# Autism Spectrum Disorders and Aluminum Vaccine Adjuvants

Lucija Tomljenovic, Russell L. Blaylock, and Christopher A. Shaw

## Introduction

Aluminum (Al) is the most abundant neurotoxic element in the Earth's crust and widely bioavailable to humans. Al is present in drinking water, food additives, cosmetics, pharmaceuticals including vaccines, and because of such ubiquity, it is increasingly found in our bodies (Tomljenovic 2011). None of this would necessarily be a problem if Al was inert in biological systems. However, in spite of a widely held belief that this is true, it is demonstrably not the case. Al is highly reactive with oxygen and carbon, two of the most abundant organic elements, yet appears to have no beneficial role in organic chemistry of any biota on the planet at any concentration. Rather, research shows that Al is toxic to all forms of life (Exley 2009). The mechanisms by which Al exerts toxicity are numerous. Al is genotoxic, prooxidant, proinflammatory, and immunotoxic (Tomljenovic 2011; Tomljenovic and Shaw 2011a, b; Blaylock 2012; Lujan et al. 2013). Additionally, Al is an endocrine disruptor; it depresses glucose metabolism and interferes with many other essential cellular processes such as calcium homeostasis, various

L. Tomljenovic (✉)
Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada
e-mail: lucijat77@gmail.com

R.L. Blaylock
Theoretical Neurosciences Research, LLC, Belhaven University, Jackson, MS, USA
e-mail: blay6307@bellsouth.net

C.A. Shaw
Departments of Ophthalmology and Visual Sciences and Experimental Medicine, and the Graduate Program in Neuroscience, University of British Columbia, Neural Dynamics Research Group, Vancouver, BC, Canada
e-mail: cashawlab@gmail.com

V.B. Patel et al. (eds.), *Comprehensive Guide to Autism*,
DOI 10.1007/978-1-4614-4788-7_89,

ATP-dependent mechanisms, membrane receptor signalling, and mitochondrial function (Agarwal et al. 1996; Tomljenovic 2011).

Experimental and clinical data have clearly identified the central nervous system (CNS) as the most sensitive target of Al's toxic effects. The neurotoxicity of Al typically manifests in learning, memory, concentration and speech deficits, impaired psychomotor control, increased seizure activity, and altered behavior (i.e., confusion, anxiety, repetitive behaviors, and sleep disturbances (Tomljenovic 2011)). Many of these altered features of CNS function typical of Al intoxication are also typical of autism. Because infants and children are routinely exposed to the immune adjuvant form of Al through vaccination programs, there are increasing concerns that such exposures might in part be contributing to the growing burden of neurodevelopmental and immune syndromes, particularly those of the autism spectrum (Seneff et al. 2012; Melendez et al. 2013). The purpose of this chapter is to discuss the mechanisms by which Al vaccine adjuvants might cause alterations to the developing neuroimmune system and, in turn, how these adjuvant-mediated mechanisms might be relevant for the pathogenesis of autism.

## Al Adjuvants: The Biological Basis for Neurological and Immune Toxicity

There is a growing body of data that support a significant role for immune molecules in the etiology of a variety of neurological disorders (Theoharides et al. 2009; Garay and McAllister 2010). For example, epidemiological data have consistently correlated the risk of schizophrenia to hyperactivation of the peripheral immune system and autoimmunity (Ortega-Hernandez et al. 2009). Further, gene association analyses reveal that there is a strong link between genetic variants of classical complement cascade major histocompatibility complex (MHC) class I and II molecules and neurological disorders such as schizophrenia (Havik et al. 2011), autism (Pardo et al. 2005), and Alzheimer's disease (Lambert et al. 2009). These observations point to a common mechanism underlying several neurological disorders that have been conventionally viewed as unrelated, namely, a disease mechanism arising from altered activity of immune-related pathways in the brain. What role might Al adjuvants play in such neuroimmune disorders? Part of the answer lies in characterization of Al as the neurotoxin (Tomljenovic 2011; Blaylock 2012). Another part is provided by recent identification of an "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA) that encompasses several adjuvant-triggered medical conditions which are characterized by a misregulated immune response (Shoenfeld and Agmon-Levin 2011; Table 1). In other words, experimental evidence indicates that Al adjuvants have all the necessary biochemical properties to induce neuroimmune disorders, including those of the autism spectrum (Table 2).

**Table 1** Autoimmune/inflammatory disorders linked to vaccines

| Disease | References |
|---|---|
| Neurodevelopmental disabilities/autism[a,b] | (Gallagher and Goodman 2010; Tomljenovic and Shaw 2011b; Seneff et al. 2012) |
| Multiple sclerosis[a,b,c] | (Authier et al. 2001) |
| Guillain-Barré syndrome[a] | (Khamaisi et al. 2004) |
| Acute disseminated encephalitis[a] | (Mendoza Plasencia et al. 2010) |
| Necrotizing encephalopathy[a] | (Aydin et al. 2010) |
| Status epilepticus and lymphocytic pneumonitis[a] | (Carvalho and Shoenfeld 2008) |
| Demyelinating leukoencephalitis[a] | (Konstantinou et al. 2001) |
| Vasculitis | (Orbach et al. 2010) |
| Macrophagic myofasciitis[a,c] | (Gherardi and Authier 2012) |
| Chronic fatigue[a,b,c] | (Exley et al. 2009) |
| Gulf War syndrome[a,c] | (Shoenfeld and Agmon-Levin 2011) |
| Arthralgia/arthritis[a,b,c] | (Shoenfeld and Agmon-Levin 2011) |
| Lupus[a,b,c] | (Orbach et al. 2010) |

[a]Linked to Al-adjuvanted vaccines
[b]Linked to hypothalamic-pituitary adrenal (HPA) axis dysfunction (Eskandari et al. 2003; Porges 2005)
[c]Specifically recognized as "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA)

**Table 2** Shared aspects between the neuroimmunotoxic effects of Al adjuvants and pathological findings in autism

| **Type of Al adjuvant effect** | **References** |
|---|---|
| Accelerated and prolonged immune system hyperstimulation | (Israeli et al. 2009; Lujan et al. 2013) |
| Autoimmunity | (Israeli et al. 2009; Shoenfeld and Agmon-Levin 2011) |
| Increased brain inflammatory markers | (Li et al. 2009; Shaw and Petrik 2009; Lujan et al. 2013) |
| Increased micro- and astrogliosis | (Petrik et al. 2007; Shaw and Petrik 2009) |
| Increased reactive oxygen species/oxidative stress | (Exley et al. 2010) |
| Disruption of the blood-brain barrier | (Khan et al. 2013; Lujan et al. 2013) |
| Increased immune cell infiltration in the brain | (Khan et al. 2013; Lujan et al. 2013) |
| Excitotoxicity/neuronal death | (Shaw and Petrik 2009; Lujan et al. 2013) |
| Cognitive deficits, decreased performance in learning tasks | (Petrik et al. 2007; Shaw and Petrik 2009) |
| Abnormal behavior, restlessness and compulsive behavior | (Lujan et al. 2013) |
| Triggering of mast cell-dependent allergic sensitization to food proteins, intestinal inflammation and diarrhoea | (Berin and Mayer 2009) |

## Al Adjuvants: A Toxicological Risk for a Developing Brain

Al is perhaps best known for its powerful immunomodulatory properties and it is precisely because of these that it has been, and remains, the most commonly used vaccine adjuvant. The immune-enhancing effects of Al were discovered in 1926 and Al has been used in vaccines ever since (Glenney et al. 1926). During the last four decades, the number of vaccinations required for preschool entry in developed countries has significantly increased (i.e., from <10 in the late 1970s to >30 in 2010 (Tomljenovic and Shaw 2011b)), and this trend is likely to continue as more vaccines are currently being approved for use.

Al has potent and multifactorial stimulatory effects on the immune system (Exley et al. 2010). Other than attenuated viruses, in the absence of Al, most antigenic compounds fail to launch an adequate immune response (Israeli et al. 2009), suggesting that a significant part of vaccine-induced immune stimulation may be driven by the Al adjuvant itself. Consequently, many routinely used vaccines contain some form of Al (Table 3).

The adjuvant-mediated immune-enhancing effect is accomplished via mechanisms that impinge on both the innate and adaptive immune systems (Eisenbarth et al. 2008; Exley et al. 2010). While the potency and toxicity of Al adjuvants should be adequately balanced so that the necessary immune stimulation is achieved with minimal side effects, such 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 (Tables 1 and 2).

Although historically vaccine Al adjuvants have been portrayed as inherently safe (Offit and Jew 2003), studies in animal models and humans have demonstrated their ability to inflict immuno-inflammatory conditions by themselves (Authier et al. 2001; Petrik et al. 2007; Couette et al. 2009; Israeli et al. 2009; Shaw and Petrik 2009; Gherardi and Authier 2012; Lujan et al. 2013). The prolonged hyperactivation of the immune system and chronic inflammation triggered by repeated exposure are thought to be the two principal factors underlying the toxicity of Al adjuvants. In particular, Al's interference with the endocrine and the immune system's regulatory pathways may trigger proinflammatory and prooxidative cascades with detrimental effects on the brain's development and function (Tomljenovic and Shaw 2011b; Blaylock 2012; Lujan et al. 2013). Because of this, there are increasing concerns that closely spaced pediatric vaccinations might be contributing to the growing burden of autistic disorders (Tomljenovic and Shaw 2011b; Blaylock 2012; Seneff et al. 2012). Al is however used in vaccines at concentrations viewed as an acceptable compromise between adjuvanticity and toxicity by the industry and regulatory agencies. Nonetheless, as listed below, there are many significant problems associated with using a neurotoxic substance such as Al as an immune stimulator in vaccinations routinely administered to the most vulnerable populations, i.e., infants, children, and pregnant women.

Compelling evidence has underscored the tight connection between the development of the immune system and that of the CNS. Thus, it appears plausible that

**Table 3** Summary of antigenic components in vaccines in the 2010 US vaccination schedule. Vaccine components were sourced directly from the manufacturers' monographs

| Age of inoculation | Birth | 2 months | 4 months | 6 months | 12 months | 18 months | 24 months | 4–6 years | |
|---|---|---|---|---|---|---|---|---|---|
| Vaccine (# of bacterial antigens/ # of live attenuated viruses) | EngerixB* (1/0) | Infanrix-IPV* (5/3) | Infanrix-IPV* (5/3) | EngerixB* (1/0) | Pedvax* (2/0) | Daptacel* (5/0) | Fluviral (0/3) | Daptacel* (5/0) | |
| | | Comvax* (3/0) | Pedvax* (2/0) | Infanrix-IPV* (5/3) | Prevnar* (14/0) | Havrix* (0/1) | | Imovax Polio (0/3) | |
| | | Prevnar* (14/0) | Prevnar* (14/0) | Prevnar* (14/0) | Imovax Polio (0/3) | | | MMR-II (0/3) | |
| | | Rotarix (0/1) | Rotarix (0/1) | | MMR-II (0/3) | | | Varivax (0/1) | |
| | | | | | Varivax (0/1) | | | Fluviral (0/3) | |
| | | | | | Havrix* (0/1) | | | | |
| | | | | | Fluviral (0/3) | | | | |
| Total # bacterialantigens | 1 | 22 | 21 | 20 | 16 | 5 | 0 | 5 | 90 |
| Total # live attenuated viruses | 0 | 4 | 4 | 3 | 11 | 1 | 3 | 10 | 36 |

*denotes vaccines containing Al adjuvants. EngerixB, hepatitis B vaccine; Infanrix-IPV, diphtheria-pertussis-tetanus-polio hexavalent vaccine; Comvax, *Haemophilus influenzae type b*/hepatitis B bivalent vaccine; Prevnar, pneumococcal vaccine; Rotarix, rotavirus vaccine; Pedvax, *Haemophilus influenza* type b vaccine; Imovax Polio, poliovirus trivalent vaccine; MMR-II, measles-mumps-rubella trivalent vaccine; Varivax, varicella virus vaccine; Havrix, hepatitis A virus vaccine; Fluviral, trivalent influenza virus vaccine; Daptacel, diphtheria-pertussis-tetanus trivalent vaccine

disruptions of critical events in immune development may also play a role in the establishment of neurobehavioral disorders (Dietert and Dietert 2008). Indeed, immune stimulation (including vaccine-induced) during critical windows of developmental vulnerability both pre- and postnatal has been shown to produce behavioral outcomes and neuroanatomical features typical of autism in animal models (Table 4).

Despite the prevalent view that peripheral immune responses do not affect brain function, overwhelming experimental evidence clearly points to the contrary (Besedovsky and Rey 2008). It is well established that there is a complex network of interactions between the brain and the immune system which plays a crucial role in immune regulation, brain function, and maintenance of general homeostasis (Fig. 1). In turn, perturbations of this "immune-neuroendocrine" network have been demonstrated in autism and a variety of other immuno-inflammatory conditions, including those linked to vaccinations (Table 1).

During prenatal and early postnatal life, the brain is extremely vulnerable to neurotoxic insults (Johnston 1995; Olney 2002). Both of these are periods of rapid structural and functional development of the CNS and thus highly sensitive to extrinsic disturbances. Any particular CNS structure or function will be most sensitive to disruption during its critical period of formation (Olney 2002; Dietert and Dietert 2008; Garay and McAllister 2010).

The immature renal system of neonates diminishes their ability to eliminate environmental neurotoxins. Because of this, children are at much greater risk of adverse reactions from Al than adults (Dorea and Marques 2010; Tomljenovic and Shaw 2011b). Moreover, the sizes of most Al-adsorbed vaccine complexes are higher than the molecular weight cutoff of the glomerulus, which would likely preclude efficient excretion of Al adjuvants (Tomljenovic and Shaw 2011b). A longer elimination period is in fact one of the major properties of effective vaccine adjuvants. Notably, long-term persistence of Al, up to 8–10 years following vaccination, has been demonstrated in adult patients suffering from adjuvant-induced autoimmune diseases (Gherardi et al. 2001).

Adjuvant Al is recognized as a plausible trigger for several serious postvaccination autoimmune/inflammatory conditions including those affecting the CNS (Authier et al. 2001; Couette et al. 2009; Passeri et al. 2011; Lujan et al. 2013). For example, deficits in cognitive and psychomotor functions, behavioral disturbances, and MRI abnormalities showing demyelinating inflammatory lesions associated with long-term retention of Al adjuvants have been observed in adult patients who received between 1 and 8 Al-containing vaccines over the course of several years (Authier et al. 2001). Yet, in spite of these observations, infants and children in most developed countries routinely receive up to 18 Al-adjuvanted vaccines through pediatric vaccination schedules (Tomljenovic and Shaw 2011b, 2012).

Because vaccine injections containing Al bypass the usual biological barriers to absorption (i.e., gastrointestinal), they confer maximal exposure (Tomljenovic and Shaw 2011a, b, 2012). Moreover, since there are no known physiological roles for Al within the human body, its accumulation can only be considered as potentially deleterious.

**Table 4** Shared aspects between autism and abnormal neuroimmune outcomes resulting from repeated peripheral immunostimulation by various stimuli in the pre- and postnatal period. Abbreviations: Poly I:C polyriboinosinic-polyribocytidilic acid, a synthetic analogue of double-stranded RNA (viral antigen); LPS *E. coli* lipopolysaccharide

| Types of abnormalities | Type of immune stimuli/time of stimulation | Species | Outcome | Autism |
|---|---|---|---|---|
| Neurobehavioral | Poly I:C/early postnatal | Mouse, rat | Deficits in social interaction, increased anxiety (Ibi et al. 2009; Konat et al. 2011) | Impaired social skills, increased anxiety, and stereotypic behavior (Theoharides et al. 2009) |
| | LPS/early postnatal | Rat | Altered responses to novel situations (i.e., reluctance to explore a novel object) (Spencer et al. 2005) | Anxiety to novel situations, preference for routine (Jansen et al. 2000; White et al. 2011) |
| | Poly I:C/early postnatal | Mouse | Cognitive dysfunction (i.e., memory deficits (Ibi et al. 2009)) | Cognitive dysfunction and mental retardation (Fombonne 1999) |
| Neuroanatomical | Poly I:C/prenatal | Mouse | Compromised neurogenesis and abnormal formation of the cerebral cortex (Soumiya et al. 2011) | Abnormal neuronal morphology and cytoarchitecture of the cerebral cortex (Herbert et al. 2003) |
| | Complete US pediatric vaccine schedule/ postnatal, according to schedule | Monkey | Failure to undergo normal maturational changes in amygdala volume (Hewitson et al. 2010) | Impaired amygdala development (Herbert et al. 2003) |
| Neurochemical | Poly I:C/early postnatal | Mouse | Increased extracellular glutamate in the hippocampus (Ibi et al. 2009) | Increased glutamate in the amygdala-hippocampal region (Purcell et al. 2001) |
| | LPS/early postnatal | Rat | Increased seizure susceptibility (Galic et al. 2008) | Increased seizures and epilepsy (Tuchman and Rapin 2002) |
| Immune | LPS/early postnatal | Rat | Abnormal cytokine profiles (Spencer et al. 2007) | Abnormal cytokine profiles (Pardo et al. 2005; Vargas et al. 2005) |
| | Al adjuvant/early adulthood | Mouse | Increased astrocyte and microglia reactivity (Petrik et al. 2007; Shaw and Petrik 2009) | Increased astrocyte and microglia reactivity (Pardo et al. 2005; Vargas et al. 2005) |
| | LPS/early postnatal | Rat | Exacerbation of inflammatory conditions (Spencer et al. 2007) | Immune hypersensitivity (Dietert and Dietert 2008) |

Recent animal experiments have demonstrated that Al adjuvant nanoparticles have a unique capacity to cross the blood-brain and blood-cerebrospinal fluid barriers and incite deleterious immuno-inflammatory responses in neural tissues (Khan et al. 2013; Lujan et al. 2013). Moreover, the bioaccumulation of Al in the brain appeared to occur at a very low rate in normal conditions, thus potentially explaining the good overall tolerance of this adjuvant despite its strong neurotoxic potential. Nonetheless, according to Khan et al. (2013), continuously increasing doses of the poorly biodegradable Al adjuvant may become insidiously unsafe, especially in cases of repetitive closely-spaced vaccinations and immature/altered blood brain barrier (BBB).

The immuno-stimulatory properties of Al have been routinely exploited for inducing mast cell-dependent food allergies in experimental animal models (Table 2). Mast cells play key roles in a wide range of inflammatory gastrointestinal pathologies in which they compromise mucosal immunity and increase intestinal permeability (Berin and Mayer 2009; Theoharides et al. 2009). Gastrointestinal dysfunction and food allergies are the most common non-neurological comorbidities in autism, and mast cell activation is strongly implicated as the underlying factor (Theoharides et al. 2009).

The above observations suggest that increasing concerns about the safety of Al-adjuvanted vaccines are warranted and deserving of more serious consideration than what has been provided to date.

## Al Adjuvants and Sequential Systemic Immune Stimulation: Implications for Neurodevelopmental Disorders

The mechanism by which peripheral (systemic) immune stimulation affects responses in the brain is critical to understanding the potential role of Al adjuvants in neurodevelopmental disorders of the autism spectrum. An important advance in understanding of the function of the normal and the diseased brain was the recognition that there is an extensive communication between the immune system and the CNS (Fig. 1). As a result of this neuroimmune cross talk, neural activity can be significantly altered in response to immune stimuli (Dantzer and Kelley 2007; Besedovsky and Rey 2008; Barrientos et al. 2012). Such stimuli lead to de *novo* production of proinflammatory cytokines within the brain by the activated microglia, the brain's resident immune cells (Barrientos et al. 2012). It should be emphasized that repeated activation of resting microglia can induce an irreversible shift of these cells to a neurodestructive proinflammatory and excitotoxic phenotype (Blaylock and Maroon 2011; Barrientos et al. 2012; Blaylock 2012).

Early observations have linked systemic immune stimulation to changes in behavior and neurological pathology, based on the common constellation of neurological outcomes associated with viral illnesses, such as the flu (i.e., impairments in cognition, memory, learning and attention, social withdrawal, irritability, and depression). Extensive research on this phenomenon, otherwise known as "sickness behavior," suggests that it is caused by elevations in proinflammatory cytokines

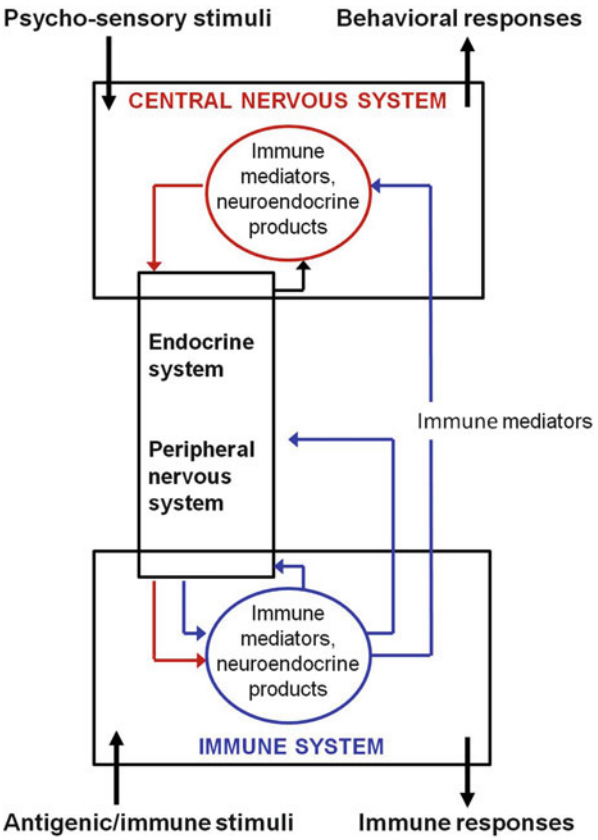


**Fig. 1** Schematic depicting the "immune-neuroendocrine" network. A multileveled network of interactions between the nervous, endocrine, and the immune system. The interactions within this network can be influenced by psycho-sensorial and immune stimuli. For example, immune cytokines released during activation of peripheral immune responses serve as afferent messengers from the immune system to the central nervous system (*CNS*) and can induce production of cytokines in the brain. In turn, CNS-derived cytokines can trigger neuroendocrine responses purposed to regulate the activity of immune cells and organs. Antigenic/immune stimuli can thus profoundly influence behavioral responses (i.e., "sickness behavior"). Likewise, psycho-sensorial stimuli can influence immune responses. The HPA axis is part of this network (denoted in *red*). The integrity of functional interactions between the neuroendocrine and the immune axis is required for homeostasis, which by necessity implies that disturbances at any level within the network have a potential to trigger deleterious neuroimmunological outcomes (Adapted from Besedovsky and Rey 2008)

such as IL-1β, IL-6, and TNF-α (Dantzer and Kelley 2007), which cause fever and alter the levels of key neurotransmitters (Dunn 2006).

Proinflammatory responses arising from peripheral immune stimuli early in the postnatal period are even more detrimental, because they also result in accumulation of proinflammatory cytokines and excitotoxic levels of the neurotransmitter glutamate within the brain, thus promoting inflammation and disrupting neural

development (Ibi et al. 2009; Du et al. 2011). Moreover, such immune stimuli can increase CNS vulnerability to subsequent immune insults and the latter can then permanently impair CNS function (Bilbo et al. 2005; Galic et al. 2008). For example, in rats, a single peripheral immune stimulus with either bacterial antigens or viral mimetics at postnatal days 4 or 14 was sufficient to cause an increase in brain inflammatory markers in the hippocampus and cortex, impairments in memory, anxiety-like behaviors, and a long-lasting increase in seizure susceptibility (Table 4). All of these outcomes are in various degrees seen in autistic children. For example, in addition to anxiety, a large proportion of autistic individuals (40 %) also suffer from seizures (Tuchman and Rapin 2002), while an even larger proportion (70 %) are cognitively impaired (Fombonne 1999).

Repeated administration of bacterial and viral antigens (most of which are adsorbed to Al adjuvants) through present vaccination schedules (Table 3) is clearly analogous both in nature and timing to peripheral immune stimulation with microbial mimetics in experimental animals during early periods of developmental vulnerability. If administered during these periods (including early postnatal), such potent immune stimuli not only produce adverse neurodevelopmental outcomes (Table 4) but can also permanently impair immune responses to subsequent immune challenges (Boisse et al. 2004; Galic et al. 2009), which in turn may compromise the ability of the host to cope with a wide range of immune and inflammatory disorders later in life (Spencer et al. 2007; Galic et al. 2009). In spite of these observations, pediatric vaccinations have been historically exonerated as a plausible cause for the growing burden of neurodevelopmental and immune abnormalities in children (Gerber and Offit 2009).

Data show that many cytokines induced by an immune response (including adjuvant-mediated) can act as "endogenous pyrogens," that is, they can induce a rapid-onset fever by acting directly on the hypothalamus without the requirement for the formation of another cytokine (i.e., IL-1β, IL-6, TNF-α (Besedovsky and Rey 2008)). While transient fever is an essential component of the early immune response to infection, the prolonged febrile response is a hallmark of many inflammatory and autoimmune diseases (Dinarello 1999). Moreover, fever-promoting cytokines produced in peripheral tissues upon immune stimulation can enter the brain via the circumventricular organs (Dinarello 1999; Eskandari et al. 2003), which are among the few sites in the brain devoid of a BBB, and thus promote brain inflammation. That persistent hyperinflammation of the CNS plays a prominent role in the development of autism can hardly be disputed in view of existing data (Pardo et al. 2005; Vargas et al. 2005).

Given thus that the very nature of peripheral immune stimulation can negatively influence brain function, the possibility that such outcomes could also occur with administration of Al adjuvants needs to be considered (Fig. 2). Cohen and Shoenfeld noted that: "It seems that vaccines have a predilection to affect the nervous system" (Cohen and Shoenfeld 1996, 699). Indeed, CNS deficits in humans are now an established side effect triggered by Al adjuvants (Authier et al. 2001; Couette et al. 2009; Gherardi and Authier. 2012). In this context, the latest research by Lujan et al. (2013) who described a severe neurodegenerative syndrome in

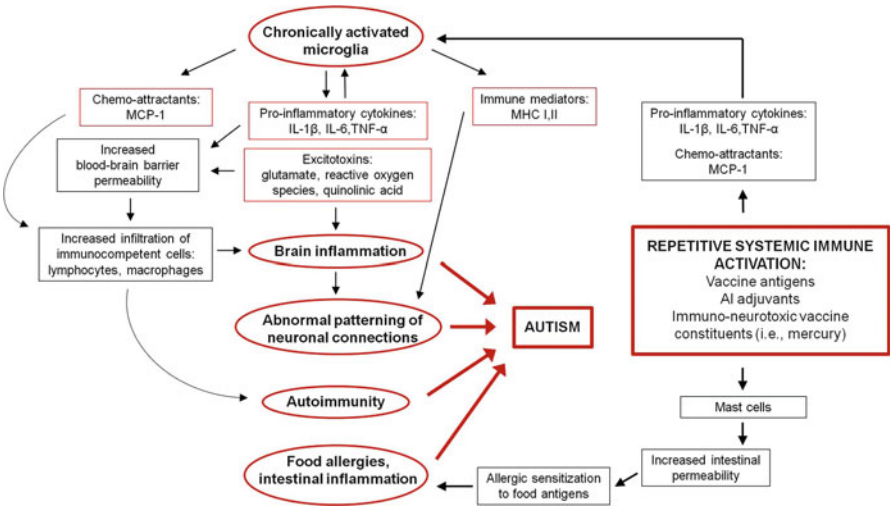


**Fig. 2** Schematic depicting how repetitive sequential immune stimulation by Al-adjuvanted vaccines may promote brain inflammation and autoimmunity, disrupt immune pathway-dependent structural and functional patterning of the brain, and trigger allergic sensitization to dietary antigens and gastrointestinal dysfunction, thus ultimately leading to autism

commercial sheep, linked to the repetitive inoculation of Al-containing vaccines, is noteworthy. In particular, the "sheep adjuvant syndrome" mimics in many aspects human neurological diseases linked to Al adjuvants (Lujan et al. 2013). Moreover, the adverse chronic phase of this syndrome affects 50–70% of flocks and up to 100% of animals within a flock. It is characterized by severe neurobehavioural outcomes (restlessness, compulsive wool biting, generalized weakness, muscle tremors, loss of response to stimuli, ataxia, tetraplegia, stupor, coma and death), inflammatory lesions in the brain and the presence of Al in central nervous system tissues (Lujan et al. 2013). These latter findings thus confirm the ones by Khan et al. (2013) who demonstrated the ability of Al adjuvants to penetrate the BBB, and further, they show that the resulting presence of Al in the brain can trigger severe neurological damage with devastating consequences. The findings by Lujan et al. (2013) and Khan et al. (2013) may also in part explain why the vast majority of reported adverse reactions following vaccinations are neurological and neuropsychiatric (Zafrir et al. 2012).

Al adjuvants activate 312 genes, 168 of which play a role in immune activation and inflammation (Mosca et al. 2008). At least 13 cytokines and chemokines are produced within 4 h of Al adjuvant injection, including proinflammatory IL-1β and IL-6 (McKee et al. 2009). Such strong peripheral immune stimuli can potentiate the brain's innate immune system responses mediated by the microglia (Fig. 2). Indeed, only two subcutaneous injections of Al adjuvants (relevant to adult human exposure) in young male mice, spaced 2 weeks apart, were sufficient to cause dramatic activation of microglia and astrocytes that persisted up to 6 months postinjection.

Moreover, micro- and astrogliosis induced by Al in mice were accompanied by motor neuron death, deficiencies in cognition, and decreased performance in learning and motor tasks (Petrik et al. 2007; Shaw and Petrik 2009). Similarly, repeated peripheral injections of 1 mg/kg of Al nanoparticles to adult rats resulted in a significant activation of astrocytes and phagocytic microglia/macrophages in various regions of rat brains, especially the perivascular area (Li et al. 2009). It should be noted that comparable amounts of Al are routinely administered to 2-, 6-, and 15-month-old infants according to the US vaccination schedule (Tomljenovic and Shaw 2011b). Furthermore, because proinflammatory insults to the cerebral vasculature compromise the integrity of the BBB (Aydin et al. 2010; Theoharides and Zhang 2011), they can increase its permeability to circulating inflammatory mediators (Prat et al. 2001), such as adjuvant-activated cytokines and immune cells. These inflammatory mediators would further act as a continuous source of microglial stimuli (Fig. 2).

Repeated activation of primed microglia amplifies their proinflammatory and neurodestructive potential (Blaylock and Maroon 2011; Barrientos et al. 2012). Of note, IL-1β, one of the key proinflammatory cytokines released by the activated microglia (Barrientos et al. 2012), is also the main cytokine induced by Al adjuvants through their stimulation of the NLRP3 inflammasome complex (Eisenbarth et al. 2008; Li et al. 2008).

An exaggerated neuroinflammatory response of the pre-sensitized microglia which is mediated by proinflammatory cytokines appears to be a key causative factor in aging-associated decline of CNS function (Barrientos et al. 2012). Excessive activation of microglia is also a prominent neuropathological feature in autism (Pardo et al. 2005; Vargas et al. 2005). Altogether, these observations indicate that a dysregulated/hyperactive innate immune response in the brain underlies both neurodevelopmental dysfunction in autism and neurodegeneration associated with aging.

In summary, the fact that peripheral immune stimuli in the postnatal period can increase vulnerability of the developing brain to subsequent immune-stimulatory insults and the latter lead to permanent CNS impairment (Bilbo et al. 2005; Galic et al. 2008) is of particular concern in view of current pediatric schedules which often require repeated and simultaneous administration of Al-adjuvanted immune complexes soon after birth and throughout the first year of life (Table 3). Of additional concern is the fact that Al in the adjuvant form can accumulate in the brain (Redhead et al. 1992; Khan et al. 2013) and the spinal cord (Shaw and Petrik 2009; Lujan et al. 2013). Repeated exposure as well as retention of Al adjuvants in these CNS areas would serve both as an initial (priming) and subsequent immune stimuli to the microglia, thus augmenting their neurodestructive potential (Fig. 3). Collectively, these observations may explain why vaccines have a marked tendency to affect the nervous system (Cohen and Shoenfeld 1996; Carvalho and Shoenfeld 2008) and why, as noted above, the common underlying component to a vast majority of Al adjuvant-containing vaccine-induced diseases of the CNS appears to be a misregulated, unrestrained, and persistent inflammatory response (Quiroz-Rothe et al. 2005; Lujan et al. 2013).

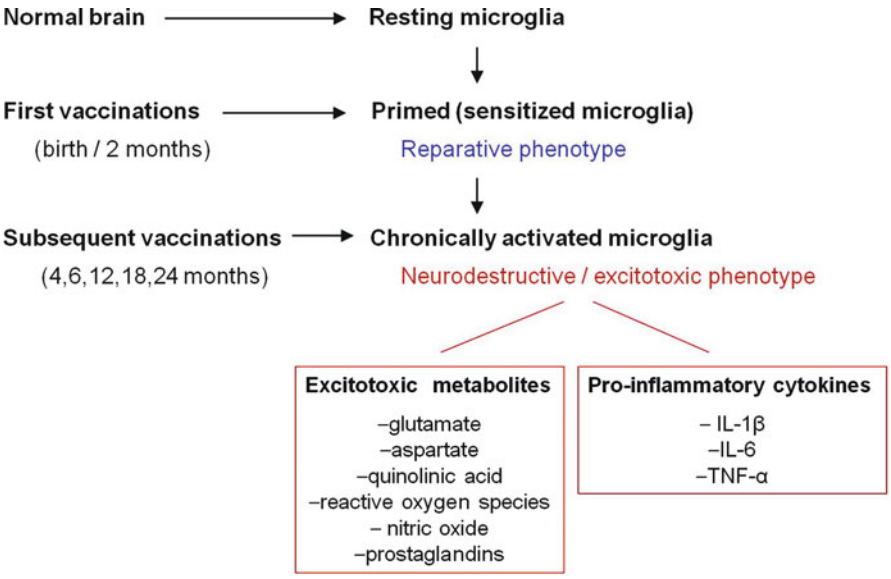


**Fig. 3** Schematic depicting how sequential vaccination induces a phenotypic shift of the microglia from the resting to the chronically activated

## (How) Do Al Adjuvants Play a Role in Perturbing the HPA Axis in Autism?

IL-1β, the key proinflammatory cytokine released following NLPR3 inflammasome activation by Al adjuvants, exhibits multifactorial effects on the immune system (Eisenbarth et al. 2008; Li et al. 2008). IL-1β is also known to activate neurons in the central nucleus of the amygdala (Buller and Day 2002), and this nucleus plays a major role in hypothalamic-pituitary adrenal (HPA) axis response to systemic immune stimulation (Xu et al. 1999). Abnormalities in the amygdala (Herbert et al. 2003) and alterations in cortisol levels indicative of a dysfunctional HPA axis are common in autistic children and may explain their limited abilities to react adequately to their social environment and their tendency towards anxious behavior (Jansen et al. 2000; Porges 2005). The HPA axis is crucial not only for regulating a broad array of psychological stress responses (Gunnar et al. 1996) but also for regulating the neuroimmune stress, which arises from exposure to bacterial and/or viral stimuli. In fact, the HPA axis is a major pathway by which the CNS regulates the immune system (Eskandari et al. 2003; Besedovsky and Rey 2008; Fig. 1). Alterations in HPA axis regulation can lead to either excessive immune activation and hence inflammatory and autoimmune diseases or excessive immune suppression and thus increased susceptibility to infectious diseases. In this context it is notable that the vast majority of autoimmune/inflammatory conditions reflective of a dysfunctional HPA axis, including autism, have been consistently linked to

vaccinations. Moreover, some of them have specifically been recognized as part of the ASIA syndrome (Table 1).

According to typical pediatric vaccination schedules in developed countries, the greatest magnitude of immune stimulation occurs at the age of 2, 4, and 12 months (Table 3). The period between 2 and 4 postnatal months is characterized by major developmental transitions in many biobehavioral systems, including sleep, temperature, respiration, and brain-wave patterns (Gunnar et al. 1996), all of which are regulated by the neuroendocrine network (Porges 2005; Besedovsky and Rey 2008) and many of which are also known to be impaired in autism (Tuchman and Rapin 2002; Polimeni et al. 2005). In this same period, the HPA axis is known to be highly reactive and capable of producing highly significant amounts of cortisol in response to routine pediatric vaccinations with Al-adjuvanted vaccines (i.e., double the basal levels (Lewis and Thomas 1990; Gunnar et al. 1996)). High levels of glucocorticoids during early postnatal period are known to be detrimental for normal brain development (Gulino et al. 2009), which argues against excessive stimulation of the HPA axis during this period. On the other hand, excessive immune stimulation after 6 months of age, when the HPA axis becomes significantly hyporesponsive to stress stimuli (Gunnar et al. 1996), may increase the risk for autoimmune and inflammatory diseases in susceptible individuals.

Cortisol, the main glucocorticoid product of HPA activity, appears to play a crucial role in priming the microglia towards a hyperactive and neurodestructive phenotype (Barrientos et al. 2012). There is compelling evidence that aging-associated decline in CNS function is in fact due to a dysregulated cortisol homeostasis, skewed towards higher cortisol levels, which then predisposes the aged brain to exaggerated proinflammatory responses to future immune insults (Barrientos et al. 2012). Pretreatment with exogenous cortisol as well as exposure to acute stress consistently produces overly sensitized microglia programmed to respond to subsequent immune stimuli with abnormally high production of proinflammatory cytokines (Johnson et al. 2002; Barrientos et al. 2012). Elevated exposure to glucocorticoids also produces excitotoxic levels of glutamate and reactive oxygen species resulting in neuron atrophy and death (Lee et al. 2002). Glutamate, the major excitatory neurotransmitter, is crucial to brain development and function (Johnston 1995). However, excess glutamate is extremely deleterious to neuronal viability and is well known to contribute to the pathophysiology of neuropsychiatric disorders including autism (Blaylock and Strunecka 2009). For example, both children and adults with autism typically show higher serum levels of glutamate (Shinohe et al. 2006; Shimmura et al. 2011) as well as a higher concentration of glutamate/glutamine in the amygdala-hippocampal region (Purcell et al. 2001). Around 2 years of age, the developing brain contains more synaptic glutamate receptors than at birth, and their number progressively declines over the next decade. Because of this, the immature brain of an infant is likely to be more susceptible to excitotoxic insults than that of a young adult (Johnston 1995).

Collectively, the above observations argue against repeated and excessive stimulation of the HPA axis in early development, especially during the first 2 years of life when the developing brain is vulnerable to excitotoxicity (Johnston 1995; Olney 2002). Moreover, vaccine-induced HPA stimulation significantly elevates cortisol levels in 2-month-old infants (Lewis and Thomas 1990; Gunnar et al. 1996) and may thus sensitize the resting microglia to adopt a neurodestructive proinflammatory/excitotoxic phenotype upon subsequent immune stimuli. In consequence, continued vaccinations (i.e., every 2 months) throughout the first year of life could result in an irreversible shift of these cells to a proinflammatory phenotype, thus creating an environment persistently hostile to developing neurons (Fig. 3).

Recently, a highly significant positive correlation between Al exposure from vaccines at 3–4 months of age and the prevalence of autism in developed countries was reported. In addition, children from countries with the highest autism prevalence appeared to have a much higher exposure to Al adjuvants, particularly at 2 months of age (Tomljenovic and Shaw 2011b). Of note, the period spanning the first 4 postnatal months coincides not only with major biobehavioral transitions as noted above but also with several critical stages of human brain development known to be impaired in autism (i.e., onset of synaptogenesis (birth), maximal growth velocity of the hippocampus (2–3 postnatal months (Avishai-Eliner et al. 2002)), and onset of amygdala maturation (8 weeks postnatal age (Rhawn et al. 1996)).

Overall, continuous and closely spaced vaccine-induced immune stimulation of the HPA axis in early development may impair a child's ability to adequately respond to any type of stress stimuli later in life, including immune stimuli. This in turn could increase the risk of socio-behavioral disturbances, increase the susceptibility to infections and immune-mediated diseases, disrupt brain development and circadian rhythms (i.e., sleeping patterns), and in general compromise the proper functioning of organs regulated by the immune-neuroendocrine network (Fig. 1). Clinical and experimental data show that all of these abnormalities, albeit in various extents, characterize autism spectrum disorders (Jansen et al. 2000; Polimeni et al. 2005; Porges 2005; Vargas et al. 2005; Dietert and Dietert 2008; Theoharides et al. 2009). The heterogeneity of autistic phenotypes may thus be a reflection of the degree/severity of HPA axis dysfunction.

## The Role of Immune Molecules in Brain Development

Until relatively recently, the brain has been viewed as immune privileged, devoid of immune signalling and expression of canonical immune mediators except in rare instances such as disease and trauma (Garay and McAllister 2010). However, research has established a central role for immune-mediated pathways in regulating brain functions during both development and adulthood. Immune molecules are intrinsically expressed by the brain at a right place and time to help shaping of the developing CNS, affecting processes such as neurogenesis, neuronal migration,

axon guidance, synaptic connectivity, and plasticity (Stevens et al. 2007; Eroglu and Barres 2010; Garay and McAllister 2010). However, the very same immune pathways that regulate proper brain development and function appear to be targeted for impairment by a variety of immune stimuli (Garay and McAllister 2010) including Al adjuvants (Tomljenovic and Shaw 2011b, 2012).

Abnormal neural connectivity is a prominent feature in autism (Belmonte et al. 2004). Early during CNS development, an excessive number of synaptic connections are formed and establishment of mature neuronal circuitry requires selective elimination of inappropriate synaptic connections (Stevens et al. 2007). Components of the innate immune system (including the glia), the MHC family of proteins, and proinflammatory cytokines are essential for the establishment and modification of these connections. The activity of these immune molecules is spatially and temporally restricted during development for the appropriate shaping of neuronal circuits (Stevens et al. 2007; Eroglu and Barres 2010; Garay and McAllister 2010). Cerebellar Purkinje cells, which are significantly reduced in autism, are a site of prominent MHC class I expression, and one hypothesis currently under investigation is that specifically timed changes in neuronal MHC class I expression could contribute to autism (Belmonte et al. 2004). MHC class II also appears to play a role in autism as indicated by its aberrant microglia-associated expression in the cerebellum of autistic patients (Vargas et al. 2005).

Immune stimulation by Al adjuvants can profoundly influence the brain's innate immune cells (microglia; Shaw and Petrik 2009) and thus perturb the levels of key immune molecules which mediate CNS development. Because of this, it is possible that Al would interfere with synaptic pruning and activity-dependent synaptic remodeling (Fig. 2). Furthermore, by accumulating in the brain (Khan et al. 2013), Al adjuvants could also act as a constant trigger of inappropriate immune responses, thus further compromising successful structural and functional patterning of the CNS during development.

An increasing number of new cases of autism that have been appearing since the early 1980s includes patients who do not show dramatic changes in brain anatomy which are typical of classic complex autism (Miles et al. 2005). The difference in severity of changes may be related to the stage at which the insult arises. Postnatal injury is more likely to produce a less obvious pathology associated with regressive autism than prenatal injury which may be associated with more severe forms of autism. In light of these above observations, it should be noted that in parallel with the increase in autism prevalence since the early 1980s in the USA, the number of vaccinations recommended prior to school entry has also been steadily increasing. This increase in the number of vaccines preceded the "autism epidemic" (i.e., from 10 in the late 1970s to 32 in 2010). During this same period, the prevalence of autism in the USA increased by as much as 2,000 % (from $<$ 5 per 10,000 to 110 per 100,000). Similar trends have been observed in other developed countries (Tomljenovic and Shaw 2011b). It is further important to note that in humans, a considerable amount of postnatal brain development (including synaptogenesis)

occurs during the first 2 years after birth (Johnston 1995), a period in which children receive the majority of pediatric vaccinations (Table 3).

## Immune Hyperstimulation: Vaccination with Al Adjuvants Versus Natural Infections

Although vaccines are credited for decreasing the risk of neurodevelopmental complications arising from natural infections in early childhood, it should be noted that in many ways the immune challenge from vaccinations may be much greater in magnitude than that arising from natural stimuli. The main reason for this is that early-life immune responses (before 6 months of age) are weaker and of shorter duration than those elicited in immunologically mature hosts. Consequently, vaccine efficacy in this vulnerable population is limited (Siegrist and Aspinall 2009). Thus, in order to provoke and sustain an adequate B-cell immune response in a neonate, strong immune adjuvants such as Al with repeated, closely spaced booster doses are needed (Table 3). In contrast, during the course of natural infections, an infant has to deal with one infectious agent (or immune stimulant) at a time (i.e., pertussis only as opposed to diphtheria, pertussis, and tetanus all at once), which would then allow for a more balanced priming of the immature immune system as well as brain recovery from the resulting neuroimmune insult.

The potential hazards from high levels of vaccination are thus twofold. First, a single vaccine may change the preprogrammed immune milieu in a neonate and thus compromise neurodevelopmental programs. Second, multiple such vaccinations are routinely administered simultaneously (Table 3), thus magnifying the inflammatory response which, although being essential for linking the innate and adaptive immune responses, is also responsible for the adjuvant's immunotoxic effects (Table 2). The repetitive taxing of the immune system by high doses of Al adjuvants may also cause a state of immune hyperactivity, a known risk for autoimmune diseases (Eskandari et al. 2003).

## Al Adjuvants, Autoimmunity, and Autism

Autoimmune manifestations, particularly those affecting the CNS, are prevalent in autistic individuals (Vojdani et al. 2002; Pardo et al. 2005). Research data also shows that simultaneous administration of as little as 2–3 immune adjuvants, or repeated stimulation of the immune system by the same antigen, can overcome the genetic resistance to autoimmunity (Tsumiyama et al. 2009; Rose 2010). These facts are overlooked in the design of pediatric vaccination schedules. For example, 2-month-old infants receive a total of 22 viral bacterial antigens (most of which are adsorbed onto Al) and four attenuated viruses following the current US vaccination

recommendations for preschool children (Table 3). Such strong immune stimuli are then more or less repeated at 4, 6, and 12 months of age. Hence, by the time children are 4–6 years old, they would have received a total of 126 antigenic compounds under the US vaccination guidelines (Table 3).

Autoimmune manifestation in autism is not limited to a few nervous system antigens. For example, Vojdani et al. (2002) demonstrated elevated levels of autoantibodies against nine different neuron-specific antigens in autistic children. Such widespread manifestation of autoimmunity is indicative 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, not only leads to neuroinflammation but is also responsible for the development of seizures (Theoharides and Zhang 2011). Al is known to harm the BBB and can increase its permeability by increasing the rate of transmembrane diffusion and by selectively altering saturable transport systems (Banks and Kastin 1989). The breakdown of the BBB by Al may also result from excessive release of proinflammatory cytokines from Al-stimulated microglia (Prat et al. 2001; Aydin et al. 2010). It is well known that activated microglia increase the permeability of the BBB to other inflammatory factors and to trafficking lymphocytes (Prat et al. 2001). In animal models, only two injections of Al adjuvants at concentrations comparable to those used in human vaccines caused significant activation of the microglia (Shaw and Petrik 2009). Yet typical childhood vaccination schedules routinely require repeated, closely spaced administration of multiple Al-adjuvanted vaccines (i.e., every 2–4 months) from birth up until 12 months of age (Table 3).

Increased production of IL-6 and TNF-α and pathologies suggestive of a BBB breakdown (i.e., brain hemorrhages and edemas) following administration of Al-adjuvanted DTP vaccines have been demonstrated by previous investigations (Fantuzzi et al. 1994; Aydin et al. 2010). Furthermore, the ability of Al adjuvants to cross the BBB (Redhead et al. 1992; Khan et al. 2013; Lujan et al. 2013) and upregulate chemoattractants such as MCP-1 (Seubert et al. 2008) could promote active recruitment of immunocompetent cells to the brain, leading to both autoimmunity and deleterious inflammatory processes (Fig. 2). Consistent with this interpretation, postmortem analysis of six children aged 4–17 months who died within 48 h of vaccination with Al-adjuvanted hexavalent vaccines revealed abnormal pathological findings in the brain, including a defective BBB, infiltration of the leptomeninx by macrophages and lymphocytes, perivascular lymphocytic infiltration, diffuse infiltration of the pons, mesencephalon and cortex by T-lymphocytes, and microgliosis in the hippocampus and pons (Zinka et al. 2006). Notably, increased microglial activation in conjunction with elevated production of proinflammatory mediators (i.e., TNF-α, IL-6, and MCP-1) and increased recruitment of macrophages to the area of the cerebellum with neurodegeneration has been demonstrated postmortem in 5–44-year-old autistic patients (Pardo et al. 2005; Vargas et al. 2005). These findings suggest that neuroglia-mediated innate immune responses play an important role in triggering cerebellar dysfunction in autism. Although there was no evidence of

leptomeningeal, parenchymal, or perivascular lymphocytic infiltration in autistic brains (unlike in the brains of infants who deceased shortly after hexavalent vaccination), suggesting a lack of T-cell- and/or antibody-specific responses in the chronic phase of autism, the researchers noted the possibility of such adaptive immune-mediated reactions during the acute onset of the disease (i.e., pre- or early postnatal stages of development (Pardo et al. 2005)).

It is important to note that all of the abovementioned neuropathological outcomes, namely, those occurring following administration of Al-adjuvanted vaccines (Zinka et al. 2006) and those observed postmortem in autistic brains (Pardo et al. 2005; Vargas et al. 2005), are consistent with the established neurotoxic and immuno-stimulatory properties of Al adjuvants which impact both innate and adaptive immune responses (Table 2; Fig. 2).

## Conclusion

Al adjuvants can accumulate in the brain where they may trigger excessive inflammatory, excitotoxic, and autoimmune reactions and thus disrupt immune-mediated developmental processes. The proinflammatory effects of Al adjuvants appear to be mediated by the hyperactivated microglia, brain's resident immune cells. Al adjuvant-induced neuroimmunotoxicity is thus consistent with the pathogenesis of immune dysfunction in autism as the latter is typified by immune hyperactivation, microgliosis, excessive neuroinflammation, and autoimmunity (Fig. 2).

## Key Terms

*Aluminum (Al) adjuvants*. Al compounds used in vaccines to stimulate the immune response to vaccine antigens.

*Immune-neuroendocrine network*. A network of interactions between the brain and the immune system which plays a crucial role in immune regulation, brain function, and maintenance of general homeostasis. The operation of this network can be altered by either peripheral immune stimuli or direct brain stimuli.

*Hypothalamic-pituitary adrenal (HPA) axis*. A major pathway by which the brain regulates the immune system. Alterations in HPA axis regulation can lead to either excessive immune activation and hence inflammatory and autoimmune diseases or excessive immune suppression and thus increased susceptibility to infectious diseases.

*Microglia*. The brain's resident innate immune cells that play key roles in supporting neurons during development and adulthood.

*Excitotoxicity*. A neurodestructive injury resulting from excess levels of excitatory neurotransmitters (i.e., glutamate) which overstimulate neurons. It typically leads to death of neurons, loss of synaptic connections, and axonal damage. Excitotoxicity is considered as a common pathological pathway in various neurological disorders including autism.

## Key Facts of Al Adjuvant Toxicity Relevant for the Pathogenesis of Autism

- Autism is a developmental multisystem disorder typified by impaired brain function, hyperinflammation, and autoimmunity.
- Aluminum (Al) is a neurotoxin and a powerful stimulant of the immune system; hence, Al has all the necessary biochemical properties to induce neuroimmune disorders including those of the autism spectrum.
- In adult humans Al adjuvants can cause serious autoimmune and inflammatory conditions including those affecting the brain, yet children are routinely exposed to much higher amounts of Al from vaccines than adults.
- In humans, a considerable amount of postnatal brain development occurs during the first 2 years after birth, a period in which the immature brain is extremely vulnerable to neurotoxic and excitotoxic insults, and in which children receive the majority of their pediatric vaccinations (most of which are adjuvanted with Al).
- Repeated exposure to Al adjuvants (i.e., every 2 months) throughout the first year of life may cause an irreversible shift of microglia to a neurodestructive proinflammatory/excitotoxic phenotype, thus creating an environment persistently hostile to developing neurons (Figs. 2 and 3).
- By perturbing the levels of key immune molecules which mediate brain development, Al could compromise successful structural and functional developmental patterning of the brain (Fig. 2).
- Al is a blood-brain barrier (BBB) toxin and research evidence strongly suggests that the BBB integrity is compromised in autism (Fig. 2).
- The immuno-stimulatory properties of Al have been routinely exploited for inducing mast cell-dependent food allergies in experimental animal models. Gastrointestinal dysfunction and food allergies are the most common non-neurological comorbidities in autism and mast cell activation is strongly implicated as the underlying factor (Fig. 2).

## Summary Points

- This chapter focuses on aluminum (Al), particularly the immune adjuvant form commonly present in vaccines, and its potential role in autism.
- Clinical and experimental data provide strong evidence that the nervous system is the most sensitive target of Al's toxicity and that the same adjuvant-mediated mechanisms that drive the immune-stimulatory effects of vaccines have the capacity to provoke serious inflammatory and autoimmune reactions.
- Infants are routinely exposed to Al adjuvants through routine vaccination programs, and there are increasing concerns that such exposures might in part be contributing to the growing burden of neurodevelopmental and immune syndromes, particularly those of the autism spectrum (Fig. 2).

- The increase in the number of pediatric vaccines preceded the increase in cases of regressive autism.
- Because vaccine injections containing Al bypass the usual biological barriers to absorption (i.e., gastrointestinal), they confer maximal exposure. Moreover, since there are no known physiological roles for Al within the human body, its accumulation can only be considered as potentially deleterious.
- Al adjuvants can accumulate in the brain where they may cause neuroinflammatory reactions and disrupt immune-mediated developmental processes (Fig. 2).
- The proinflammatory effects of Al adjuvants appear to be mediated by the hyperactivated microglia, brain's resident immune cells. Al adjuvant-induced neuroimmunotoxicity is thus consistent with the pathogenesis of immune dysfunction in autism as the latter is typified by immune hyperactivation, microgliosis, neuroinflammation, and autoimmunity (Fig. 2).

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