

Aluminum's Role in CNS-immune System Interactions leading to Neurological Disorders

Shaw CA^{1,2,3*}, Kette SD⁴, Davidson RM⁵ and Seneff S⁶

¹Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, 828 W. 10th Ave., Vancouver, British Columbia, V5Z1L8, Canada

²Program Experimental Medicine, University of British Columbia, Vancouver, V5Z1L8, Canada

³Program in Neurosciences, University of British Columbia, Vancouver, V5Z1L8, Canada

⁴Independent researcher, Hudson, FL 34667, USA

⁵Internal Medicine Group Practice, PhyNet, Inc., 4002 Technology Center, Longview, TX 75605, USA

⁶MIT Computer Science and Artificial Intelligence Laboratory, 32 Vassar Street, Cambridge, MA 02139, USA

Abstract

Multisystem interactions are well established in neurological disorders, in spite of conventional views that only the central nervous system (CNS) is impacted. We review evidence for mutual interactions between the immune and nervous systems and show how these seem to be implicated in the origin and progression of nervous system disorders. Well-established immune system triggers leading to autoimmune reactions are considered. Of these, aluminum, a known neurotoxicant, may be of particular importance. We have demonstrated elsewhere that aluminum has the potential to induce damage at a range of levels in the CNS leading to neuronal death, circuit malfunction and ultimately, system failure. Aluminum is widely used as an adjuvant in various vaccine formulations and has been implicated in a multisystem disorder termed "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA). The implications of aluminum-induced ASIA in some disorders of the CNS are considered. We propose a unified theory capturing a progression from a local response to a systemic response initiated by disruption of water-based interfaces of exposed cells.

Keywords: Aluminium; CNS; Neurological disorders; Autoimmunity

Introduction

One characteristic of conventional reductionist approaches in the biological sciences is that various systems tend to be viewed in isolation from each other. That this occurs generally is not really in dispute, but the impact of such an approach often obscures relationships that almost certainly would prove seminal to a clearer understanding of various disease states. Examples from the neurological disease literature abound. For example, Lou Gehrig's disease (ALS), Parkinson's disease and Alzheimer's disease are frequently viewed as totally unrelated and completely distinct from each other, even though there are extremely clear cases that prove the opposite: ALS-parkinsonism dementia complex (ALS-PDC) of Guam and the Western Pacific often combines the features of all, albeit with the ALS phenotype usually preceding the loss of neurons in other CNS fields [1]. Parkinson's disease and ALS frequently feature aspects of Alzheimer's like dementia [2-5]; fronto-temporal dementia can have motor neuron loss, etc., as part of the long-term spectrum of disease expression [6]. The risk of ALS is significantly increased in people who suffer from asthma, celiac disease, early diabetes, multiple sclerosis, myasthenia gravis, hypothyroidism, Sjögren's syndrome, systemic lupus erythematosus (SLE) and ulcerative colitis [7].

Even within a particular disorder, entirely different organ systems may be involved. In ALS, patients often exhibit changes in skin characteristics, in addition to motor neuron losses, features that have been known since Charcot's seminal work in 1880 [8]. A series of studies by Japanese investigators have examined in detail the possible links between changes in dermis and epidermis and motor neuron loss in ALS [9,10]. Western scientists have generally ignored these data, in spite of the obvious linkage provided by embryology that both systems are ectodermal in origin [11-14]. These skin changes can even be demonstrated in animal models of ALS [15].

Other organ disorders can also be features of Parkinson's, Alzheimer's and ALS-PDC. The extent of central nervous system

(CNS) involvement of clearly multisystem disorders, chronic fatigue syndrome, Gulf War Illness, etc. shows just how widespread such multisystem effects may be [16-18].

Nowhere is the possible link more obvious than in disorders that involve both the immune system and the nervous system, and we will argue in this paper that more than their just being juxtaposed in the same disorder, there are powerful interactions between natural immune and abnormal autoimmune functions and normal development and pathologies, respectively, of the CNS. Moreover, we will argue that some common triggers of autoimmunity may be key contributors to neurological disease by direct toxic actions, as well as indirectly *via* autoimmune responses. In particular, we will focus on the clear multi-level multisystem toxicant role of aluminum (Al) [19].

Evidence for CNS-immune interactions

A recent review by Besedovsky and A del Rey [20] describes in detail how immune/CNS interactions may occur through the release of various cytokines. Cytokine release in the periphery can directly impact neurons in the CNS by binding to a range of cytokine receptors on neural cells, resulting in changes in neuronal activity. The relationship is reciprocal, such that cytokine release from neural cells of all types can serve signaling functions to immune cells outside the CNS [21].

***Corresponding author:** Shaw CA, Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, Research Pavilion, 828 W. 10th Ave., Vancouver, British Columbia, V5Z1L8, Canada, E-mail: cashawlab@gmail.com

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During CNS development, cytokines released in the periphery are thought to shape neuronal circuitry and function [22]. Such impacts of the immune system have been linked to both normal and abnormal CNS development [19], in the latter case to autism spectrum disorder (ASD). Such stimulation could arise naturally by way of immune system activation by various pathogens, or by iatrogenic immune activation through vaccination.

Autoimmunity in neurological disease

Although space precludes a full description of the literature, there is now abundant evidence for an autoimmune component to the classical age-related neurological diseases, including ALS [23-27], Alzheimer's [25,28-32] and Parkinson's diseases [28]. It remains, however, uncertain whether the immune markers found in affected regions of the CNS are causal or secondary to the resulting loss of neurons [23-32]. This same consideration applies to the typical presence of activated microglia at the site of most CNS lesions and whether the neuroinflammatory response is primary or secondary to neuronal degeneration. In the case of microglia, the situation is doubtlessly complicated by microglia's dual roles as neuroprotective cells or scavengers, a role that depends on a range of other factors [21].

Multiple sclerosis and gut bacteria

There has been a recent surge in interest in the concept of gut bacterial dysbiosis as a mediator of autoimmune disease [33]. Multiple sclerosis (MS), an inflammatory disease that leads to demyelination in the CNS, is mediated by autoreactive T cells that become antigenic towards myelin [34]. The immune cell attack on myelin leads to altered axonal conductance and the slowing or failure of neuronal signaling [35]. It has been proposed that the autoimmunity in MS might arise out of molecular mimicry from a pathogenic protein with sequence homology to peptide sequences in myelin [36]. However, extensive search has not yet produced a candidate pathogen for MS. A recent study searched a database of reported sequences from all known human bacterial and viral agents for possible matches to three established encephalitogenic peptides. Intriguingly, mimics were detected for several bacteria that are ordinarily benign residents in the gut [37]. These data may suggest that a leaky gut syndrome, in conjunction with distressed microbiota, may lead to MS via antigenic exposure to DNA debris from common gut bacteria.

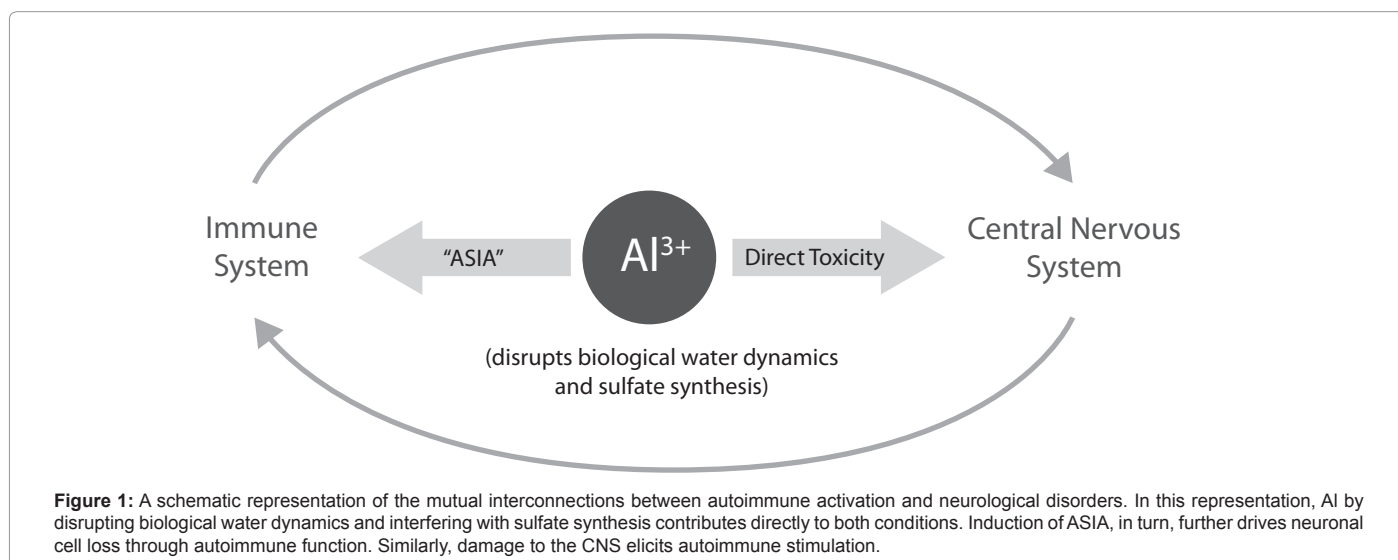
Guillain-Barre disease is a disorder involving the Schwann cell myelin sheath of peripheral nerves. As the nerves become progressively demyelinated, neural conductance may slow and then cease. A now large literature suggests that the overall mechanisms of action are autoimmune in nature. In the case of Guillain-Barre, a well-known trigger appears to be vaccination [38-40]. If correct, Guillain-Barre would be part of the spectrum of disorders, now termed "autoimmune/autoinflammatory syndrome induced by adjuvants" or "ASIA".

Autoimmune/autoinflammatory syndrome induced by adjuvants (ASIA)

Shoenfeld et al. [41,42] reviewed the large body of evidence that clearly demonstrates adjuvant administration preceding the onset of immune-mediated diseases, including siliconosis, Gulf war syndrome and a rapidly emerging entity termed macrophagic myofasciitis (MMF) (Figure 1) [43]. Collectively, these illnesses present similar clinical features, which are now designated being part of the ASIA syndrome. Many of these appear to arise due to the use of Al adjuvants, e.g. MMF in humans [43]. Similar outcomes have been reported in sheep also following Al adjuvant exposure from vaccines. Concerning the latter, Lujan et al. reproduced an autoinflammatory illness experimentally among sheep immunized against blue tongue and showed that Al was present in the CNS of affected animals. Notably, the impact of the adjuvant Al was more severe in winter months, suggesting an interaction with other stress factors.

Other vaccine adjuvants appear capable of inducing autoimmune reactions in humans, as well. Nohynek et al. provided evidence of a significant increase in adolescent narcolepsy in Finland, following vaccination with a lipid-based adjuvant in the H1N1 influenza vaccine [44,45], data that have now apparently been reproduced in several other northern European countries. Whether these outcomes truly reflect negative impacts of the particular adjuvant on the CNS or whether other components of the vaccine alone or in combination with the adjuvant were responsible, remains uncertain. As above with the Lujan et al. results, it may be notable that such impacts occurred during winter months.

Shoenfeld et al. [41,42] have also demonstrated that a variety of other compounds apart from vaccine adjuvants are also capable of inducing ASIA syndrome.



Aluminum adjuvants: History of use and impact on CNS structure and function

Aluminum has been used in vaccine formulations since 1926 after the discovery that it potentiates the immune response to the target pathogen [46,47]. Perceptions of Al safety that abound in the medical literature are largely based upon a lack of recognized adverse events over the past 70 years [48], rather than randomized, true-placebo-controlled clinical trials, or the now abundant experimental animal literature [49]. A meaningful conclusion that unlimited use of Al is safe in vaccines cannot be made. Adverse events are significantly under-reported, and physician bias often influences the reporting process. Quite often, the requisite inquiry as to whether a vaccination preceded an acute illness is not asked. Autoimmune reactions to aluminum in vaccines are not of sufficient frequency to facilitate prospective randomized control trials. Causation is difficult to establish in general, when so many factors could be in play, although the use of the Hill criteria certainly helps the process of sifting causality from coincidence [49,50]. Some researchers have opined that the latency period of autoimmune disease makes it difficult to infer causation retrospectively, but this may not be a valid critique, since there is still a clear sequence of events from presumed causal factor to disease outcome.

Al adjuvants are used in childhood vaccines against diphtheria, tetanus, pertussis, hepatitis B, anthrax, *Haemophilus influenzae* and human papilloma virus, amongst others [48,51]. A child may be injected with as much as 4.225 mg of elemental Al by the age of 12 months [52]. Our review of currently licensed vaccine package inserts in the United States is consistent with this figure. Mitkus et al. [52] reported that this dosage is within the U.S. Agency for Toxic Substances and Disease Registry's minimum risk levels for infants, extrapolating data from a volunteer study of adults using radioactive aluminum tracer [53], and a toxic autokinetic study performed on rabbits [54]. Mitkus et al. [52] used the creatinine clearance differential between children and adults to estimate total Al body burden of infants following vaccination. The estimation is based upon an assumption that Al excretion parallels creatinine clearance, an assumption that is unlikely to be correct either on theoretical or experimental grounds. In the first instance, rapid excretion of Al would nullify the very basis of having it as an adjuvant in the first place. Experimentally, the notion that Al adjuvants are rapidly excreted is challenged by the recent work of Khan et al. [55].

There is a growing body of data to suggest that Al is *biosequestered* by albumin, transferrin and macrophages of the reticuloendothelial system after intramuscular injection. According to Ganrot [56], insoluble metal hydroxides are thought to mainly be taken up by the reticuloendothelial cells, while soluble salts of the trivalent ions are mainly bound to the skeleton or excreted in the urine. Ubiquitous heparan sulfate proteoglycans (HSPGs), which decorate the glycocalyxes of our cells membranes, are likely to act as multi-dentate chelators--biosequestrants--of Al [57-59]. Moreover, "cationized" bovine serum albumin (cBSA) and "cationized" human serum albumin (cHSA) have long been known to have enhanced endocytic uptake via adsorptive transcytosis by the blood brain barrier [60-62]. cBSA has been found to be present in subepithelial immune deposits in children with idiopathic membranous glomerulonephropathy [63]. The Flarend rabbit study [54] showed that absorption following intramuscular Al particulate injections into the blood is not instantaneous, and only some of the Al was absorbed from the injection depot over the first 28 days. These data are supported by the recent study by Khan et al. [55] suggesting that the initial trajectory for Al hydroxide is into the lymphatic system. There has been a concerted effort to reduce the Al burden in parenteral

feedings to premature infants due to the observation that 4-5 µg/kg per day of Al can induce neurodevelopmental delays [64]. In spite of this, there seems not to be an equal or adequate concern about the potential risks of injected Al whose clearance from the CNS may be extremely slow [55]. The overall impact of Al used as an adjuvant in vaccines has been addressed in detail elsewhere [51]. In addition, these same authors have provided some evidence for a causal role in ASD based on anecological study of US government databases [65].

Outline of the article

In the remainder of this paper, we will develop what we believe to be a novel proposal for an inflammation cascade subsequent to exposure of tissues to Al and other neurotoxicants. Briefly, the cascade can be outlined as follows:

(a) Aluminum disrupts water-based cellular homeostasis and causes a crisis for the exposed cell.

(b) The cell sends out "death alarm" messages, which draw in macrophages and other immune cells, initiating an inflammatory cascade.

(c) The highly stressed cell dies *via* necrosis rather than a "programmed cell death," and releases its DNA into the interstitial tissues.

(d) This extracellular DNA is picked up as an antigenic signal by immune cells and leads directly to autoimmune disease.

(e) In parallel, sulfate synthesis and sulfate transport are disrupted due in part to Al contamination of the pineal gland and other sensitive nuclei in the midbrain.

(f) The entire biological system switches from a sulfate-based to a phosphate-based management strategy for maintaining water interfaces, leading to hyperparathyroidism.

In the following three sections, we will introduce the three principle components of this cascade, the local disruption of cellular homeostasis, the systemic cascade response leading to widespread sulfate deficiency, and the calcium-signaling-based switch from sulfate to phosphate as an anionic buffering solution. We first briefly review in Section 4 the literature on the biophysical role of water in biological systems, emphasizing how this role gets disrupted by Al. Section 5 will address the systems level cascade response to such disruption, leading to impairments in the supply of biosulfates to the tissues, systemically. We will discuss various disease manifestations of this impairment and propose an essential role for the pineal gland. Section 6 describes the final stage of the cascade when calcium phosphate based signaling cascades launched by a hyperactive parathyroid gland replace magnesium sulfate for the role of buffering water and maintaining its homeostasis in the cells, in the vasculature and in the tissues.

Section 7 will provide some specific examples from the literature of various diseases and conditions that we think also fit the model, we are proposing here. The Discussion will review the sequence of events and summarize our main findings and conclusions.

A Biophysically Based Pathway to Immune Dysfunction and Autoimmune Disease (Section 4)

There is a vast and growing literature on the special physical properties of water, and we have selected for the brief review here only some of the most compelling papers on this subject.

Biological water is an active participant

It is well established that water is essential to life. However, it is the unique biophysical properties of water that make it essential. It is becoming increasingly clear that water is an active participant in most biochemical reactions, rather than simply the medium in which the reaction takes place. Sulfates are members of a distinguished class of molecules-kosmotropes-which have the property that they order neighboring water molecules into a dynamically-structured arrangement that is far more viscous than the bulk water (variously referred to as the "exclusion zone" or the "coherence domain"), and that also exhibits other unusual properties with respect to responses to electromagnetic fields, exclusion of solutes and the mobility of protons and electrons [66]. The interface between this dynamically structured water and the bulk water has interesting physical properties, as do the dynamically-structured water itself, and biological systems almost certainly exploit these properties to energize their reactions. There is not space here to provide anything other than a brief overview of this vast topic.

In 1987, Bak et al. [67] showed that dynamical systems with spatial degrees of freedom naturally evolve into a self-organized/ordered critical structure—a metastable state—a state which is barely stable. Such systems often, but perhaps not always, demonstrate power-law behavior over vastly different time scales [67]. Biological water dynamics fits the criteria for such self-ordered/self-assembling systems in that it demonstrates the combination of dynamical minimal stability and spatial scaling predicted to lead to a power law for temporal fluctuations [68-70].

A novel hypothesis for dynamically-structured water at the interphase

In the remainder of Section 4, we will incorporate by reference and expand upon the data reviewed in two recent review articles [71,72]. We will briefly present a novel hypothesis in which the cumulative disruption of dynamically-structured biological water at the interphase [73] of neurolemmal membranes, induced by certain *polycationic* inorganic surfactants, e.g. various Al^{3+} species eventually exceeds a critical threshold, resulting in loss of macromolecular recognition, immune dysfunction and autoimmune disease.

Nanoclusters of biological water at the interphase are thought to represent clusters of minimally-stable states, which are defined dynamically as the spatial regions over which small local perturbations, e.g. induced by exogenous interfacial water stressors, such as Al^{3+} , will propagate. The neurotoxicity of Al^{3+} begins with the disruption of hydrogen-bond cooperativity and quantum coherence of water at the interphase of neurolemmal membranes, which consequently exceeds the threshold of self-ordered criticality necessary to maintain membrane potentials and action potentials [71,72]. The minimally stable states of interphase water at neurolemmal membranes are upset by the "noise" or "turbulence" propagated through the scaling clusters by means of a "domino" effect. Al^{3+} is thought to induce long-wavelength perturbations, which cause a cascade of energy dissipation on all length scales. Nanoclusters of water and ensembles of coherence domains comprise the "clusters" of minimally-stable states, which can be defined dynamically as spatial regions over which a small local, long-wavelength perturbation, e.g. induced by an exogenous interfacial water stressor, such as Al^{3+} , will propagate.

Many researchers have long sought data to show that the brain operates at a critical state to benefit from the maximal dynamic range

of processing, fidelity of information transmission, coherence between multiple "nested" biosemiotic levels [19] and information capacity [74-80]. A very appropriate marker of criticality may prove to be the percolation transition of interphase water at neurolemmal membranes, e.g. at the interphase of neuronal myelin [81,82]. Experimental studies of the conductivity of hydrated biosystems provide direct evidence for the formation of a spanning network of hydration water *via* the percolation transition [83]. The percolation transition and charge transfer of water may play crucial roles in biological function [71]. Several instances have been reported where the percolation transition of water occurs at the hydration level where various forms of biological activity develop. Based on percolation theory, the percolation transition of water at the interphase of myelin is likely to be the point at which neurological conductivity of charge occurs [82,83], with similar albeit shorter range conductivity occurring with unmyelinated axons.

Water dynamically couples the neuronal network to the environment

A widely-held orthodox view of the etiology of immune dysfunction and autoimmune disease is that a combination of environmental, genetic and immunological factors may play roles in their pathogenesis. Today, environmental exposures, molecular mimicry and genetic predisposition [84,85] are frequently invoked etiologies. By itself, however, genetic reductionism utterly fails to explain most of the autoimmune diseases of today, including those for neurodevelopment disorders, such as ASD. Similarly, most age-dependent neurological disorders as cited in the Introduction cannot be reduced to gene mutations, in spite of prolonged efforts to do so.

In regard to ASD, Vargas et al. [86] published evidence of innate immune cell activation in brain tissue of autism patients; in particular, activated glial cells were identified microscopically, indicating that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients. Of note, the Vargas microscopic data appears to provide support for a much earlier study by Gallez and Coakley [87], who demonstrated that interfacial instability at cell membranes accompanied cell "*activation*." Specifically, the average number of waves per wavy cell rim "...decreased when cell surface charge was depleted, when polyvalent cations were in the suspending phase, and when cationic drugs were present and increased in the presence of anionic drugs".

According to our novel alternative biophysical view of the etiology of autoimmune disease, various Al^{3+} species cumulatively induce exogenous interfacial water stress (EIWS) [71], which causes:

- (a) Immune cell activation, phagocytic activity, inflammatory cytokine release;
- (b) Decrease in neurolemmal membrane potentials and failure of action potentials [88,89];
- (c) Loss of macromolecular recognition [68];
- (d) Loss of proton and charge conduction of neurolemmal membranes [90];
- (e) Unfolded DNA Response (UDR) [72];
- (f) Unfolded Protein Response (UPR) [72];
- (g) Thrombohemorrhagic phenomena [91];
- (h) Loss of self-ordered criticality [74,76,77].

The self-ordered criticality of biological water

Branching cascades of neuronal network activity have been likened to chain reactions and avalanches, such as those seen in events like earthquakes, forest fires, landslides, power grid collapses and nuclear chain reactions. In 2003, Beggs and Plenz [75] showed that *in vitro* propagation of spontaneous activity in cortical networks obeys a power law, and is described by equations that govern avalanches [62]. They proposed that these so-called "neuronal avalanches" may represent new modes of neuronal network activity [74-76], which differ profoundly from oscillatory, synchronized or wave-like network states. They further proposed that *in the critical state*, the branching network may satisfy competing demands of information transmission and network stability [75]. Previously, Paczusi et al. [78] showed that the spatial and temporal distribution of similar cascades or avalanches were well-described by power laws. The power law dependency indicates that the systems are in a critical state [67], and that the dynamics can be seen at many different scales [78]. Today, it seems clear that actual neuronal networks display critical behavior, and that criticality is a robust feature of neuronal organization. The percolation transition of biological water at the interphase of neurolemmal membranes is, in our opinion, very likely to be the minimum requirement for a neuronal system to show criticality.

Neuronal networks are thought today to be dynamically-coupled to their environment [77]. Biological water at the interphase of neurolemmal membranes is the likely mediator of the dynamical coupling between neuronal networks and their environment. Dissipative structures are not true organizational systems [77]. Al^{3+} directly impairs self-ordered criticality of biological water dynamics and increases entropy [71]. According to Taylor et al. [74], a brain at or near criticality would have maximum dynamic range, enabling it to react and adapt to the dynamics of the surrounding environment and maintain balanced neuronal activity [74]. Quantum coherent, cooperatively hydrogen-bonded, nanoclusters of water at the interphase of biological membranes necessary for the percolation transition of neurolemmal membranes. Dynamically-structured water at the interphase is essential in (a) capturing and transducing extremely low frequency EM energy from the environment, (b) dynamically-coupling the neuronal network to the environment, and (c) maintaining the network in a metastable critical native state. Myelin is endowed with sulfoglycolipids such as sulfatide and HSPGs, which are essential in generating current and separating charge. Myelin lipids and proteins demonstrate surface fractality over many scales [69,81].

The point of criticality occurs at the percolation transition of interphase water at neurolemmal membranes. The detailed spatial and temporal embedding may be found in the ultrafast electron crystallography of interfacial water by Pal and Zewail [68], where it was found that macromolecular recognition is dependent on biological water dynamics in the 20-40 picosecond range [68,82]. Loss of macromolecular recognition would logically be expected to precede molecular mimicry, immune dysfunction and the onset of autoimmune disease. Neuropathological states can thus be conceptualized as the breakdown of, or deviation from, the metastable critical state of biological water dynamics at the interphase of neuronal membranes.

The notion has been proposed that the brain may self-organize to a critical state [63]. A new marker of criticality, which may have considerable utility in the neurosciences, is the self-ordered criticality [77] of biological water dynamics at the interphase of neuronal membranes. Support for this proposal is found in the work

of Johansson and Sukhotskaya [69], who showed that self-organized water demonstrates an allometric power law scaling.

Pioneering studies with implications for neuroimmune disease

Inoue et al. [88] and Ueda et al. [89] conducted a series of experiments, which suggested remarkably that water, properly maintained, can contain solutes and hold cellular resting potentials even in the absence of a plasma membrane. A complex coacervate of protoplasmic droplets obtained from *Nitella* cells were shown to have an interfacial tension of 0.04 dyne/cm. These protoplasmic droplets not only exhibited an inside-negative resting potential of from -70 to -90 mV, equal to those seen in many normal excitable living cells, but they were also electrically excitable, generating an action potential in response to a short pulse of electric current. According to Ling [92], in his polarized multilayer (PML) theory of cell water (including his subsidiary hypothesis of coacervation), coacervates have exceedingly low interfacial tension because the coacervate surface contains a great deal of water, albeit polarized and oriented in parallel arrays.

Ling [92] cites the low interfacial tension of living sea-urchin eggs (0.08 dyne/cm or even lower), of *Nitella* endoplasm droplets (0.04 dyne/cm), and of gelatin-gum Arabic coacervate (0.0023 dyne/cm), which when viewed in toto, strongly suggest that the living cell membrane is "just like the bulk-phase protoplasm comprising in the main fully-extended proteins and multilayers of polarized-oriented water". With increasing temperature, Ueda et al. [89] showed that the interfacial tension of the protoplasmic droplets isolated from *Nitella* cells decreased discontinuously from 10 dynes/cm to the order of 10^{-4} dynes/cm at about 34°C. These changes were reversible. Ueda et al. [89] also observed that the addition of multiply-charged inorganic cations in the test solution led to an abrupt depolarization of the membrane potential at a definite concentration for each ion species, wherein the critical salt concentration was inversely-related and strongly dependent on the valence of the cations (Th^{4+} , La^{3+} , Ba^{2+} , Sr^{2+} , Na^{+}) added. When the drop was allowed to stand for 10 minutes in the depolarized state, for example, in a 10 mM solution of various polycationic inorganic salts (UO_2^{2+} , Fe^{3+} , Hg^{2+} , Cu^{2+} , Cd^{2+} , Ag^{+} , etc.), the protoplasmic "streaming" movement in the drop was suppressed and led to an irreversible change of the drop, an unambiguous sign of toxicity. In 1991, Tsuchiya et al. [93] noted that interactions of actin and myosin molecules participate in generation of the motive force for protoplasmic streaming. If these experiments were repeated with Al^{3+} , it seems likely that similar depolarization of the membrane potential and loss of motive force for streaming would be observed. Supportive evidence from plants comes from the observation that Al depolymerizes microtubules and depolarizes the membrane in root cells of intact Arabidopsis seedlings [94].

The aforementioned studies have clinical ramifications for toxicant exposures of mammalian species, whose neuronal motility is intrinsic to the formation of the central and peripheral nervous systems during development [95]. Herein, lies in substantial measure, the clinical relevance of the preclinical electrophysiology research by Ling and others. Similar inorganic polycationic surfactants to Al^{3+} with high charge-densities are also toxicants under the definition of the National Cancer Institute. The novel hypothesis presented in this section gives a short overview of how such metal ions synergistically and cumulatively induce inflammation, immune dysfunction, autoimmunity and cancer.

Taken together, the aforementioned research of Ling, Inoue, and Ueda suggests that coacervation and phase transitions in aqueous heterogeneous media may provide much of the physical basis for

water-driven, coherent, dynamical, multiscale, cellular self-assembly, self-ordering and biosequestration, which enables the generation and maintenance of the membrane potential and action potential in the neurological tissue of mammals, including humans.

Evidence presented here and elsewhere [71], suggests that a metastable self-ordered critical state of neural tissue ensues once a certain threshold of hydration occurs in a relatively “dry” environment, such as that found sheltered within the blood-brain and blood-cord barrier [83]. As will become apparent later in this paper, we believe that biosulfates play a critical role in maintaining this healthy state. The 3D percolation transition of interfacial water within the interphase of aqueous myelin is predicted to be the threshold of criticality within neural tissue, throughout the entire human nervous system, both central and peripheral.

In summary, the hypothesis outlined herein, is not *per se* incompatible with the widely held conventional view of the etiology of immune dysfunction, and autoimmune disease being a result of molecular mimicry and genetic predisposition. However, it differs substantively in the view towards the role of biological water in the disease process. This distinction is not insignificant, and it should be said that the distinction has a large and *rapidly* growing published physical basis.

A Systems Biology Pathway to Immune Dysfunction and Autoimmune Disease (Section 5)

We believe, based on the observed effects of Al-containing vaccines and in consideration of the known biophysical and biochemical properties of Al, that one of the most devastating consequences of exposure to the ions or complexes of this element in certain cell types is a near-permanent switch from sulfate synthesis to nitrate synthesis. This switch will have systemic consequences, as discussed below. There is not sufficient space here to cover all the details.

The affected cell types are those that contain nitric oxide synthase (NOS), which include epithelial cells [96], endothelial cells in the vasculature [97], red blood cells (RBCs) [98], skeletal muscle cells [99,100] and neurons [101]. NOS play an important role in the pathophysiology of many diseases and conditions, such as the metabolic syndrome and cardiovascular disease [102-104]. Endothelial NOS (eNOS) is found in epithelial and endothelial cells, RBCs and muscle cells, whereas neuronal NOS (nNOS) is present in muscle cells and neurons.

There is a large literature on eNOS [105-109], with respect to complex regulation of its synthesis of nitric oxide (NO), a signaling gas that regulates vascular tone. It has recently been proposed that eNOS is a “moonlighting” [110] enzyme, which synthesizes sulfate when it is attached to the membrane at caveolae upon sunlight exposure and synthesizes nitrate when it is free in the cytoplasm, serine-phosphorylated and activated by calmodulin following calcium binding [105,111]. Seneff et al. [111] argued that, with a single enzyme synthesizing both sulfate and nitrate, the cell can exercise tight control over titration between excess presence of kosmotropes (structure making molecules) or chaotropes (structure breaking molecules) [112] in the blood, as it is essential to keep these two influences in perfect balance.

Since sulfate is a kosmotrope and nitrate is a chaotrope, tight regulatory control over the synthesis of these two molecules can restore balance when other circulating molecules such as Al, disrupt it, which is a strong cationic kosmotrope. Al induces iNOS synthesis in the

cerebellum in rats [113], an effect that is not potentiated by iron. We hypothesize that it is due to Al's kosmotropic properties, which require balancing through immediate and intense production of the chaotrope, nitrate.

In the remainder of this section, we will first briefly review the argument that NOS produces sulfate when it is attached at the plasma membrane. We will discuss the important need for sulfate in maintaining levels of cholesterol sulfate and heparan sulfate in many tissues in the body. We will follow this with a discussion of how Al, both through its direct ability to mimic calcium and the ability of aluminum-fluoride complexes (AlF_x) to mimic phosphate, induces NOS to switch from sulfate to nitrate synthesis, while simultaneously inducing many other metabolic adjustments in the cell.

Sulfate synthesis by nitric oxide synthases

It has been very well established that the NOS isoforms synthesize NO, an important signaling gas, which is oxidized within a few seconds to nitrite and nitrate [114]. In the case of RBCs, this presents a puzzle [98], because hemoglobin is a potent NO scavenger and nitrosylation of hemoglobin, similar to the effect of carbon monoxide, would impair its ability to transport oxygen. RBCs, in fact, do not use their eNOS to synthesize NO, except perhaps under extreme pathological conditions. This is clear because (1) eNOS remains bound to the membrane rather than in the cytoplasm in RBCs, and (2) RBCs exclude the substrate for NO, L-arginine and have an enzyme that actively breaks down any minute amounts that gain entry.

The proposal in Seneff et al. [111] that eNOS is a dual-purpose enzyme solves two problems for RBC's: it explains (1) why they contain abundant Enos, and (2) how they can obtain sulfate to be combined with cholesterol, yielding cholesterol sulfate. Cholesterol sulfate is produced by RBCs, and it plays a vital role in their membrane by protecting them from hemolysis [115,116], and helping to maintain the blood's highly negative zeta potential [71,72,111,117].

Epithelial cells also produce abundant cholesterol sulfate, which becomes a major component of the corpus striatum--the outermost layer of skin composed of enucleated cells that maintains a tight barrier to protect from water loss and microbial invasion. Cholesterol sulfate stimulates the synthesis of filaggrin, an essential protein in the highly cross-linked mesh in the corpus striatum, essential to its proper functioning [118]. Deficiencies in filaggrin are associated with conditions like atopic dermatitis that are observed in adverse reactions to mercury-and Al-containing vaccines. Deficient filaggrin can explain skin-related pathologies associated with CNS disorders. Atopic dermatitis also has immune components in that IgE levels are elevated [119].

Endothelial cells need sulfate to produce sulfated proteoglycans that make up the glycocalyx, which is essential for protection from vascular leaks. The glycosaminoglycan (GAG) sulfate anions present in heparan sulfate, chondroitin sulfate and keratin sulfate are essential in maintaining the structured (gelled) form of water [120] in the region surrounding not only the cells lining the vascular walls, but also most cells in the body. Loss of sulfates in these GAGs results in extensive impairment in cell function. Under sulfated GAGs in the intestinal wall and the intestinal vasculature are implicated in intestinal disorders, such as colitis and Crohn's disease [121].

Both neurons and muscle cells require large amounts of energy and Seneff et al. [111] argued that these cell types take advantage of heparan sulfate in membrane-bound syndecans, as a way to temporarily store

excess glucose. The sulfation step is necessary to prevent glycation damage to vulnerable proteins in the vicinity. Heparan sulfate is constantly synthesized and stored outside the cell as GAGs, and then later endocytosed into lysosomes over an elapsed interval of 4 to 6 hours [122]. The subsequent breakdown of the glucose in the lysosome provides a buffered source of energy to the cell. The amount of eNOS found in muscle cells is inversely related to obesity [99], and to nutritive flow into skeletal muscles [99]. With insufficient sulfate, these cells become insulin resistant, because they can no longer store part of the glucose they take in these GAGs. A similar strategy probably exists in neurons and its impairment may be responsible for the "Type III" insulin resistance that has been proposed as an early indicator of dementia [123], and which has been linked to Alzheimer's disease [124].

Heparan sulfate in neurons also plays an important role in neurite outgrowth [125], which would, therefore, be impaired if sulfate supplies were insufficient, potentially contributing to the pathology in autism and in various dementias. It also participates in long-term potentiation in the hippocampus [126], a process thought by some to be part of memory formation. Mice engineered to be impaired in the ability to sulfate heparan-sulfate chains in the brain suffered from all of the pathologies associated with "mouse-autism" [127]. Structural pathologies in the hippocampi were associated with depletion of heparan sulfate in the lateral ventricles in the brains at autopsy of mice exhibiting a mouse-model of autism [128]. Similar heparan sulfate deficiencies were also observed in postmortem analyses of human brains of individuals with autism [129]. A study of Alzheimer's brains post-mortem assessing the distribution of various lipids found that sulfatide, the only sulfated lipid, was uniquely under-represented in the Alzheimer's brains compared to normal controls [130]. Sulfatide was depleted up to 93% in the gray matter. These studies point to a deficiency in sulfate in the brain as a contributing factor in both autism and Alzheimer's disease.

Aluminum disrupts sulfate synthesis

As discussed above, the synthesis of sulfate by NOS when it is attached to the plasma membrane is highly plausible as a means for cells to supply themselves with adequate sulfate. Cells often need to supply their own sulfate due to sulfate's anionic kosmotropic property [111]. Because free sulfate transport is highly precarious, the body maintains an upper limit of less than 0.5 mM concentrations of free sulfate in the blood [131]. Any amounts above this level are exported through the kidneys. Cholesterol sulfate delivery by RBCs to the tissues during their passage through capillaries is likely an important means to supply the tissues with both cholesterol and sulfate. Unlike cholesterol, cholesterol sulfate freely migrates from one plasma membrane to another through water-based media because it is amphiphilic, i.e. both hydrophilic and lipophilic. In addition, the cholesterol in cholesterol sulfate supports a firm anchor within the membrane of an RBC during transit, ameliorating the kosmotropic effects of sulfate.

We can anticipate two ways in which Al would disrupt sulfate synthesis by eNOS, and in fact, cause eNOS to be locked into a nitrate-synthesis mode, with potentially devastating consequences. Most simply, Al^{3+} is a strong kosmotrope, which will influence the endothelial cells to switch to nitrate synthesis as a counterbalancing electrolyte. However, further considerations lead us to consider a more significant possibility. Al^{3+} is highly attracted, electrostatically to the negative charge of the sulfates in the GAGs of the glycocalyx. Al^{3+} would be expected to subsequently gain entry *via* calcium transporter channels, as a Ca^{2+} analogue. Once inside a cell, Al^{3+} binds to calmodulin with

a 10-fold higher affinity than Ca^{2+} [132]. Through a well-established signaling cascade, this would cause eNOS to detach from the membrane and stop producing sulfate [105].

Furthermore, Al^{3+} readily binds to fluoride to form AlF_x complexes (mostly AlF_3 and AlF_4^-). Fluoride is likely to be present in the blood due to nearly universal water fluoridation programs and fluoridated toothpaste. AlF_x is an excellent mimetic of phosphate, so much so that it has been effectively utilized to elucidate phosphorylation signaling cascades [133]. Like phosphate, AlF_x induces a GTP-mediated signaling cascade, through the mimetic $GDP-AlF_x$. Unfortunately, the $G\alpha^*GDP^*AlF_4^-$ complex is a very stable molecule that resists deactivation by hydrolysis and remains in the active state indefinitely [134]. This initiates a pronounced inflammatory response that may partially explain Al's adjuvant activity to promote an antigenic response. What this means for eNOS is that it becomes and remains phosphorylated, and therefore, produces sustained excessive amounts of NO, at the expense of sulfate.

Depression, Alzheimer's and the pineal gland

Seasonal affective disorder (SAD) may affect more than 10 million Americans [135]. In addition to depression, patients often experience fatigue, hypersomnia, carbohydrate craving and weight gain. Exposure to bright light, especially in the morning, is an established therapy [136].

SAD is likely tied to impaired melatonin synthesis in the pineal gland, a small gland located directly behind the eyes in the center of the brain. It produces the neurotransmitter melatonin, which plays an important role in the sleep-wake cycle. Melatonin is sulfated during transport, and we hypothesize that sulfate transport is a critical role of melatonin, such that it can supply sulfate to neurons distributed throughout the brain.

In a study of the amount of Al present in various brain tissues postmortem, more than twice as high a concentration of Al was found in the pineal gland, as in any of the other tissues examined, which included pituitary, cerebellum and cortex [137]. Mercury also accumulates in the pineal gland in occupationally exposed miners [138]. The amount of melatonin sulfate excreted in the urine is markedly reduced in association with occupational mercury exposure in miners, despite the fact that the amount of melatonin in the blood is sharply elevated [138]. Melatonin suppresses the synthesis of NO by NOS isoforms in the presence of calcium [106,108], which suggests that it enhances the synthesis of sulfate, which is needed for its transport.

An experiment on mice that involved exposing dams to Al orally during gestation and lactation at a level that did not noticeably impair their health was very informative in terms of the consequences to the offspring of the pregnancy [139]. The pups suffered from deficits in sensory motor reflexes, delays in the *opening of the eyes* and dose-dependent disturbances in serotonin and dopamine synthesis. Since serotonin is the precursor to melatonin, this translates into deficiencies in melatonin, which might be caused by impaired sulfate supply, as serotonin is also sulfated in transport. An experiment on rats exposed to Al with or without melatonin supplements demonstrated that melatonin protects from the oxidative damage in the cerebellum and cerebral cortex associated with Al exposure [140].

NOS activity exists in the pineal gland in both presynaptic nerve fibers and in pinealocytes, as well as in the endothelial cells of the blood vessels supplying the gland [106]. Norepinephrine is released at night from the nerve endings in the pineal gland, and such release is blocked by light exposure, which also markedly suppresses pineal NOS activity

[141]. Thus, NOS in the pineal gland produces NO mainly at night. We propose that during daylight and upon sunlight exposure, it produces sulfate instead. Strong support for this hypothesis comes from the fact that sunlight induces 3-O sulfation of heparan sulfate proteoglycans in the pineal gland, catalyzed by a heparan sulfate sulfotransferase [142]. The sulfate produced by day can be used to sulfate the melatonin produced by night.

The pineal gland may also supply sulfate to the *Substantia nigra*, a proximal midbrain nucleus that produces dopamine. Dopamine 3-O-sulfate is present in considerable amounts in mammalian plasma, and it is converted to norepinephrine through enzymatic action of dopamine- β -hydroxylase, thus making the sulfate anion bioavailable [143]. Thus, the pineal gland may play a significant role in supplying sulfate to neurons in the brain, mediated by sunlight exposure and transport *via* melatonin and dopamine, a role that would be disrupted by Al accumulation. The pineal gland becomes calcified during aging, and it has been shown that such calcification is especially severe in association with Alzheimer's disease [144].

An accumulation of fluoride in the pineal gland has been identified in association with aging [145]. Excitotoxicity has been proposed as a central mechanism in fluoride toxicity, in part due to its ability to readily complex with Al to form AlF_x complexes [146]. Increased Al content was found in melanin-containing neurons of the *Substantia nigra* in two out of three Parkinson's disease patients compared to none in controls [147]. This midbrain nucleus lies in close proximity to the pineal gland in the mesencephalon. Al and fluoride, especially in combination, would be expected to disrupt sulfate synthesis in the pineal gland.

A Neuroendocrine Pathway to Immune Dysfunction and Autoimmune Disease (Section 6)

Burnatowska-Hledin et al. [148] provide an excellent summary of the implications of hyperparathyroidism in the toxicity of Al, whether ingested in foods or in antacids, or present in dialysis fluid of patients with end-stage kidney disease. Hyperparathyroidism--excess production of parathyroid hormone (PTH)--leads to deposition of Al in brain and bone, as well as in the parathyroid gland itself. Al inhibits parathyroid hormone release, resulting in a euparathyroid state in dialysis patients with Al-related vitamin D-resistant osteomalacia. These authors argued that Al organ toxicity would be likely to occur not only in patients with impaired renal function, but also, more generally, in anyone expressing hyperparathyroidism. We develop this idea in this section, relating it, in particular, to vitamin D deficiency and insufficient sun exposure.

Aluminum in vaccines and environment as an autoimmune stimulant

Modern vaccines, such as acellular pertussis, are highly processed antigens and hepatitis B contains a viral surface antigen mimic produced from recombinant DNA in yeast cells. However, these production methods render the processed antigens unrecognizable to the immune system as pathogenic. Thus, processed antigen does not reliably stimulate satisfactory acquired immunity. Therefore, adjuvants have become increasingly essential in vaccine formulations to maintain efficacy. For example, the processing of whole organism, *Bordetella pertussis* to an acellular antigen requires that the antigen be adsorbed on the surface of Al hydroxide or Al phosphate particles for the vaccine to be considered effective. Likewise, recombinant hepatitis B vaccine

antigen must be adsorbed on Al hydroxide or amorphous Al hydroxy phosphate sulfate (AAHS) particles, which are then injected.

Mechanism of impaired excretion of aluminum

There is a strong link between Al toxicity and renal failure [149,150]. HogenEsch reviewed Al adjuvant safety and noted that Al toxicity is common in chronic kidney disease [48]. Aluminum causes renal dialysis dementia in part due to elevated parathyroid hormone activity in association with kidney disease [151]. Excess parathyroid hormone results in hypercalcemia and Al can be retained as well, as a consequence of its ability to mimic calcium. Parathyroid hormone inhibits normal urinary excretion and enhances gastrointestinal absorption of Al [152,153]. Therefore, it is not kidney disease per se that causes plasma Al to accumulate.

There are numerous reasons why parathyroid hormone activity or iPTH levels can be elevated besides chronic kidney disease. Thus, susceptibility to Al toxicity extends far beyond a select group of patients with chronic kidney disease. These include primary hyperparathyroidism, as well as physiologic hyperparathyroid state or secondary hyperparathyroidism. Causes of secondary hyperparathyroidism may include vitamin D insufficiency or deficiency, vitamin D resistant rickets, genetic variation of vitamin D receptor and others.

Parathyroid hormone levels can fluctuate physiologically relative to the availability of vitamin D in maintaining plasma calcium concentration within narrow bounds, and potentially impart a variable susceptibility to Al toxicity. Sun deprived populations, such as those who reside in Northern locations or those with darker skin [154,155] have incrementally higher prevalence of 25(OH)D3 insufficiency and secondary hyperparathyroid (2hPTH) state. Sufficient exposure to increasing Al dosage can ultimately intoxicate individuals with otherwise normal calcium homeostasis when parathyroid hormone becomes more predominant in maintaining plasma calcium.

Cannell [156] presented a compelling association of vitamin D deficiency and autism. Al is the only component listed in vaccine package inserts known to have a toxicokinetic profile modulated by parathyroid hormone activity. The causal cascade of aluminum toxicity in chronic kidney disease [157,158] would differ from sun deprivation only in that diseased proximal renal tubule cells are not able to convert 25(OH)D3 to 1,25(OH)2D3 [159,160].

Thus, the impaired ability to excrete aluminum may be more a function of parathyroid hormone activity than creatinine clearance. Movsas et al. [64] performed an experiment on 15 preterm infants at the age of 2 months by injecting 1200 μ g of aluminum in a single day and measuring serum and urine aluminum 24 hours before and after the injections. The investigators observed that the urine Al concentration remained unchanged. On that basis, they concluded Al in the vaccines is safe. Hillman et al. [161] found that parathyroid hormone is elevated in pre-term and full-term infants at 48 hours and up to 7 days. The pre-term infants had higher PTH than full term infants. Furthermore, Bishop et al. [162] found that feeding pre-term infants with solutions containing Al compared to Al-depleted solutions is associated with neurological impairment, using the Bayley Scales of Infant Development at 18 months of age. Therefore, Movsas et al. [64] view that urinary Al concentration remained unchanged after injecting 1200 μ g of Al into the infants is not reassuring when reviewing these papers.

PTH is the major systemic calcium regulating hormone, but it also induces both eNOS expression and eNOS activity, increasing the

production of NO from L-arginine, and therefore, of nitrate [163]. This is likely mediated by the protein kinase A and C pathways. Thus, this is entirely consistent with our prior discussion of a switch on the part of eNOS from sulfate synthesis to nitrate synthesis in association with calcium uptake in a cell. Any disease process that results in elevated PTH potentially renders individuals susceptible to Al toxicity, in part, perhaps even in large part, due to Al's ability to mimic calcium.

Aluminum adjuvant specificity

Al hydroxide and Al phosphate are the most common adjuvants licensed for use in vaccines in most countries, including the United States. The selection of Al phosphate or hydroxide is based upon the electrostatic properties of the antigen [164]. Al phosphate particulates are known to have a neutral surface charge in a medium with pH of about 5.0, whereas Al hydroxide has a neutral surface charge in a medium with pH of about 11.4. The higher isoelectric point of Al hydroxide can be reduced using phosphate substitution by ligand exchange on the surface of the aluminum particles [165]. The degree of phosphate substitution creates an optimal isoelectric point for the given isoelectric point of a manufactured antigen to maximize electrostatic adsorption [166]. Negatively charged antigen has a higher electrostatic affinity for Al hydroxide particles having a more positive surface charge. Conversely, a positively charged antigen will have a higher electrostatic affinity for Al phosphate particles having a more negative surface charge. Aside from pH specificity, adjuvants are used with a variety of antigens to potentiate immunostimulation. We have not found a basis to assume the adjuvant effect of Al is specific only to manufactured antigens, or an explanation why self-antigens at the injection depot or distant sites of Al biosequestration would somehow be excluded from the effect [19].

Various versions of the DTaP vaccine allow us to examine any differences in the adverse reactions between Al hydroxide and Al phosphate. From comparing reactions to these two variants in the Vaccine Adverse Event Reporting System (VAERS) database, it can readily be seen that Al phosphate favors a systemic reaction (seizures, abdominal pain, diarrhea, nausea, throat irritation), whereas Al hydroxide favors a local reaction (edema and erythema at the injection site). We hypothesize that this difference reflects the fact that Al hydroxide tends to bind to negatively charged membrane-bound sulfates at the injection site, whereas Al phosphate, being negatively charged, is relatively more mobile and migrates through the lymph system to finally infiltrate midbrain centers that control homeostasis, such as the pineal gland.

Aluminum clearance and kinetics

Crowther and Marriott [167] showed that, on oral ingestion, ions of higher valency, e.g. Fe^{3+} and Al^{3+} were bound with increasing avidity to a sulfate-bearing glycoprotein component of pig gastric mucosa. Jouhanneau et al. [168] studied the gastrointestinal absorption, tissue retention and urinary excretion of dietary aluminum in rats by using ^{26}Al and found that (a) the median fraction of ^{26}Al retained in the brain was 3.8×10^{-8} (range, $0.8-6.5 \times 10^{-8}$; mean \pm SD, $3.7 \pm 1.1 \times 10^{-8}$ (n=6), (b) the amount of ingested Al retained by bones in young rats was as great as that excreted in urine, and (c) the accumulation in the skeleton appeared to be relatively permanent.

A very efficient phosphate binder, aluminum hydroxide, was introduced in the seventies as standard phosphate binder therapy in uremic patients receiving dialysis treatment, but was abandoned in favor of calcium-containing phosphate binders because of its *significant*

negative effects on bone metabolism and cognitive function [169]. In comparing the pharmacokinetics of aluminum and lanthanum, this group noted that absorption of orally administered aluminum from the gastrointestinal tract amounted to from 0.01% to 0.10%, and that aluminum was mainly eliminated *via* the kidney, with negligible biliary excretion. Also of note, this group found that when Al hydroxide (2.4 g/day) was co-administered with citrate, Al excretion increased from 70 to 120 mg/day up to 350 to 603 mg/day [170].

In a recent pilot study (N=15), Movsas et al. [64] found significant declines post vaccination in serum iron (58.1%), manganese (25.9%), selenium (9.5%) and zinc (36.4%) levels, as well as a significant increase in serum copper level (8.0%). These authors noted that the trace elements play important roles in neurodevelopment and the immune system. Zinc and iron are both needed by iNOS, whose increased synthesis likely reflects an acute immune response to Al and would deplete serum stores of these minerals. The selenoprotein, iodothyronine deiodinase (DIO) catalyzes the conversion of thyroxine (T4) to triiodothyronine (T3), releasing iodide, which like nitric oxide, is a strong chaotrope [171]. An increased synthesis of DIO to further offset the kosmotropic influence of Al might explain selenium depletion, but these depletions may also represent competition by Al^{3+} for binding sites via molecular mimicry of Fe^{3+} , Mn^{2+} and Zn^{2+} , thereby altering their pharmacokinetics post vaccination. Further research is warranted.

In vivo phosphate substitution as a mechanism for autoimmune stimulation

Although elution of antigen from Al particles at the injection depot had been thought to diminish their efficacy [172], vaccines have been found to remain effective in inducing antibody titers despite desorption [173]. Furthermore, Al adjuvant more effectively potentiates immune stimulation after desorption of antigen in the interstitial fluid than if the antigen remains more strongly adherent to the adjuvant particles [174]. The strength of adsorption is greater by ligand exchange than by electrostatic attraction, and the force of attraction of phosphates to Al hydroxide adjuvant by ligand exchange greatly exceeds that of electrostatic repulsion forces [175]. Al hydroxide and to a lesser degree, Al phosphate, have free hydroxyls and are subject to ligand exchange with phosphorylated antigens [173]. It is conceivable that phosphorylated self-antigens can be substituted by ligand exchange *in vivo*, leading to autoimmune reactions following T-cell activation.

Adaptations to environmental aluminum and toxic threshold

Although Al constitutes 8% of the earth's crust and is ubiquitous in the environment, living organisms are usually relatively well adapted to survive its toxic properties. Roots of plants have the ability to resist low concentrations of Al in more alkaline soil. This defensive mechanism can be overwhelmed when Al concentration in the soil exceeds a toxic threshold or the soil becomes acidic, following acid rain [176], leading to plant death and removal from the food chain.

Animals and humans, likewise have the ability to resist Al toxicity by ingestion. Ordinarily, gut absorption of Al is 0.1-0.4% [53]. However, this protective mechanism is limited and can be overcome by unnaturally high dietary aluminum, exposures such as Al-containing dialysis fluids, a hyperparathyroid state or concomitant ingestion of oral vitamin D and citrate. Kirschbaum and Schoolwerth [177] reported severe encephalopathy among women with renal failure who were given oral citrate and Al hydroxide as an antacid.

During July of 1988, the drinking water supply in Camelford,

England became contaminated with Al sulfate and many residents became ill [178]. They suffered from loss of concentration, memory loss and poor psychomotor performance [179]. The outbreak of illness that followed was initially dismissed as hysteria and heightened awareness by way of media publicity [180]. However, Altman et al. [179] performed a more rigorous evaluation of 55 affected residents three years after the incident. By comparing the results of psychological testing and visual evoked potentials with fifteen closely age-matched sibling controls living outside the area, they proved that affected individuals suffered cerebral dysfunction not related to anxiety [179]. Bondy [181] reviewed neurotoxicity of environmental Al and cited several epidemiologic reports, associating Al content of drinking water with increasing prevalence of neurological disease. Campbell et al. [157] proposed that long-term low dose oral Al exposure in drinking water that does not necessarily result in acute toxicity is associated with increased inflammatory response in the brain, and that minimal chronic exposure confers long-term risk of age-related neurodegeneration and neuro-inflammatory disease.

Aluminum has been found in pyramidal neurons in hippocampal tissue from confirmed Alzheimer's patients postmortem [182]. Impaired memory and attention disorder developed during old age in rats chronically exposed to aluminum in their drinking water, and the degree of impairment was highly correlated with the percentage of aluminum-loaded pyramidal cells in their entorhinal cortex ($p < 0.05$) [183]. This was associated with an increased synthesis of amyloid precursor protein, a well-established marker of AD [184].

Aluminum toxicity by inhalational and dermal exposure

Al can also be toxic by way of inhalational exposures. Inhalational exposures typically occur as a result of occupational activity. Inhalational exposures have been reported with Al smelting and among agricultural workers exposed to road dust. Al chlorohydrate is aerosolized in deodorants and can be inhaled. Dermal exposure to Al occurs with use of deodorants [158].

ASIA: A Unifying Diagnosis (Section 8)

In this section, we will discuss two examples of autoimmune reactions that we believe can be explained by the reaction cascade we presented in the Introduction. We will first describe how three seemingly unrelated conditions, adverse reactions to vaccines, preeclampsia and autism, can be explained by nitrate overload and sulfate depletion subsequent to an acute reaction to Al exposure. Then, we show how physical somatic conditions that are often associated with neurological disease can be explained by a system-wide deficiency in cholesterol sulfate.

Anaphylaxis, preeclampsia and autism

Anaphylaxis is an allergic reaction associated with severe hypotension as the initiating symptom. It is believed to affect from 1 to 15% of the US population, but the prevalence has increased significantly in recent times [185]. A study on a mouse model of anaphylactic shock used pertussis toxin plus Al hydroxide as the sensitizing agent [186]. Surprisingly, it was identified conclusively that eNOS rather than iNOS was the NOS isoform responsible for the excess synthesis of NO that induces hypotension and the subsequent acute cascade. The authors wrote: "In contrast to the unsubstantiated paradigm that only excessive iNOS-derived NO underlies cardiovascular collapse in shock; our data strongly support the unexpected concept that eNOS-derived NO is the principal vasodilator in anaphylactic shock".

In an example [115], an intricate relationship among preeclampsia, pernicious anemia, autism and acute adverse reactions to vaccines was demonstrated and supported by analyses of the Vaccine Adverse Event Reporting System (VAERS) database maintained by the US Centers for Disease Control. Preeclampsia is a condition characterized by hypertension, proteinuria and elevated serum homocysteine, which develops in the third trimester of pregnancy. Preeclampsia can be life threatening to the mother and the fetus, and is a strong predictor of autism in the fetus. It is commonly treated with magnesium sulfate, and/or heparan sulfate, both of which would help boost sulfate levels in the vasculature.

In the VAERS database, a comparison between reactions that contain mentions of anemia-related symptoms and those that do not reveal that the "anemic profile" in the reaction is predictive of autoimmune symptoms associated with autism, such as eczema ($P=0.01$) and asthma ($P=0.0005$), as well as being highly predictive of autism itself ($P=0.0007$). Seneff et al. [115] argued that excess nitric oxide released into the serum in response to the Al and the antigen leads to a dramatic drop in blood pressure and anaphylactic shock. Hemolysis is a natural *sequitur* and this releases hemoglobin, which can rapidly neutralize the excess NO. The bioavailability of sulfate is greatly reduced due directly to the loss in sulfate supply from both the switch in eNOS from synthesizing sulfate to synthesizing nitrate and the reduced population of sulfate-providing RBCs (via cholesterol sulfate)—the anemia arising from hemolysis. Those who are vulnerable are already deficient in sulfate, such that the added stress of the vaccine induces an acute reaction. The depleted sulfate supply may explain the eczema and asthma, as well as the increased risk of autism, as described above.

Since vitamin D3 synthesis and the metastable state of interphase water of neurolemmal membranes depend upon sunlight stimulus, as does eNOS' synthesis of sulfate [111], insufficient sunlight exposure would lead to impaired vitamin D3 synthesis and impaired sulfate supply. As shown in Table 1, there is a correlation between autism rates in the 50 states of the US and several different parameters related to climate, in such a way that exposure to UV light protects from autism. Autism rates were computed on the basis of data available on the Web at <http://nces.ed.gov/ccd/bat/> for individuals enrolled in the exceptional student education (ESE) autism category in grades 1-6 in 2007, with total student enrolment in grades 1-6 serving as the normalizing factor. These data were compiled according to the U.S. Department of Education (USDE), Individuals with Disabilities Education Act (IDEA). Weather information for the 50 states individually is readily available on the Web.

Asthma, dermatitis and eosinophilic esophagitis

Aluminum hydroxide attracts eosinophils to the injection site, even in the absence of any antigenic stimulation, a response that is mediated

| Parameter | Correlation |
|--------------------------|-------------|
| Latitude | 0.22 |
| Rainfall | 0.16 |
| RMS (Rainfall, Latitude) | 0.34 |
| Temperature | -0.16 |
| Elevation | -0.28 |

Table 1: Pearson correlation coefficients (Correlation) for various measures of climate for the 50 states in the US compared with autism rates according to the US IDEA data. RMS() is the root mean square (geometric mean) of the two parameters. The larger correlation with autism shows that rainfall and latitude are largely independent (additive) factors. High elevation results in higher exposure to UV, which may be protective against autism.

by T cells [187]. It also elicits and activates IL-4 expressing eosinophils that prime B cell responses to generate antigen-specific IgM [188].

Dysphagia (difficulty swallowing) is a common problem affecting up to 22% of patients in primary care [189], and a characteristic feature of Alzheimer's, Parkinson's and ALS. Eosinophilic esophagitis (EE) is a newly recognized condition as of the mid 1990's [190]. It is characterized by eosinophil infiltration into the esophagus, which is manifested as dysphagia in adults and refractory reflux symptoms in children [191]. There has been an alarming recent increase in the incidence of EE in Western countries [192-195]. Yakoot [195] proposed that EE and allergic bronchial asthma may be two expressions of the same disease in two different organ systems.

EE is associated with a Th2 immune profile and synthesis of the cytokine IL-13, which has direct cytotoxic effects on epithelial cells. Eosinophils are characteristic of a Th2 response, and eosinophil recruitment is mediated by IL-13. Vaccination with formalin-inactivated respiratory syncytial virus (RSV) can lead to enhanced morbidity and mortality following a subsequent natural infection with the virus, due to enhanced eosinophil recruitment [196]. RSV is the leading cause of lower respiratory tract disease in children.

IL-13 down-regulates filaggrin expression in skin keratinocytes [197]. Perturbed barrier function, leading to increased skin permeability, microbial invasion and autoimmune diseases, can be explained by impaired filaggrin expression, and this can lead to increased susceptibility to atopic dermatitis (eczema) [198], EE [199], asthma and various food allergies [198].

Mutations in the gene encoding filaggrin play a significant role in ichthyosis vulgaris, eczema, and in other atopic diseases, such as asthma and allergic rhinitis [200]. Certain single nucleotide polymorphisms [SNPs] increased the risk for eczema by more than 3-fold, and of concurrent asthma. Filaggrin is strongly expressed in the cornified epithelium in the nasal vestibular lining.

Patients with atopic dermatitis have low levels of cholesterol sulfate in the skin, and this is associated with pathological desquamation (skin peeling), characteristic of dermatitis [201]. Mercury poisoning can also cause such desquamation, along with hypertension, failure to thrive and developmental regression [138]. This suggests that mercury may interfere with cholesterol sulfate synthesis in the skin. A case example of contact dermatitis from occupational exposure to Al supports our hypothesis that Al may induce atopic dermatitis via cholesterol sulfate inhibition [202]. Reduced filaggrin synthesis consequential to impaired cholesterol sulfate synthesis likely increases risk to these allergic conditions, especially in genetically susceptible individuals.

The lung epithelium possesses both constitutive and inducible NOS activity, and the synthesis of NOS isoforms is enhanced under inflammatory conditions [107]. Asthma is characterized by epithelial damage in the lung, along with increased cytokine production and increased synthesis of nitric oxide from iNOS, brought on by inflammatory agents [203]. Asthmatic patients produce significantly more nitric oxide in exhaled air compared to controls [204].

Discussion

In this paper, we have developed a systems-level hypothesis to explain the commonly observed links between immune disorders and neurological disorders. Furthermore, we have implicated chronic and acute aluminum exposures as playing a critical role in the pathology of both of these systems level diseased states. We argue that the initial

exposure of cells localized to the site of an injection containing Al adjuvant can lead to a breakdown in their water-based membrane potential and electrical supply, as well as disrupting their ability to metabolize glucose. Distressed cells launch an immune response cascade, which causes the release of cytokines and inflammatory agents that can be destructive to neighboring cells. Membrane destruction of acutely stressed cells leads to the release of antigenic DNA debris into the tissues, which can eventually lead to autoimmune disease due to activation of T cells [205].

At the molecular biosemiotic level, in Section 4, we have presented a brief overview of a novel hypothesis wherein the onset of immune dysfunction and autoimmune disease is postulated to begin with exposure to EIWS, wherein local "unwetting", "stretching" and hydrophobic "collapse" of interfacial water occurs, and for which considerable support is currently provided by a large and rapidly-growing body of published scientific literature. Macromolecular recognition has been shown empirically to depend critically on biological water dynamics in the 20-40 picosecond timescale. We refer to long wavelength noise or turbulence by sub-nanometer scaled particles as manifestations of EIWS. EIWS is thought to impact multiple biosemiotic levels simultaneously. Biological water is proposed to quantum coherently and fractally mediate the dynamical-coupling between the neuronal networks and their environment on multiple scales of time and space.

While EIWS results on the macro-scale in immune dysfunction and autoimmunity, EIWS results on the micro-scale in disruption of the percolation transition of biological water at the interphase of neuronal membranes, thereby lowering membrane potentials, and in certain circumstances, completely eliminating action potentials. This model of immune dysfunction is based on biological water dynamics at the interphase of neuronal membranes, percolation theory and avalanche mathematics, which require for optimal function, the unique molecular level properties of both (a) sufficient hydration levels, and (b) the sulfoglycolipids and HSPGs at the neuronal membranes, to facilitate the storing of incident radiant energy from sunlight as entropy loss and charge separation [206]. We suggest that such a model will provide a potentially useful biophysical parameter for assessing the criticality of the native metastable critical state of neural function found in the CNS and peripheral nervous systems. We suggest further that there will be proven a strong correlation between loss of self-ordered criticality of biological water, with the polysystemic clinical manifestations described recently by Shoenfeld and others, as ASIA.

At the systems biology level in Section 5, we identified the molecule eNOS as coordinating an intricate balancing between sulfate and nitrate buffering in the blood in order to maintain a healthy ratio between chaotropic and kosmotropic influences. Al, as a strong cationic kosmotrope, is highly disruptive of blood homeostasis in this regard, as well as through its disruption of zeta potential. An important contributor to susceptibility is inadequate sun exposure to both the eyes and the skin, because, as argued [111], sunlight catalyzes the synthesis of sulfate by eNOS.

We have proposed here for the first time to our knowledge, a novel role for the pineal gland in synthesizing sulfate upon sunlight exposure and in transporting this sulfate to various parts of the nervous system *via* neurotransmitters such as melatonin and dopamine. Sleep disorders are associated with many neurological diseases, such as Alzheimer's disease, Parkinson's disease, autism and depression, and Alzheimer's is associated with both low bioavailability of sulfatide, a sulfated lipid and calcification of the pineal gland, which would impair its ability to

synthesize sulfate. Al accumulates in high concentration in the pineal gland, and this likely relates to calcification. Al gains entry by acting as calcium mimetic, as evidenced by the fact that the depolarization and disruption of microtubules observed in plant roots exposed to Al is prevented by calcium channel blockade [94].

The capacity to produce vitamin D3 in the skin decreases with aging [207], and we believe this can be attributed in part to the impaired ability to produce sulfate because of an increasing Al burden. Sulfate is needed for efficient transport of vitamin D3 and of cholesterol, which is also produced in the skin. We have argued that Al disrupts this function by its biophysical effects on water. The overuse of Al-containing high-sun protection factor (SPF) sunscreens contributes to the problem both by blocking the UV light and by Al's role in disrupting eNOS' sulfate synthesis. Correlations between reduced sun availability and autism rates in the 50 states of the US are consistent with this hypothesis. Impaired sulfate synthesis leads to systemic dysfunction manifested not only as neurological impairment, but also as diverse somatic conditions such as eczema, asthma, impaired gut function, diabetes, kidney disease and heart disease, due to deficiencies in cholesterol sulfate and other sulfated biomolecules. This provides a direct link between somatic and neurological aspects of autoimmune diseases.

Depending on a combination of genetic predisposition and the cumulative burden of environmental toxic exposures, the brain may or may not be spared when sulfate supplies become deficient. Even within the brain, it depends on which parts of the brain are most affected as to which neurological disease will emerge. Parkinson's disease defects are mostly concentrated in the *Substantia nigra* (the source of dopamine) [208], whereas Alzheimer's affects mainly the cortex, at least initially [209], and ALS may focus on the motor neurons in the spinal cord, brain stem and motor cortex [210]. However, all of these conditions have somatic complications that are explained by deficiencies in sulfate and by excessive activation of calcium phosphate pathways through an overactive parathyroid gland.

As discussed in Section 5, an increase in bone fragility and parathyroid function follows directly from vitamin D3 insufficiency [207]. We propose that this is due directly to the need to replace sulfate with phosphate as an ionic kosmotrope for maintaining water homeostasis in the cells. Patients suffering from hyperparathyroidism have a higher incidence of impaired glucose tolerance, along with elevated serum levels of calcium [211]. These are connected by the fact that excessive PTH leads to a leaching of calcium phosphate from the bones in order to supply it to the tissues as a substitute for magnesium sulfate [212]. Sulfate supply is depleted due to the interference of toxic chemicals like Al on sulfate synthesis and sulfate transport [213]. In addition, sulfate depletion then leads to glucose intolerance due to the important role sulfate plays in the storage of glucose in the extracellular matrix [111].

In this article, we have demonstrated the multiple deleterious roles that Al plays across all levels of organization, beginning at a molecular level and culminating in systems-wide dysfunctions. Of particular relevance for the etiology of CNS disorders, Al acts directly to alter neural cell function. As well, Al disturbs immune function, and thus indirectly attacks the nervous system through autoimmune actions. The combined weight of these two actions may explain the diverse forms of many developmental and age-related neurological diseases. These observations may provide more than sufficient reasons to consider how we can limit human exposure to this element from whatever source. Of particular concern in this regard is to limit the exposure to the most vulnerable populations: the very young and the very old.

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