COVID.[143,144] Clinical heterogeneity in post-infection outcomes may also be partially driven by variability in host immune-mediated responses including variability in HLA composition. Indeed, HLA has been implicated in COVID course and severity.[145-151] In silico studies have documented Class I[147,148] and Class II HLA[151] alleles with high and low binding affinity to SARS-CoV-2 peptides, presumably resulting in, respectively, enhanced and reduced ability to present viral antigens to immune cells. To our knowledge no studies have documented HLA-associations with long-COVID; however, we have posited that reduced binding affinity due to HLA-antigen incongruence may contribute to persistent COVID antigens and subsequent downstream long-COVID sequelae.[150] Several lines of evidence document immune system disruption that may be associated with HLA including lower levels of certain antibodies soon after infection, suggesting deficient adaptive immune system response,[152] and evidence of SARS-CoV-2 viral antigen in the gut[73] and in several other extrapulmonary tissues including the brain, up to 7 months after symptom onset.[72] Since antigen persistence is known to result in immune system disruption and inflammation, it is possible that the prolonged

inflammation in patients with long-COVID[3,153-155] may be partially attributable to viral antigen persistence.

ME/CFS and HLA

Early, small scale attempts to identify ME/CFS and HLA associations yielded mixed and inconclusive results. However, a recent study on 426 adults suffering from ME/CFS and 4511 healthy controls provided the first clear association of this disorder with specific HLA alleles.[28] All participants were genotyped for the 6 classical HLA genes (Class I: A, B, C; Class II: DBP1, DQB1, DRB1) at high, 4-digit resolution and the HLA composition between patients and controls compared. Two risk alleles were identified, one from HLA Class I (C*07:04) and another from Class II (DQB1*03:03), as well as one protective allele from Class I (B*08:01). This latter allele frequently occurs with 3 other alleles on the haplotype C*07:01-B*08:01-DRB1*03:01-DQB1*02:01, which was also found to be less prevalent among the ME/CFS patients studied, as compared to the controls. Although the emphasis of that study is on the risk alleles, the identification of protective alleles is more interesting from the perspective of our persistent antigen hypothesis, because it is their presence that may be instrumental in eliminating the offending pathogens and thus preventing the disease. In our view, in the absence of those alleles, chronic

inflammation due to the persistence of the offending antigen(s) and accompanying tissue breakdown would trigger autoimmunity (mediated by C*07:04 and/or DQB1*03:03) and perpetuate the symptomatology of the disorder.

GWI and HLA

An intriguing issue is why serum from healthy GW veterans exerts the same neutralizing effects as antibodies against anthrax antigen? We have previously surmised that healthy Gulf War veterans were able to make antibodies against the antigens in vaccines administered to them whereas GWI veterans were unable to do so due to lack of immunogenetic protection, resulting in the persistence of antigens.[1,47] Since antibody production hinges on HLA Class II molecules, we compared HLA composition in GWI veterans and healthy Gulf War veterans and found that veterans with GWI can be distinguished from healthy Gulf War veterans by the lower prevalence of 6 HLA Class II alleles in GWI veterans, indicating lack of protection against GWI[47] (DPB1*01:01, DPB1*06:01, DQB1*02:02, DRB1*01:01, DRB1*13:02). This protective effect was further documented by the finding that the severity of GWI symptoms was negatively associated with the frequency of these alleles such that lower allele frequency was associated with greater symptom severity.[47] The 6 Class II HLA alleles shown to protect against GWI (ie, those that were present in healthy Gulf War veterans but not GWI veterans) were shown to bind with high affinity to antigens from vaccines administered to Gulf War veterans.[156] As such, it was concluded that healthy Gulf War veterans were able to mount a sufficient immune response to vaccine antigens whereas individuals lacking immunogenetic protection against those vaccine antigens are unable to mount a sufficient immune response resulting in antigen persistence.[1]

Downstream effects of antigen persistence include inflammation, autoimmunity, and cell death.[44,81,82] Numerous studies have documented immune system disruption[47,157-159] and altered brain function[36,160] in genetically vulnerable Gulf War veterans.[47] In addition, studies have documented elevated peripheral inflammation in GWI.[161,162] Furthermore, C-reactive protein, a marker of inflammation, is associated with cortical thinning, hippocampal atrophy, and decreased white matter integrity in veterans with GWI.[38,39] With regard to autoimmunity, one-quarter of veterans with GWI are positive for lupus anticoagulant (LAC), an autoantibody against phospholipids or phospholipid-binding proteins that results in formation of blood clots in vivo.[163] Furthermore, LAC-positivity is associated with significant brain atrophy in GWI veterans.[164] Remarkably, the presence of specific Class II HLA has been shown to spare brain atrophy,[165] a characteristic of GWI,[37] and prevent inflammation and cortical thinning.[40] Finally, evidence indicates that brain function in veterans with GWI is indistinguishable from that of classic autoimmune disorders.[166] The latter, coupled with evidence of immune system disruption and a large number of symptoms involving the brain, has led to the conclusion that GWI is most accurately considered a neuroimmune disorder.[166-168]

In summary, persistent harmful antigens do not have to be confined to viruses or other pathogens but they can come from other sources, such as the anthrax vaccine. Interestingly, there is a parallel in the mechanism of antigen persistence that, at least partly, underlies that persistence - namely a diminished (or lack of) protection by the HLA system due to a pathogen effect or genetic makeup. For example, viruses (eg, HIV, HHV, influenza) downregulate the expression of HLA molecules,[169-173] whereas an individual may lack specific HLA molecules possessing high binding affinity to specific harmful antigens (given that 1 carries only 12 classical HLA alleles), therefore becoming vulnerable to those antigens. The latter case is exemplified in GWI where the lack of 6 protective HLA Class II alleles has been documented,[47] among which DRB1*01:01 and DRB1*13:02 have been shown to have very high affinity of binding to anthrax vaccine antigen PA63.[156]

Summary and Implications

Here we briefly reviewed evidence suggesting that 3 distinct chronic conditions (long-COVID, ME/CFS, and GWI) are associated with immune system dysfunction and result in similar symptoms involving multiple organ systems including the brain. While long-COVID is attributable to infection with the SARS-CoV-2 virus, the etiology of GWI and ME/CFS are uncertain though infectious agents have been implicated. We suggest that all 3 chronic conditions are, in part, associated with persistent antigens resulting from HLA-antigen incongruence. We discuss this point further in that what follows.

Why do pathogen antigens persist?

As we reviewed above, pathogenic antigens that persist are at the root of many chronic disease conditions affecting the brain and other organs. A fundamental question then is, why do such antigens persist? Obviously they entered the body, typically as component parts of a pathogen (eg, virus or other microbe), but could not be eradicated from it. Such entrance triggers an immune response at basically 3 different levels, including (a) activation of the innate immune system to mobilize white blood cells (macrophages and polymorphonuclear cells) and demarcate infection by initiating local inflammatory response, (b) engagement of HLA Class I molecules (present in nucleated cells) which bind to small peptide fragments (9-10 AA length) of the cleaved offending protein, and migrate to the cell surface to activate CD8+ cytotoxic lymphocytes which kill the infected cell, and (c) engagement of HLA Class II molecules (present in antigen presenting cells – APC) which bind to longer peptides (12-22 AA length) of the offending protein, and migrate to the cell surface to activate CD4+ lymphocytes which transport the peptide(s) to the B cells and initiate antibody production. This defense scheme aims (a) to immediately

delimit the infection, (b) to kill quickly (apoptosis) the infected cells, and (c) to initiate production of antibodies which will inactivate persisting offending antigens (after a couple of weeks) and prevent reinfection in the future by the same pathogen. All these 3 lines of defense are crucial for dealing successfully with infections, as evidenced by the severe detrimental consequences when these defenses fail, as discussed above.

Between the 2 extremes of a perfectly healthy organism and a severely immunosuppressed one, there lies a wide spectrum of conditions where immune defenses are not at their best and the organism is at relative risk. For example, suppression of white cell formation of various degrees (and consequently low white blood cell count) can occur as a side effect of pharmacotherapy of various diseases, reduction of CD4+ *T* lymphocytes is caused by human immunodeficiency virus, suppression of lymphocyte function can be caused by substances produced in tumor cells, etc. Such cases are collectively labeled as "immunocompromised," in contrast to the term "immunocompetent" denoting a healthy immune system. It is not surprising that antigens can, and usually do, persist in immunocompromised individuals simply because the immune mechanisms to eliminate them are deficient and hence only partially successful in that aspect. However, antigens can persist in fully immunocompetent individuals—but why? There are 2 main reasons for this, not mutually exclusive, namely (a) properties of the pathogen, and (b) genetic makeup of the host. With respect to pathogens, a typical case concerns human herpesviruses (HHV) which notoriously can persist in the body in a latent state for a long time due to various mechanisms. For example, HHV1 invades sensory nerves at the periphery shortly after infection, it is transported retrogradely to the cell soma where it can remain latent for long periods of time, and get reactivated periodically (due to various reasons), in which case it is transported anterogradely to the periphery, exits the axon and infects epithelial cells.[174] In a different "strategy," human betaherpesviruses HHV6A and HHV6 evade the adaptive immune response by downregulating the production of HLA Class I and Class II molecules,[169,170] among other mechanisms.[175] This mechanism targeting the production of HLA molecules is particularly interesting because its effect is essentially the same as the lack of specific HLA molecules for genetic reasons. As mentioned above, an individual carries 6 HLA Class I and 6 Class II classical alleles. Each one of these alleles has a very specific structure, such that it can connect with high affinity only to peptides with amino acid sequence that matches that in the groove of the HLA molecule. Given that specificity, it is not surprising that a given HLA molecule can match with high affinity only to a limited number of peptide structures, ultimately coming from a limited number of pathogens. The vast polymorphism of the HLA system assures the evolutionary benefit conferred to the population but it means little to the individual who carries only 12 HLA alleles. In contrast to the HHV6 downregulation of HLA molecules indiscriminately, the genetic HLA makeup of the individual essentially protects

only against certain pathogens and, conversely, renders the individual susceptible to other pathogens for which HLA specificity is absent (lack of protection). Given the large variety of pathogens (mostly viruses) and the very limited coverage afforded by 12 HLA alleles, it is not surprising that a good number of pathogens may persist after infection simply because of a mismatch of their proteins to the specific set of 12 HLA alleles the individual possesses. In fact, this is the main point of this review, namely that antigen persistence may be attributed, to a major degree, to the lack of HLA molecules suitably specific to match with those antigens. In that sense, in our view, antigen persistence, with all associated manifestations such as chronic inflammation, is due in part to the lack of protection afforded by HLA against those antigens. This is illustrated schematically in Figures 2 and 3. It is noteworthy that lack of HLA protection in GWI has been documented[47] and associated brain mechanisms identified.[160,166] Conversely, it was found that the presence of the GWI-protective HLA allele DRB1*13:02 protects from subcortical brain atrophy in GWI.[165]

Can persistent antigens alone cause disease?

The cell culture studies on GWI reviewed above have demonstrated the presence of vaccine-related persistent antigens in the blood of veterans with GWI 30 years after their enlistment. One question is to what extent the harmful effects of these persistent protein antigens on the N2A neuroblastoma culture are associated with symptom severity in those patients. We found that this is indeed the case [132]; specifically, the severity of neurocognitive-mood GWI symptom was positively associated with apoptosis, an effect that was highly statistically significantly ($r = .837$, $P < .001$).[132] This result validates the in vitro culture testing approach which could be applied to other disease conditions. Interestingly, the possible pathogenicity of pure viral protein fragments (without nucleic acids) was reported recently for SARS-CoV-2[176] where it was shown that intratracheally instillation of the S1 subunit of SARS-CoV-2 spike protein (S1SP) in K18-hACE2 transgenic mice that overexpress human ACE2 and examined signs of COVID-19-associated lung injury 72 hours later. Indeed, mice instilled with S1SP exhibited a decline in body weight, dramatically increased white blood cells and protein concentrations in bronchoalveolar lavage fluid (BALF), upregulation of multiple inflammatory cytokines in BALF and serum, histological evidence of lung injury. In contrast, K18-hACE2 mice that received either saline or SP exhibited little or no evidence of lung injury. This is the first demonstration of a COVID-19-like response by an essential virus-encoded protein by SARS-CoV-2 in vivo and is in keeping with the finding that SARS-CoV-2 spike glycoprotein is proinflammatory.[177] Given the considerations above concerning the potential of persistent protein antigens to produce disease, it would be important to know whether such antigens
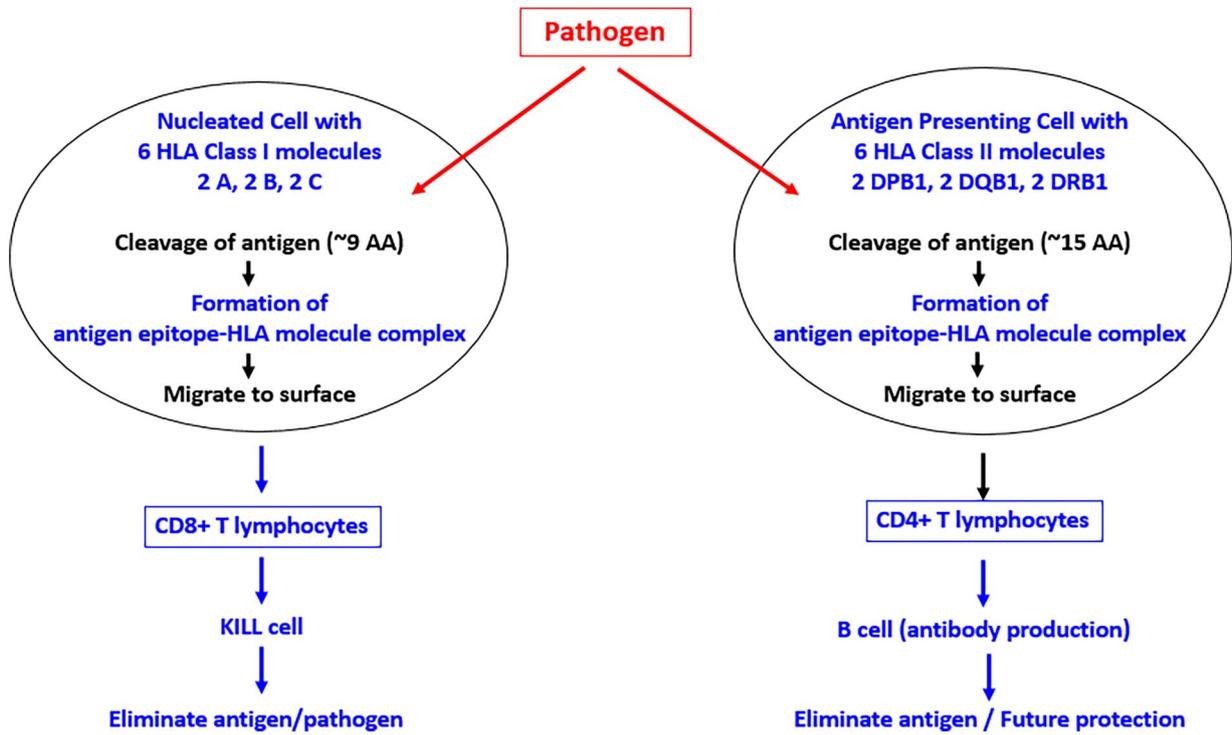
Figure 2. Schematic diagram to illustrate the normal HLA mechanism of antigen processing. See text for details.
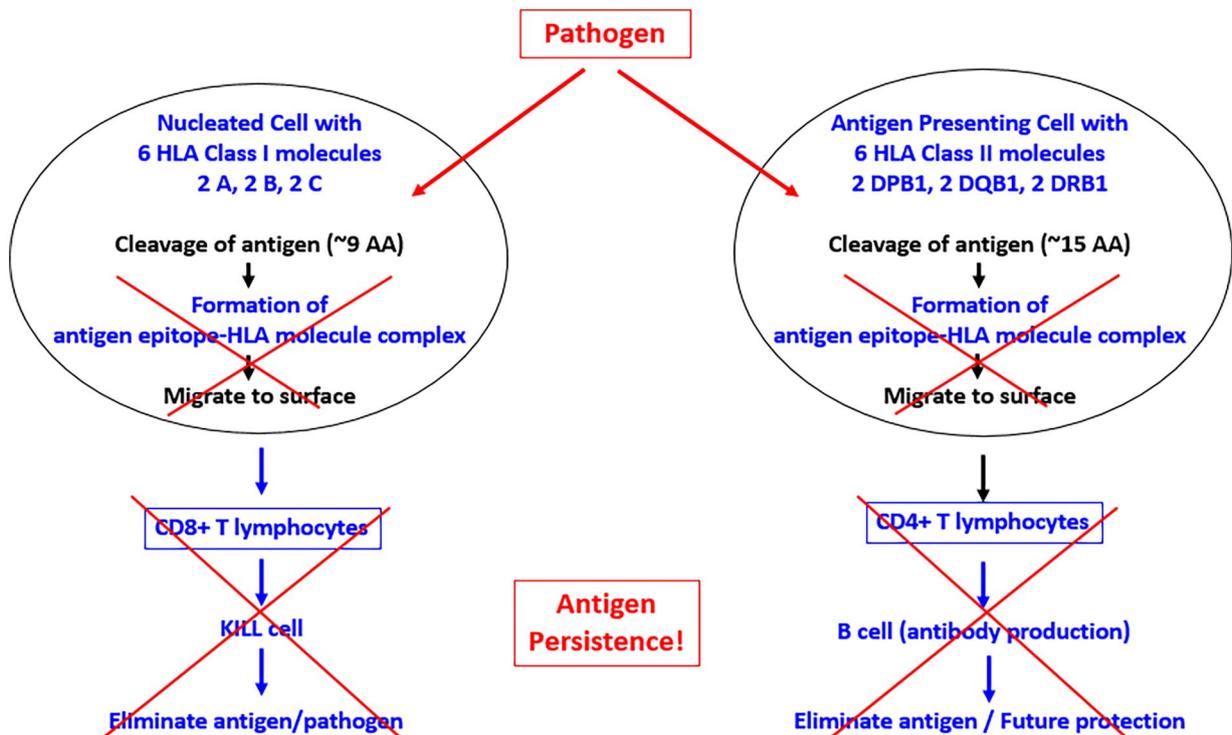
Figure 3. Schematic diagram to illustrate the altered antigen processing in the absence of HLA match. See text for details.

can be transmitted from person-to-person. In this context, it is interesting that a multisystem syndrome resembling GWI has been reported in spouses of veterans with GWI,[178] in a prevalence independent of the deployment status of the GWI spouse. This finding raises the issue of potential transmissibility of GWI persistent antigens.
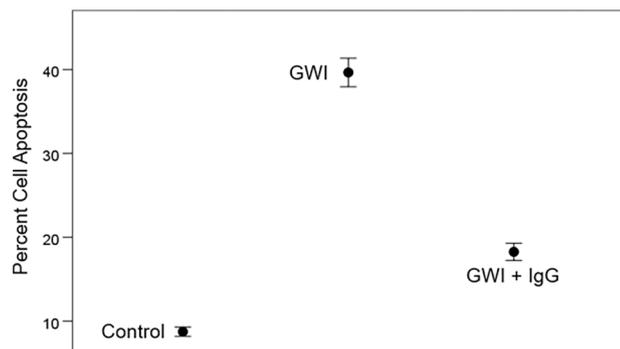
Figure 4. Beneficial but limited effect of added pooled human IgG to GWI serum in reducing apoptosis. The limited effect is presumably due to the expected lack of antibodies in IgG of rare diseases (anthrax, botulism, plague, rabies, etc.) to which the general population is not typically exposed and for which no vaccines are given routinely. Bars are SEM. From Rouse and Kaistha.[63]

Potential interventions

Current treatments for the 3 chronic diseases discussed above are essentially focused on the treatment of symptoms. The ideal treatment would be the eradication of the offending persistent antigens to arrest the ongoing damage and prevent future damage. (a) With respect to long-COVID-19, the obvious objective is to eradicate the SARS-CoV-2 virus from the body, a feat waiting to be accomplished. (b) With respect to ME/CFS, the pathogenic persistent antigens are suspected but unknown and, therefore, no radical intervention at removing them is possible. Interestingly, an early attempt at intervention by administering pooled human IgG did not have a beneficial effect.[179] (b) With respect to GWI, the case is different for GWI because persistent antigens have been identified circulating in the blood of GWI patients.[50-52] Theoretically, these antigens could be eliminated by administering specific antibodies but this is infeasible, especially since these antigens include the anthrax PA63 antigen. The addition of pooled IgG to the N2A culture did reduce the apoptosis induced by GWI serum but did not fully eliminate it (Figure 4). This was expected since pooled IgG from the general population is not likely to contain antibodies against anthrax, botulinum toxin, plague, and, perhaps, against cholera, yellow fever and rabies,[52] that is for rare diseases for which no routine vaccines are given. Nevertheless, there is a potentially beneficial intervention aiming at the removal of those antigens from the blood, namely plasma exchange. Since disease symptoms are likely due to chronic inflammation (induced and maintained by the persistent antigens) and associated immune reaction(s) (eg, release of cytokines), it is reasonable to expect that the removal of the offending antigens would be beneficial in ameliorating the symptoms and reduce the persistent antigen load. As mentioned above, plasma exchange would be most beneficial in GWI, where, for example, the harmful anthrax antigen was introduced and persisted because of genetic HLA vulnerability but which did not replicate. On the other hand, this procedure

would be only temporarily beneficial in cases where a virus becomes latent by "hiding," as is the case of HHV1 which invades nerve cells and does not circulate in the absence of overt infection. Therapeutic Plasma Exchange is a relatively safe procedure, which is performed in certain diseases, notably in myasthenia gravis,[180] where it effectively clears the blood from circulating autoantibodies against the neuromuscular junction.[181] It is an invasive and expensive procedure, and with potential serious side effects, as reported in early studies.[182,183] However, recent applications of TPE in severe COVID-19 cases has proved beneficial and without significant side effects[184-188] probably due to improvements in the procedure (eg, intravenous calcium replacement and administration of chlorpheniramine during TPE to reduce side effects).[188]

Author Contributions
APG and LMJ contributed equally to writing and editing the paper.

Significance Statement
In this review we highlight the influence of persistent antigens and lack of human leukocyte antigen protection as contributing to three conditions characterized by persistent long-term symptoms affecting the brain and other systems.

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