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ETIO PATHOGENESIS OF AUTOIMMUNITY

Aluminum in the central nervous system (CNS): toxicity in humans and animals, vaccine adjuvants, and autoimmunity

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Abstract We have examined the neurotoxicity of aluminum in humans and animals under various conditions, following different routes of administration, and provide an overview of the various associated disease states. The literature demonstrates clearly negative impacts of aluminum on the nervous system across the age span. In adults, aluminum exposure can lead to apparently age-related neurological deficits resembling Alzheimer's and has been linked to this disease and to the Guamanian variant, ALS–PDC. Similar outcomes have been found in animal models. In addition, injection of aluminum adjuvants in an attempt to model Gulf War syndrome and associated neurological deficits leads to an ALS phenotype in young male mice. In young children, a highly significant correlation exists between the number of pediatric aluminum-adjuvanted vaccines administered and the rate of autism spectrum disorders. Many of the features of aluminum-induced neurotoxicity may arise, in part, from autoimmune reactions, as part of the ASIA syndrome.

Keywords Autism · ALS · Alzheimer's · Neurodegeneration · Immune response

Introduction

We live in what one leading researcher on the chemistry of aluminum has called "the Aluminum Age" [1]. Aluminum, the third most abundant element in the Earth's crust and the most abundant metal, is one of the most remarkable elements in the periodic table. Objects made with aluminum are strong, durable, light and corrosion resistant. Aluminum is an excellent conductor of electricity. For these reasons, aluminum currently finds its way into virtually every aspect of our daily lives. Aluminum is used in cans and cookware, aluminum foil, housing materials, components of electrical devices, airplanes, boats, cars and numerous hardware items of all descriptions [2].

With aluminum geologically bound up in various molecular complexes, it is only in the last century that has become available for human use and, importantly, become bioavailable [2, 3]. In terms of bioavailability, aluminum is now found in drinking water due to its action as a flocculant, is a common additive in various processed foods, is added to cosmetics of many types, and, increasingly, shows up pharmaceutical products (Table 1). Notably, in regard to the latter, various aluminum salts are used as vaccine adjuvants. As a result of all of this, aluminum in the human environment is increasingly found in our bodies (Fig. 1) [4–7].

Aluminum is extremely reactive with carbon and oxygen, two of the leading elements of life on Earth. For this reason, the widespread use of bioavailable aluminum may have immense and far reaching implications for the health of humans and animals. In fact, much evidence shows that

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Table 1 Estimates of daily and weekly intakes of aluminum in humans (Adapted from 9)

Major sources of Al exposure in humans	Daily Al intake (mg/day)	Weekly Al intake (mg/day)	÷ PTWI * (1 mg/kg body weight; for an average 70 kg human, PTWI = 70 mg)	Amount delivered daily into systemic circulation (at 0.25 % absorption rate*)
Natural food	1–10	7–70	0.1–1	2.5–25 μg
Food with Al additives	1–20 (individual intake can exceed 100)	7–140 (700)	0.1–2 [10]	2.5–50 μg (250 μg)
Water	0.08–0.224	0.56–1.56	0.008–0.02	0.2–0.56 μg
Pharmaceuticals (antacids, buffered analgesics, anti-ulceratives, anti-diarrheal drugs)	126–5000	882–35,000	12.6–500	315–12,500 μg
Vaccines (HepB, Hib, Td, DTP)	0.51–4.56	NA	NA	510–4560 μg**
Cosmetics, skin-care products and antiperspirants***	70	490	NA	8.4 μg (at 0.012 % absorption rate)
Cooking utensils and food packaging	0–2	0–14	0–0.2	0–5 μg

* PTWI (provisional tolerable weekly intake) is based on orally ingested Al; generally, only 0.1-0.4 % of Al is absorbed from the gastrointestinal tract; however, Al may form complexes with citrate, fluoride, carbohydrates, phosphates and dietary acids (malic, oxalic, tartaric, succinic, aspartic and glutamic), which may increase its gastrointestinal absorption (0.5-5 %). Co-exposure with acidic beverages (lemon juice, tomato juice, coffee) also increases Al absorption as well as conditions of Ca^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+} deficiency

** A single dose of vaccine delivers the equivalent of 204-1284 mg orally ingested Al (0.51-4.56 mg), all of which is absorbed into systemic circulation

*** The risk of antiperspirants is both from dermal exposure and inhalation of aerosols. Inhaled Al is absorbed from the nasal epithelia into olfactory nerves and distributed directly into the brain

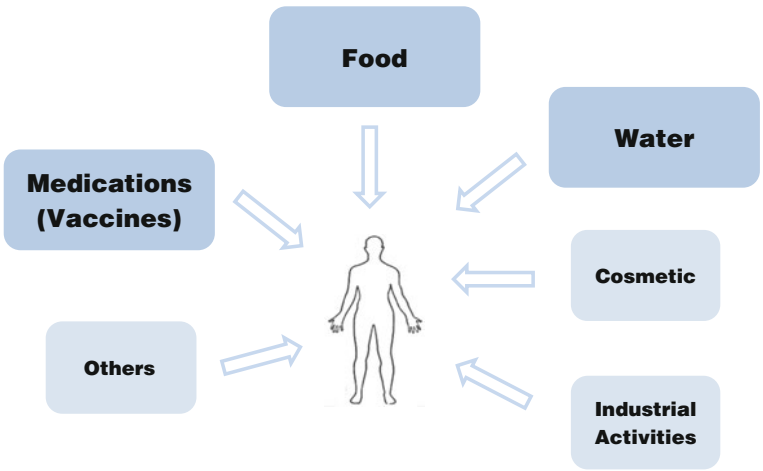

Fig. 1 Aluminum in the human environment. The schematic shows some of the key sources of bioavailable aluminum that are suspected, or demonstrated, to negatively impact human health

aluminum seems to be toxic to all forms of life on Earth, and where it appears in terrestrial biochemistry, it is invariably deleterious [1].

The notion that aluminum is toxic is hardly novel: Dr. William Gies, with 7 years of experimental testing in humans and animals on the effects of oral consumption of aluminum salts use in baking powders and food preservatives, had this to say in 1911:

> These studies have convinced me that the use in food of aluminum or any other aluminum compound is a dangerous practice. That the aluminum ion is very toxic is well known. That aluminized food yields soluble aluminum compounds to gastric juice (and stomach contents) has been demonstrated. That such soluble aluminum is in part absorbed and carried to all parts of the body by the blood can no longer be doubted. That the organism can 'tolerate' such treatment without suffering harmful consequences has not been shown. It is believed that the facts in this paper will give emphasis to my conviction that aluminum should be excluded from food. [8].

One hundred and one years after Gies' prophetic concerns, the notion of aluminum toxicity, in particular in relation to a spectrum of neurological diseases such as Alzheimer's,

ALS and autism spectrum disorders (ASD), requires a reevaluation based on the science of the last century. A now abundant literature shows that exposure of humans and animals to aluminum from various sources can have deleterious consequences on the developing and adult nervous systems (summarized in part in ref. [9]). These impacts may depend in large part on various factors, for example, the form(s) of aluminum, the route of administration, and the concentration and duration of exposure. Included in this latter category is the issue of dietary versus injected aluminum, the latter a key component of many current vaccines. In addition, the final impact of aluminum will likely depend on a number of biological variables including age, gender and the potential and largely yet unidentified genetic susceptibility factors enhancing aluminum toxicity.

The current review will briefly highlight the studies which have demonstrated aluminum toxicity in the nervous system in humans and in animal model systems, discuss the potential CNS neurotoxic role of aluminum vaccine adjuvants, and finish with a consideration of the potential negative contribution of aluminum to autoimmune reactions in disease.

Aluminum and its harmful biochemical interactions with animals and humans

As noted above, aluminum is abundant but has not typically come into direct contact with humans until relatively recently [10]. This situation changed dramatically during the last half of the nineteenth century when aluminum salts began to be used routinely in the dyeing of fabrics and in food preservation [2, 9, 11, 12]. Aluminum now routinely shows up in infant formula (where it may represent a contaminant or a deliberate additive in the production process [13], in cheese, bakery products, ready-made cake mixes, soft-drinks, etc., as well as in less processed products such as coffee and tea [9, 14]). It may also enter the body through the use of aluminum cookware and packaging [11]. Aluminum also shows up in various cosmetics, as an antiperspirant in many commercial deodorants, and in a variety of medicinal formulations [2, 5, 9, 15]. Antacids also often contain high levels of aluminum hydroxide [2, 16].

Much of the aluminum that enters the human body comes through food. A smaller amount enters through the skin, such as in antiperspirants. Both of these routes would put aluminum into the circulatory system relatively quickly, and most of this aluminum is typically rapidly removed by the kidneys [9]. The exceptions for such excretion are those who lack patent kidney function, infants until age one [17–19] and the elderly [18, 19]. It is these three groups that are most susceptible to aluminum accumulation in the body.

Vaccines and aluminum

Aluminum is added to vaccines to help the vaccine work more effectively [20], but unlike dietary aluminum which will usually clear rapidly from the body, aluminum used in vaccines and injected is designed to provide a long-lasting cellular exposure [18, 19]. Thus, the problem with vaccine-derived aluminum is really twofold: It drives the immune response even in the absence of a viral or bacterial threat and it can make its way into the central nervous system.

The origin of aluminum salts in vaccines has a curious, and largely unknown, history: In the early part of the twentieth century, vaccine researchers frustrated by low antibody titers in experimental vaccines added various compounds in the hope of making the vaccines more effective. In 1926, Glenney et al. [21] first experimented using aluminum salts as "helpers," hence the term adjuvant. Aluminum worked so well at increasing antibody titers that it became the primary vaccine adjuvant in use, a circumstance which has continued to the present day. Unfortunately, the potential for aluminum to be harmful to various organ systems, including the central nervous system, does not appear to have been rigorously tested [19].

Safety concerns for aluminum in vaccines are twofold: First, the very real toxicity of aluminum compounds to be discussed below. The second is the more general issue of the type of immune response elicited, in particular if the aluminum adjuvant induces either allergic or abnormal autoimmune responses. Such responses are now considered by some investigators to play a role in Guillain–Barre disease, multiple sclerosis and Gulf War syndrome (see [22] for references).

Aluminum and neurological disease

ALS

Amyotrophic lateral sclerosis (ALS) is a progressive disease of still unknown origin that targets the motor neurons in the brain and spinal cord. Typically, at end-stage disease, both sets of motor neurons have undergone degeneration with resulting loss of motor function. Death typically occurs by respiratory failure. The typical age for the onset of ALS starts is mid-50 s to 70 s, and the survival time after diagnosis ranges from 3 to 5 years. Many ALS victims show a significant loss of cognitive function as well at the latter stages of the disease.

About 90 % of all ALS cases (sporadic ALS) arise from unknown factors, while 10 % are "familial" with a variety of genes involved, notably mutations in the genes coding for the protein superoxide dismutase (SOD). Of the 90 % of sporadic cases, a current view is that environmental

toxins, alone or in synergy with still unknown "susceptibility" genes, are to blame. What these toxins might be remains controversial [23].

Some of the strongest evidence for an environmental toxin causing ALS has come from studies of the two confirmed clusters of ALS: ALS–parkinsonism dementia complex (ALS–PDC) in Guam and the Western Pacific and the ALS associated with Gulf War syndrome (GWS). In regard to the first, neurologists on Guam after World War II noted an extremely high incidence of what appeared to be almost classical ALS among the indigenous Chamorro population. A second disorder, PDC, described a form of parkinsonism with an associated dementia. Approximately 10 % of all patients in Guam developed both the ALS and PDC disorders, usually with the ALS features appearing first [24].

The cause of the disorder in Guam was eventually narrowed down to various putative environmental toxins, including toxins from the seed of the cycad palm which the Chamorro people once frequently ate, and abnormally high aluminum in the soil and water in southern Guam [25]. These data remain controversial but clearly point to a potential link between aluminum and ALS.

GWS (or illness) represents a spectrum of disorders primarily in military personnel in service during the Persian Gulf War (1990–1991). This set of disorders is now considered to fall into a broader category of autoimmune disorders termed "autoimmune syndrome induced by adjuvants" or ASIA 20, 26, 27. GWS is characterized by symptoms such as fatigue, muscle and joint pains, emotional disorders, posttraumatic stress reactions, headaches, and memory loss [28, 29]. Syndrome 1 includes excess fatigue and concentration and memory problems, anxiety, depression, and sleep disorders. Syndrome 2 includes blurred vision, concentration and memory problems, irregular heartbeat, loss of balance and dizziness, speech difficulties, sudden loss of strength, and tremors and shaking. Syndrome 3 includes generalized muscle aches, joint aches, numbness in the hands and feet, and swelling in the joints and in the extremities. Syndrome 2 is particularly of interest for the neurological disease community since four of the seven symptoms are consistent with early phases of ALS (loss of balance and dizziness, slurred speech, sudden loss of strength and muscle weakness, especially the arms and legs, and tremors and shaking).

The suggestion that ALS might be part of GWS became clear in 2003. First, the numbers of ALS cases in military personnel were three times higher in GWS patients than in the general population. Secondly, GWS/ALS victims tended to be younger than those with classical ALS, specifically 20–30 s instead of the normal North American onset age of 50–70 s. The age shift was consistent with a pattern familiar from the variety of forms of ALS–PDC on Guam: As incidence levels increased, the age of onset tended to decrease.

Studies of Gulf War ALS and GWS in general have suggested a variety of putative environmental factors as causal or contributing (exposure to depleted uranium [30, 31], nerve gas [32, 33], organophosphates [34, 35], vaccines [36], heavy metals [37] and bacterial infections [38, 39]). Some genetic susceptibility factors have also been considered and could work in concert with the various toxic substances listed above [23].

In recent years, increased scrutiny has focused on vaccines, in particular the anthrax vaccine which contained aluminum as an adjuvant [40]. Soldiers from the United Kingdom who also received the anthrax vaccine with aluminum showed increased psychological distress and chronic fatigue compared with those who did not get the vaccine [41]. French soldiers participating in the war did not receive the anthrax vaccine but did show some GWI-related disorders (respiratory, neurocognitive, psychological and musculoskeletal), but no ALS symptoms were reported [42]. As above, many of the features of the disease place it firmly within the ASIA family of disorders.

To explore the ALS component among GWS patients, we injected aluminum hydroxide compared to a more novel vaccine adjuvant, squalene, into young, male colony mice. We compared the outcomes in these animals to those that received both adjuvants and to those that had only saline injections [43, 44]. We tested the mice with various motor and cognitive behavioral tests over a period of 6 months. The mice injected with aluminum hydroxide showed a 50 % decrease in muscular strength and endurance compared with control mice (Fig. 2). Aluminum-injected mice also showed a 138 % increase in anxiety levels, and mice injected with aluminum and squalene had significant late-stage long-term memory loss. A second study confirmed a clear loss of spatial memory capabilities in aluminum-injected mice [44] (Fig. 3).

Mice injected with aluminum hydroxide showed a significant increase in cell death in the spinal cord and motor cortex (Figs. 4, 5), primarily affecting the motor neurons as well as neuroinflammation in the spinal cord and motor cortex as evidenced by increases in activated reactive astrocytes (Fig. 6) and microglia (data not shown).

These studies demonstrated that severe behavioral motor deficits and the loss of motor neurons throughout the nervous system resulted when an aluminum vaccine adjuvant was applied to an animal model. The effects closely resembled the damage we had seen in the motor areas of mice used to model ALS–PDC of Guam and, in addition, resembled the pathological outcomes in human ALS [23].

The available data on GWS thus seem to point at aluminum in vaccines as one of the strongest links to ALS in GWS. The neurological signs and symptoms, especially

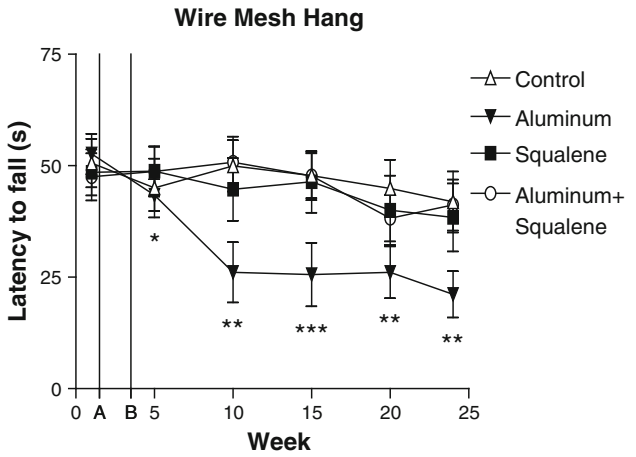

Fig. 2 Behavioral outcomes in outbred male colony mice injected with two vaccine adjuvants. The studies used aluminum hydroxide, the most common vaccine adjuvant, or squalene a precursor to cholesterol. A third treatment group combined aluminum and squalene. Control mice were injected with saline. All injections were subcutaneous. The data show the outcomes of the wire-mesh hang test for motor strength. Mice injected with aluminum hydroxide showed a significant and sustained decrease in muscular strength and endurance (–50 %) compared with the controls mice. Mice injected with squalene or both adjuvants did not show a significant decrease in muscular strength. A = first injection, B = second injection. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; one-way ANOVA). (Adapted from 43)

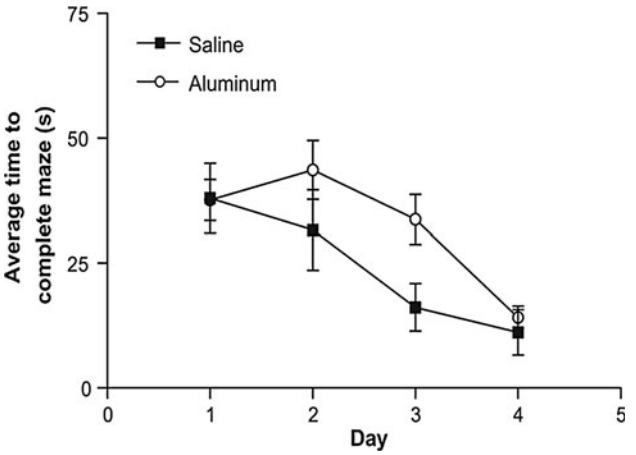

Fig. 3 Water maze test as an evaluation of learning and memory. Mice injected with aluminum hydroxide (6 injections) on average took significantly longer to complete the maze compared to saline-injected mice (two-way ANOVA. *$p = 0.0389$). (From [44])

those for the ALS subgroup, are also a good match to other signs and symptoms of aluminum neurotoxicity. For example, dialysis solutions containing aluminum have been linked to an Alzheimer's-like disorder termed "dialysis-associated encephalopathy/dementia" (DAE) (see below). In animals, aluminum neurotoxicity appears to be particularly harmful to neurons that make the neurotransmitter

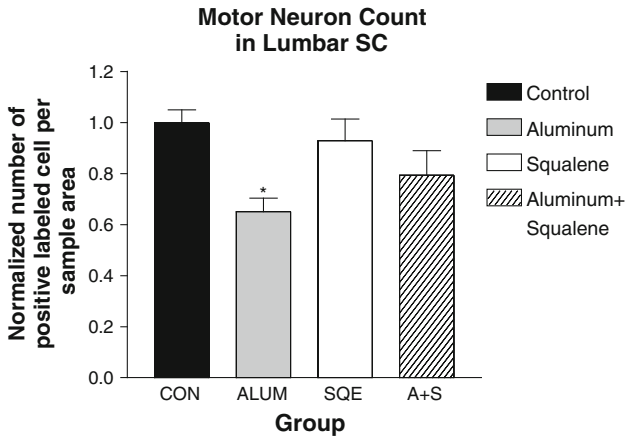

Fig. 4 Motor neuron death following aluminum hydroxide injections in outbred male colony mice. Mice injected with aluminum hydroxide showed a statistically significant decrease in motor neuron number (35 %) compared with the controls. There was no significant difference in motor neuron counts between all other groups compared with the controls. Data are mean ± S.E.M ***$p < 0.05$ versus control mice using one-way ANOVA. (From [43])

acetylcholine, for example, motor neurons in the brain and spinal cord.

Recently, two other groups have reported similar findings using aluminum hydroxide injections in mice (R. Gherardi; N. Agmon-Levin pers. comm.). Also, recent veterinary studies of apparent neurological disorders in Spanish sheep have linked the various behavioral deficits and CNS pathologies observed to aluminum-adjuvanted vaccines [45].

Macrophagic myofasciitis and the fate of aluminum adjuvants in the body

Additional evidence exists for aluminum's role in various central nervous system disorders, including multiple sclerosis associated with aluminum hydroxide injections that produce a persistent muscle inflammatory response termed macrophagic myofasciitis [22, 46, 47]. Other studies using even smaller amounts of aluminum hydroxide describe the pathway of aluminum from the muscle into the brain. In brief, these studies show that aluminum nanoparticles are carried from the site of injection in the muscle to the draining lymphatic system. Once there, the aluminum is carried into the central nervous system by circulating macrophages [46].

Alzheimer's disease

The potential link between aluminum, in various forms, and Alzheimer's disease has been the subject of speculation for decades. The first case of Alzheimer's disease was

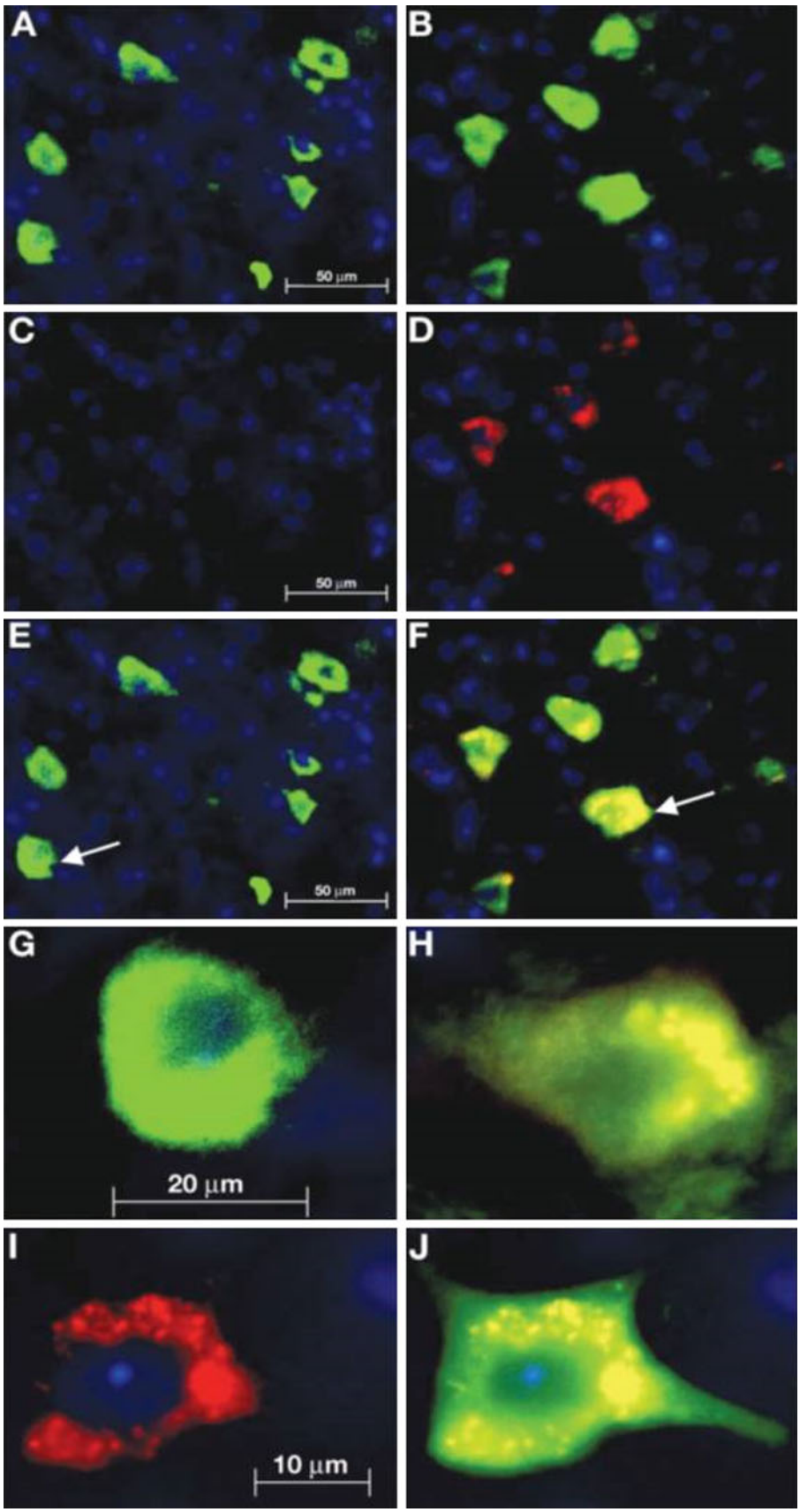

Fig. 5 Histological evaluation of aluminum hydroxide injection in mouse spinal cord. Control (**a**) and aluminum-injected (**b**) mouse motor neurons are fluorescently labeled with NeuN (*green*) and activated caspase-3 (*red*) (**c**, **d**, respectively) in the ventral horn of lumbar spinal cord. *Yellow* labeling is a merged image showing colocalization (**e**, **f**). The *blue fluorescence* is the nuclear marker DAPI. The data show that aluminum-injected motor neurons are undergoing programmed cell death (apoptosis). Magnification × 40 A–F. *White arrows* indicate neuron enlarged in (**g**, **h**). Enlargement of neurons **e**, **f** at ×100 magnification. **i**, **j**, Enlargement of another activated caspase-3-positive motor neuron at × 100 magnification. **j** Scale bar = 50 μm. **g**, **h**, *Scale bar* = 20 μm. **i**, **j**, *Scale bar* = 10 μm. (From [43]) (Color figure online)

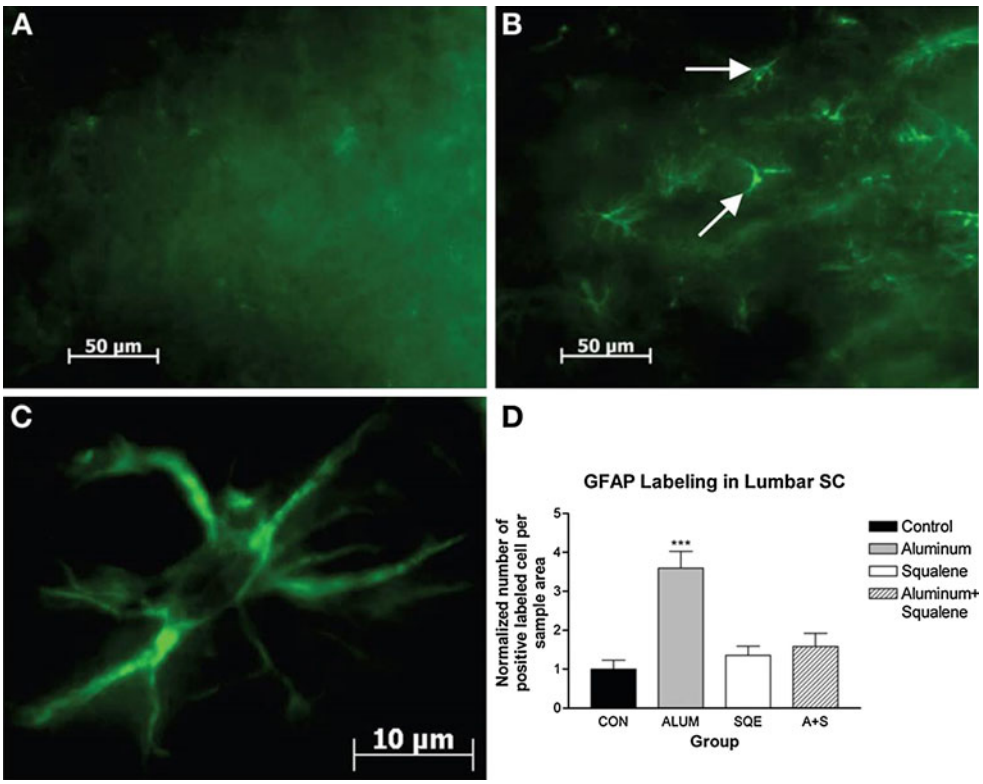

Fig. 6 Activated astrocytes labeled with glial fibrillary acidic protein (GFAP) in ventral horn of lumbar spinal cord of control (**a**) and aluminum-injected mice (**b**). Sections from mice injected with aluminum hydroxide show increased GFAP labeling and a greater number of astrocytes (*white arrows*) compared with controls (**a**, **b** ×40 magnification). *Scale bar* = 50 µm. **c** Astrocyte from aluminum-injected mouse observed under ×100 magnification. *Scale bar* = 10 µm. **d** Normalized cell counts for GFAP labeling of astrocytes in ventral horn of lumbar spinal cord ($n = 32$, eight per group). The largest increase in GFAP-positive cells occurred in the aluminum treatment group. Data are mean ± S.E.M ***$p < 0.001$ versus control mice using one-way ANOVA. (From [43])

reported in Frankfurt, Germany, about 20 years following the initial widespread use of aluminum products [9].

A rare disease as late as the 1920s, Alzheimer is now one of the most prominent neurodegenerative disorders and a leading cause of dementia, impacting some 24.3 million people worldwide (see [9] for references), with the increase is not solely attributable to a burgeoning aging population. Alzheimer's disease is characterized by a general loss of cognitive function, including memory. The brains of Alzheimer's victims contain amyloid "plaques" and neurofibrillary tau protein "tangles," and in various parts of the brain, there is significant neuronal loss. Various studies have shown the presence of aluminum associated with neurofibrillary tangles of neurotoxic tau protein [7, 48]. Although such association could be coincidental, the link certainly suggests a role somewhere in the disease process. Although discounted in recent years, the notion that aluminum could be a contributing factor in Alzheimer's disease has begun to regain momentum. An extensive review published in 2011 [9] documents the extent to which aluminum is toxic to plants, animals and humans.

An example of the potential role of aluminum in Alzheimer's disease arose with descriptions of "dialysis-associated encephalopathy" (DAE) where patients with insufficient kidney function received dialysis fluids inadvertently contaminated with high levels of aluminum [49]. The overall list of DAE features included, in sequence, speech abnormalities, tremors, impaired psychomotor control, memory loss, impaired concentration, behavioral changes, epileptic seizures and coma [49–52]. The condition generally progressed to coma and death within 3–7 years following the sudden overt manifestation of clinical symptoms in patients who had been on long-term dialysis treatment [9, 49]. High levels of aluminum in the brain were demonstrated in DAE patients as well as amyloid β accumulation [53, 54].

Patients showed rapid improvement when aluminum was removed from the dialysis fluid. It is significant that DAE as a clinical syndrome vanished once aluminum was removed from the dialysis solutions [49, 51]. It is of interest that later epidemiological studies examining ground water and Alzheimer's incidence levels found a link between dietary consumption of aluminum and the disease [55–57].

A number of studies have linked elevated aluminum levels to an increased risk of cognitive impairment and Alzheimer-type dementia [55, 57–59] especially in

conditions where silica content is low [59, 60]. Campbell et al. [61] showed that exposure to even low levels of aluminum (0.01, 0.1 and 1 mM) in drinking water for 10 weeks increased inflammatory processes selectively in mouse central nervous system. Other animal studies by Walton and others in aged rats showed significant cognitive impacts and pathological features following prolonged exposure to aluminum chloride. Other behavioral changes in rats exposed to aluminum at human dietary levels included confusion and repetitive behaviors [12, 62, 63].

Aluminum and Autism Spectrum Disorders (ASD)

The term "Autism spectrum disorders" describes a range of brain disorders that arise in infants or young children. Autism is typically characterized by delays in speech development and social functioning [64] that may never reach "normal" levels of function. By some estimates, in North America, there has been a sharp increase in the prevalence of autism by as much as 2000 % since the early 1990s [18]. A countervailing viewpoint is that autism has not changed in its yearly incidence over the last 20 years and that any apparent increases are due to (a) new and broader diagnostic criteria, (b) physicians more adept at diagnosing the condition [65] and/or (c) enhanced awareness by parents and pediatricians leading to a tendency to characterize unrelated conditions as ASD, (d) an increase in the general population, and (e) a changing gene pool. Of these, we note that (a) diagnostic criteria have not changed yearly although ASD has increased yearly; (b, c) the evidence to support these assertions appears to rests on assumptions rather than solid data; (d) the increase in the population of the United States since 1992 is closer to 35 %, not 2000 %; and (e) the occurrence of a massive shift in the genetics of the general population in a time span of only a few decades is highly unlikely.

The most conclusive data clearly show that autism prevalence has been increasing with time as shown by higher prevalence among younger groups [64, 66]. If autism rates have indeed increased since 1992, it seems reasonable to believe that some environmental factor, in combination with various genetic factors, may be responsible. What that environmental factor(s) is remains largely unknown, but the increase in various toxins in the human environment seems a likely starting point.

Clearly, as with GWS, there will be many such toxins to consider with a focus on those to which children might reasonably be exposed. Given the almost universal increase in the number of vaccines children routinely receive during their formative years [9, 18], and given the demonstrated neurotoxicity of at least some vaccine ingredients, much speculation has focused on two key vaccine components. These include mercury in the form of the preservative ethyl mercury (trademarked as thimerosal) and aluminum, the most common vaccine adjuvant as documented above [18, 67–69]. As mercury's potential role in ASD has been widely discussed in the literature [70–74], it will not be further discussed in the present review.

According to the Food and Drug Administration (FDA), vaccines represent a special category of drugs since they are generally given to healthy individuals, thus placing special emphasis on vaccine safety. The FDA sets an upper limit for aluminum in vaccines at no more than 850 μg (microgram)/dose; however, this amount was selected from data showing that aluminum in such amounts only enhanced the immunizing power of the vaccine (as cited in [18]). The FDA does not appear to have done any testing on the toxicological and safety issues of aluminum in vaccines [75].

Recently, Tomljenovic and Shaw [18] conducted a study to compare the Centers for Disease Control and Prevention (CDC) recommended vaccine schedules for children's vaccines in the United States (1991–2008) to changes in autism rates during this same period (US Dept. of Education) (original references in [18]).

The data sets, graphed against each other, show a pronounced and statistically highly significant correlation between the number vaccines with aluminum and the changes in autism rates (Fig. 7). Further data showed that a significant correlation exists between the amounts of aluminum given to preschool children and the current rates of autism in seven Western countries. Those countries with the highest level of aluminum-adjuvanted vaccines had the highest autism rates. This correlation was the strongest at 3–4 months of age, a period of rapid growth of the child's central nervous system, including synaptogenesis, maximal

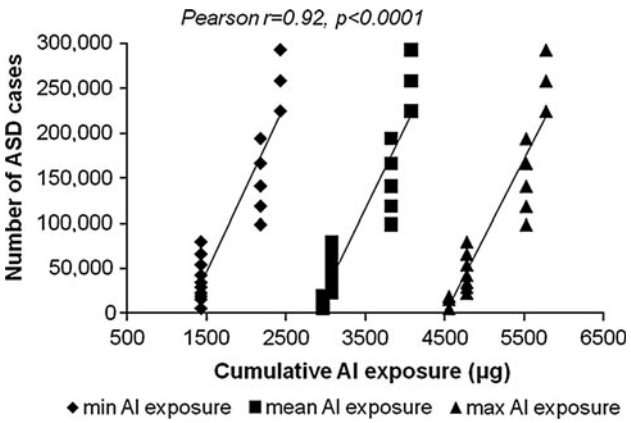

Fig. 7 Correlation between the number of children with ASD (6–21 years of age) and the estimated cumulative aluminum exposure (μg) from pediatric vaccines in the period from 1991 to 2008 (US data, see citations 18; adapted from [18]). The data satisfied eight of nine of the so-called Hill criteria for causality [81]

growth velocity of the regions of the brain responsible for short-term memory and the onset of growth of the amygdala, the latter involved in social interactions [76]. In addition, the period between 2 and 4 months in human infants also sees the development of neural systems regulating sleep, temperature, respiration and brain wave patterns [77]. Many of these brain functions are impaired in autism [78–80].

The observed correlation between the number of aluminum-adjuvanted vaccines and ASD was further tested using Hill's criteria and met eight of nine of these indicating that vaccines containing aluminum are highly likely to be at least partially causal for autism [81].

There are other links between aluminum exposure/toxicity and ASD. These include the following: A pilot study showed higher than normal aluminum levels in the hair, blood and/or urine of autistic children [6]; children are regularly exposed to higher levels of aluminum in vaccines per body weight than adults [18]; practically, nothing is known about the pharmacokinetics and toxicodynamics of aluminum in vaccines in children [82]; and aluminum in vaccines has been linked to serious neurological impairments, chronic fatigue and autoimmunity [26, 27, 83–85].

Animal studies support the human results. For example, as also cited above, injection of aluminum adjuvants at levels comparable to those that are administered to humans in vaccines has been shown to cause motor neuron death impairments in motor function and losses in spatial memory capacity in young mice (as cited above in [43, 44]). As well, injections of aluminum vaccines in 4-week-old mice were followed by a transient peak in brain aluminum levels on the second and third days after injection [86].

A common assertion made about aluminum in children's vaccines is that children obtain much more of this element from their diets, and hence, the small amount in most vaccines does not represent a significant risk factor for ASD [87]. However, this assertion contradicts basic toxicological principles because injected aluminum bypasses the protective barriers of the gastrointestinal tract and thus will likely require a lower dose to produce a toxic outcome [18]. In the case of aluminum, only $\sim$0.25 % of dietary aluminum is absorbed [88], while aluminum hydroxide (the most common form of aluminum used in vaccines) when injected may be absorbed by the body at nearly 100 % efficiency over time [89]. In addition, although the half-life of aluminum consumed through the diet is short (approximately 24 h), the same cannot be assumed for aluminum in vaccines because the molecular size of most aluminum in vaccines (24–83 kDa (137)) is higher than what the human kidney or other bodily filtering systems can process ($\sim$18 kDa [44] and indeed is contradicted by the results of Gherardi et al. [47].

Autoimmunity: do aluminum adjuvants play a role?

It is of interest to note that a typical vaccine formulation contains all the necessary components for the induction of an autoimmune disease. For example, vaccines contain antigens that may share mimetic epitopes with self-antigens ("molecular mimicry") and immune adjuvants, the most common of which is aluminum. Injection of an antigen itself in the absence of an adjuvant is typically insufficient to trigger an autoimmune reaction as noted by Glenney et al. years ago. In fact, in the absence of aluminum, most vaccine antigens (with the exception of live-attenuated viruses) fail to elicit an adequate immune response [20, 90, 91], suggesting that a significant part of vaccine-induced immune stimulation is driven by the aluminum adjuvant itself.

While the potency and toxicity of aluminum adjuvants should be adequately balanced so that the necessary immune stimulation is achieved with minimal side effects, such a balance can be difficult to accomplish in practice. This is because the same mechanisms that drive the immune stimulatory effect of adjuvants have the capacity to provoke a variety of autoimmune and/or inflammatory adverse reactions including those associated with the ASIA syndrome [26, 27, 67] Indeed, the immunotoxic effects of vaccine adjuvants are generally recognized to be a consequence of hyperstimulation of immunological responses [92, 93].

It is perhaps not surprising then to find that a potent "adjuvant effect" can overcome genetic resistance to autoimmunity. For example, while coadministration of coxsackievirus B3 (CB3) and *E. Coli* lipopolysaccharide (LPS) induces severe autoimmune myocarditis in C57BL/10 mice which are genetically resistant to autoimmunity, LPS alone has no such effect [94]. Similarly, while injection of C57BL/10 mice with myosin in combination with complete Freund's adjuvant fails to induce heart disease, coadministration of myosin, complete Freund's adjuvant and LPS has the opposite effect [94]. The fact that coadministration of as little as 2–3 immune adjuvants can overcome the genetic resistance to autoimmune diseases is often overlooked in the current design of vaccination schedules. For example, 2-month-old infants receive a total of 22 viral bacterial antigens (most of which are adsorbed onto aluminum) and 4 attenuated viruses following the current US vaccination recommendations for preschool children [67].

As noted above, autism incidence appears to have increased dramatically in the last few decades, and this increase is strongly correlated with an increase in the number of required pediatric vaccinations, most of which contain some form of aluminum. Autoimmune manifestations, particularly those affecting the CNS, are prevalent in autistic

individuals and are not restricted to only few CNS antigens. For example, Vojdani et al. [95] demonstrated elevated levels of autoantibodies against nine different neuron-specific antigens in autistic children. Such widespread manifestation of autoimmunity is indicative blood–brain barrier (BBB) disruption, as this would enable unrestrained access of immunocompetent cells to many different CNS antigens. There is substantial evidence that the BBB is indeed disrupted in autism and that this disruption, thought to be caused by environmental inflammatory stress triggers, leads to neuroinflammation and autoimmunity. Aluminum is known to damage the BBB and can increase its permeability by increasing the rate of transmembrane diffusion and by selectively altering saturable transport systems [96–98]. The breakdown of the BBB by aluminum may also result from excessive release of pro-inflammatory cytokines from aluminum-stimulated microglia [99, 100]. The ability of aluminum adjuvants to cross the BBB [47, 86] and up-regulate chemoattractants such as MCP-1 [91] could promote active recruitment of immunocompetent cells to the brain, leading to both widespread autoimmunity and deleterious inflammatory processes.

Compelling evidence for a causal role of aluminum adjuvants in triggering serious autoimmune disorders has been presented by Quiroz-Rothe et al. [92] who described a case of postvaccination polyneuropathy resembling Guillain–Barré syndrome in a dog. In this case, there was an apparent cause–effect relationship between vaccination and onset of clinical signs associated with the presence of antibodies against myelin. The authors noted that the vaccines used were obtained by cultures in renal cells and did not contain nervous tissue antigens. Thus, either viral or other vaccine antigens, or the adjuvants included in the vaccines, might have triggered the formation of anti-myelin antibodies by over stimulation of the dog's immune system. However, the fact that two different vaccines from two different manufacturers were involved strongly suggests a polyclonal activation induced by the vaccine adjuvants without the participation of myelin as the more probable pathogenesis.

Other controlled studies in dogs vaccinated with commercially available rabies and canine distemper vaccines showed a significant increase in the titers of IgG antibodies reactive with 10 autoantigens, an effect not observed in unvaccinated dogs [101]. Although molecular mimicry or a "bystander activation" of self-reactive lymphocytes could be the cause for these autoimmune manifestations, the relatively large number and variety of autoantigens observed (as in the cases of autistic children) point to a polyclonal activation or adjuvant reaction. Moreover, this adjuvant effect, associated with the development of a wide range of autoantibodies, has been typically associated with vaccines containing higher levels of adjuvants [102].

Altogether, these observations are consistent with both the neurotoxic and immunotoxic properties of aluminum. First, aluminum can compromise the integrity of the BBB, thus exposing the CNS to circulatory immunocompetent cells and pro-inflammatory mediators. In turn, aluminum stimulates the recruitment of these same immune mediators to the brain. As shown by the recent studies of the Gherardi group, aluminum adjuvant nanoparticles, taken up by monocytes after injection, first translocate to draining lymph nodes, then travel across the BBB and eventually accumulate in the brain where they can cause significant immune-inflammatory adverse reactions [47].

In summary, the above research clearly shows that hyperstimulation of the immune system by various adjuvants, including aluminum, carries an inherent risk for serious autoimmune disorders affecting the CNS. In this regard, the fact that the levels of adjuvants typically administered to vulnerable populations (i.e., infants and preschool children) have never undergone appropriate toxicity evaluations in animal models may be a cause for concern as highlighted by the various reevaluations of the clinical literature [67].

Emerging issues

The current review has demonstrated a range of neurological disorders that might arise due to exposure to aluminum. Two broad categories have emerged from this analysis: neurodevelopmental and age-related neurodegenerative. While these outcomes appear to be temporally distinct, there are clear caveats to both category and time of occurrence. For example, although ASD is clearly a neurodevelopmental disorder, neuronal damage may also occur. In regard to this aspect, we do not yet know whether such neuronal damage will serve as a precursor to the neurodegenerative diseases associated with aging.

One aspect that separates the two ends of the aluminum-induced neurological disorder spectrum is the route of administration, for example, injection versus oral. The first can be expected to have relatively rapid effects that, depending on age, can range from days to years. The latter may take years to reach a critical body burden or to trigger the end-state outcomes that are likely the result of a cascade of various pathological events. But, as above, these may not be stringent distinctions. For example, injected aluminum adjuvants in adults can trigger forms of cognitive impairment [103].

It is not really a matter of much debate that aluminum in various forms can be neurotoxic. Rather, the questions that remain are these: How crucial to the various age-related neurological deficits are routes of administration and genetic susceptibility? What role does gender play in sensitivity to aluminum toxicity and why? And, finally, can the

forms of aluminum-induced neurological deficits discussed be subsumed under the broad rubric of autoimmune disorders?

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