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SPECIAL ARTICLE

Mechanisms of aluminum adjuvant toxicity and autoimmunity in pediatric populations

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Immune challenges during early development, including those vaccine-induced, can lead to permanent detrimental alterations of the brain and immune function. Experimental evidence also shows that simultaneous administration of as little as two to three immune adjuvants can overcome genetic resistance to autoimmunity. In some developed countries, by the time children are 4 to 6 years old, they will have received a total of 126 antigenic compounds along with high amounts of aluminum (Al) adjuvants through routine vaccinations. According to the US Food and Drug Administration, safety assessments for vaccines have often not included appropriate toxicity studies because vaccines have not been viewed as inherently toxic. Taken together, these observations raise plausible concerns about the overall safety of current childhood vaccination programs. When assessing adjuvant toxicity in children, several key points ought to be considered: (i) infants and children should not be viewed as "small adults" with regard to toxicological risk as their unique physiology makes them much more vulnerable to toxic insults; (ii) in adult humans Al vaccine adjuvants have been linked to a variety of serious autoimmune and inflammatory conditions (i.e., "ASIA"), yet children are regularly exposed to much higher amounts of Al from vaccines than adults; (iii) it is often assumed that peripheral immune responses do not affect brain function. However, it is now clearly established that there is a bidirectional neuro-immune cross-talk that plays crucial roles in immunoregulation as well as brain function. In turn, perturbations of the neuro-immune axis have been demonstrated in many autoimmune diseases encompassed in "ASIA" and are thought to be driven by a hyperactive immune response; and (iv) the same components of the neuro-immune axis that play key roles in brain development and immune function are heavily targeted by Al adjuvants. In summary, research evidence shows that increasing concerns about current vaccination practices may indeed be warranted. Because children may be most at risk of vaccine-induced complications, a rigorous evaluation of the vaccine-related adverse health impacts in the pediatric population is urgently needed. *Lupus* (2012) **21,** 223–230.

Key word: adjuvants; aluminum; autoimmunity; immunotoxicity; inflammation; neurotoxicity; vaccine safety

Introduction

Aluminum (Al) is highly neurotoxic and has been shown to impair both prenatal and postnatal brain development in humans and experimental animals.[1–2] In addition to its neurotoxic properties, Al is a potent stimulator of the immune system, which is the very reason why it is used as an adjuvant.[3–8] Given this, it is somewhat surprising to find that in spite of over 80 years of use, the safety of Al adjuvants continues to rest on assumptions rather than scientific evidence. For example, nothing is known about the toxicology and pharmacokinetics of Al adjuvants in infants and children.[9] On the other hand, in adult humans long-term persistence of Al vaccine adjuvants can lead to cognitive dysfunction and autoimmunity.[6,10] Yet, in spite of these observations children continue regularly to be exposed to much higher levels of Al adjuvants than adults, via routine childhood vaccination programmes.[3,11]

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An additional concern to using a neurotoxic substance such as Al as an adjuvant in pediatric vaccine formulations is the fact that infants and young children should not be considered simply as "small adults" when it comes to toxicological risk. In spite of this, a review of the literature to date relating to Al-toxicology indicates that the vast majority of previous research and testing has been dedicated to Al exposure in adults.[12] If a few vaccines administered to adults can result in adverse outcomes associated with the "ASIA" syndrome, is it reasonable to assume in the absence of experimental evidence that the current pediatric schedules, often exceeding 30 vaccinations in the first 4 to 6 postnatal years,[3,13] are safe for children? The purpose of this review is to address the mechanisms of Al adjuvant toxicity with special reference to the developing neuro-immune system and the "ASIA" syndrome in order to shed light on this unresolved and hotly debated question.

Al adjuvants: a toxicological risk to a developing child?

Some 15 years ago, Cohen and Shoenfeld made an important observation: "It seems that vaccines have a predilection to affect the nervous system."[14] Furthermore, according to Israeli and co-workers, alongside their supportive role in vaccine-induced immune responses, vaccine adjuvants were found to inflict, by themselves, illnesses of an autoimmune nature.[5] With regard to these statements, as well as the ensuing discussion, five key observations ought to be considered. First, there are critical periods in brain development during which even subtle immune challenges (including those induced by vaccinations) can lead to permanent detrimental alterations of brain and immune function.[15–17] Indeed, a single Al-adjuvanted hepatitis B vaccine administered to newborn primates within 24 h of birth is sufficient to cause neurodevelopmental delays in acquisition of neonatal reflexes essential for survival.[17] Second, through multiple vaccinations preschool children are regularly exposed to significant amounts of Al adjuvants.[3,18] Such high exposures to Al repeated over relatively short intervals during critical neurodevelopmental periods constitute a significant neuro-immunotoxicological challenge to neonates and young children.[18] Third, despite the prevalent view that peripheral immune responses do not affect brain function, overwhelming research evidence clearly points to the contrary. Namely, it is now firmly established that there is a bidirectional neuro-immune cross-talk which plays crucial roles in immunoregulation, brain function, and maintenance of general homeostasis.[19,20] In turn, perturbations of the neuro-immune axis have been demonstrated in a variety of autoimmune/inflammatory diseases encompassed in the "ASIA" syndrome.[21–24] Fourth, the very same components of the neuro-immune regulatory system that demonstrably play key roles in both brain development and immune function (e.g., immune cytokines),[19–20,25] are heavily targeted by Al adjuvants (Table 1). Fifth, experimental evidence demonstrates that a strong adjuvant effect can overcome genetic resistance to autoimmunity.[26]

Thus, the possibility needs to be considered that repeated immune system stimulation with multiple vaccines during critical periods of brain development could result in adverse neurodevelopmental outcomes and or/autoimmunity.[18]

Mechanisms of immune stimulation by Al adjuvants: what are the risks?

The success of Al as a vaccine adjuvant is due to its potent and multifactorial stimulatory effects on the immune system (Table 1). In fact, with the exception of attenuated viruses, in the absence of Al most antigenic compounds fail to launch an adequate immune response,[5,27–28] suggesting that a significant part of the immunostimulatory effects of vaccines may be driven by the Al-adjuvant itself. 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 is difficult to achieve in practice. This is because the same mechanisms that drive the immunostimulatory effects of adjuvants have the capacity to provoke a variety of adverse reactions, including those associated with the "ASIA" syndrome (Table 1).

There are additional problems with using a neurotoxic substance such as Al as an immune stimulator in pediatric vaccinations. First, during prenatal and early postnatal development the brain is extremely vulnerable to neurotoxic insults. Not only are these highly sensitive periods of rapid brain development but also, the blood–brain barrier is incomplete and thus more permeable to toxic substances during this time.[11,12,29] Additionally, the immature renal system of neonates significantly compromises their ability to eliminate environmental toxicants.[11,12] For all these reasons, children are

at much greater risk of adverse reactions from Al adjuvants than adults.

Although vaccines are often credited for decreasing the risk of neurodevelopmental complications arising from natural infections in early childhood, it should be noted that immune stimulation induced by vaccinations may be much greater in magnitude than that resulting from natural infections. 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.[30,31] Thus, to provoke and sustain an adequate B-cell immune response in neonates, strong immune adjuvants such as Al, as well as repeated closely spaced booster doses are needed.[31] In contrast, during the course of natural infections, children are in most cases exposed to one pathogenic agent (or immune stimulant) at a time (i.e., measles only as opposed to measles,

mumps, and rubella all at once). This allows for a more subtle priming of the immature immune system, as well as brain recovery from the potential neuro-immune challenge.

The inability of an immature immune system to mount a robust immune response to certain antigens stems in part from an inherent anti-inflammatory phenotype of neonatal splenic macrophages which fail to produce sufficient amounts of pro-inflammatory cytokines (such as interleukin (IL)-1 and IL-6, both of which are induced by Al adjuvants; Table 1). These cytokines are needed for adequate stimulation of antibody-producing B-cells.[32] This attenuation of pro-inflammatory cytokine production by neonatal macrophages may be an important developmental program of the neonate, rather than a defect because the anti-inflammatory phenotype may be beneficial to the neonate at a time when

Table 1 Shared aspects between autoimmune/inflammatory conditions and immunostimulatory properties of Al vaccine adjuvants

Condition			Al adjuvant	
Disease	Th shift	Inflammatory profile	Inflammatory profile	General immunostimulatory effects
Arthritis*†	Excessive Th1[20]	Increased IL-1, IL-6, IL-12, TNF-α, IFN-γ, MIP-1α and oxidative stress[20,55,63]	Increases cytokines (IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-18, TNF-α), chemokines (IL-8, MCP-1, MIP-1α, MIP-1β), ROS, and nitric oxide (NO)[8,27,28,41,42,55]	Stimulates recruitment of mono-cytes, macrophages and granu-locytes to the injection site
Autoimmune thyroid disease	Excessive Th1[20]			Induces differentiation of mono-cytes to antigen presenting cells (APCs)
Inflammatory bowel disease (IBD)/ Crohn's disease (CD)	Excessive Th1[55]	Increased NLRP3 inflammasome complex signalling and NLRP3-dependent overproduction of IL-1β, IL-6, IL-18, TNF-α and reactive oxygen species (ROS) in MS, EAE, Type 1 diabetes mellitus[64–66] and animal models of IBD[67]	Activates the NLRP3 inflam-masome complex and NLRP3-dependent cytokines[7,8]	Activates APCs
Type 1 diabetes mellitus*	Excessive Th1[20]			Promotes antigen uptake and processing by APCs and enhances antigen-specific T-cell responses
Multiple sclerosis (MS)*† and experimental autoimmune encephalomyelitis (EAE)	Excessive Th1[20]			Increases the expression of MHC class I and II and associated co-stimulatory molecules on peripheral blood monocytes
Systemic lupus erythematosus (SLE)*	Excessive Th2[20,56]	Increased IL-10, IL-18, IL-6, IFN-γ, TNF-α[20,56,68]		Activates the complement cascade
Macrophagic myofas-ciitis (MMF) and chronic fatigue syndrome (CFS)*†	Excessive Th2[57–59]	Increased IL-4, IL-6, B-cell hyperlymphocytosis, infiltration of large periodic acid-schiff (PAS)-positive macrophages, and CD8+ T lymphocytes in the absence of conspicuous muscle fibre damage[57,59,69]		Generally stimulates Th2 responses but can also induce a Th1 shift and activate cytotoxic T lymphocytes (CTLs) in the presence of other Th1 stimulators (i.e., lipopolysaccharide (LPS), CpG, recombinant influenza protein antigen)[27,73–75]
Gulf War Syndrome (GWS)*†	Mixed Th1/Th2[60]	Increased IFN-γ, IL-5, IL-6[60]		Activates astrocytes and microglia[76]
Autism spectrum disorders (ASD)*	Both Th1 and Th2 shifts have been reported[61,62]	Increased IL-1β, IL-4, IL-5, IL-6, TNF-α, IL-8, MCP-1, MIP-1β, MHC class II[61,70,71] Increased astrocyte and microglia reactivity[70,72]		

*linked to Al-adjuvanted vaccines.[6,35,38,77–79]
†specifically recognized as 'Autoimmune/inflammatory syndrome induced by adjuvants' ('ASIA').[6]

tissue development is taking place at a rapid pace.[32]

The risks from current childhood vaccination schedules are thus twofold. First, a single vaccine may disrupt the delicate balance of immune mediators required for normal brain development and thus compromise neurodevelopmental programs. Second, such multiple vaccinations are routinely administered simultaneously (Table 2), thus magnifying the inflammatory response which, although being essential for linking the innate and adaptive immune responses, is also responsible for adjuvant's immunotoxic effects.[4] 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.[6,33,34]

Consistent with all of the above, in an epidemiological study examining the impact of hepatitis B vaccination in male children, Gallagher and Goodman[35] showed that those receiving a single vaccine during the first month of life had a threefold greater risk of neurodevelopmental disorders compared with those vaccinated later or not vaccinated. Further evidence from case reports validates the highly contentious hypothesis that multiple vaccinations may precipitate developmental regression, at least in susceptible individuals.[36] Finally, routine vaccination in children has been associated with a variety of autoimmune conditions, including tranverse myelitis,[37] insulin-dependent diabetes mellitus (IDDM),[38] multiple sclerosis. (MS)[39] and anti-N-methyl-D-aspartate receptor (NMDA) receptor encephalitis.[40]

Al vaccine adjuvants and autoimmunity

A major difficulty in understanding how the Al-adjuvant effect could account for the vast heterogeneity of autoimmune manifestations described in the "ASIA" and related syndromes, relates to the fact that most of these conditions are driven by an overactive Th1 immune response (Table 1). Although Al

Table 2 Summary of vaccine ingredients according to the current US vaccination schedule[80]

	Birth	2 m	4 m	6 m	12 m	18 m	24 m	4–6 y	
Vaccine (#antigen)	EngerixB (1)	Infanrix-IPV (5) Comvax (3) Prevnar (14)	Infanrix-IPV (5) Pedvax (2) Prevnar (14)	EngerixB (1) Infanrix-IPV (5) Prevnar (14)	Hiberix (2) Prevnar (14)	Daptacel 5	–	Daptacel (5)	
Total # antigens	1	22	21	20	16	5	–	5	90
Viral attenuated vaccine (#attenuated viruses)	–	Infanrix-IPV (3) Rotarix (1)	Infanrix-IPV (3) Rotarix (1)	Infanrix-IPV (3)	Imovax Polio (3) MMR-II (3) Varivax (1) Havrix (1) Fluviral (3)	Havrix (1)	Fluviral (3)	Imovax Polio (3) MMR-II (3) Varivax (1) Fluviral (3)	
Total # attenuated viruses	0	4	4	3	11	1	3	10	36

Vaccine ingredients were sourced directly from the manufacturer's monographs. EngerixB, HBsAg adsorbed on 250 µg Al hydroxide; Infanrix-IPV, diphtheria toxoid, tetanus toxoid, pertussis toxoid, FHA, pertactin, inactivated polioviruses Type 1 (Mahoney), Type 2 (MEF1) and Type 3 (Saukett), Al hydroxide; Comvax, Hib capsular polysaccharide PRP conjugated to OMPC of *Neisseria meningitidis* serogroup B, HBsAg, Al hydroxyphosphate sulphate; Prevnar, *Streptococcus pneumonia* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F saccharides, diphtheria CRM_{197} carrier protein, Al phosphate; Rotarix, live attenuated RIX4414 strain of human rotavirus of the G1P[8] type; Pedvax, 7.5 µg of Hib PRP, *N. meningitidis* OMPC, Al hydroxyphosphate sulphate; Hiberix, Hib capsular polysaccharide PRP conjugated to tetanus toxoid; Imovax Polio, inactivated polioviruses Type 1 (Mahoney), Type 2 (MEF1) and 32 Type 3 (Saukett); MMR-II, measles virus, Enders' Edmonston strain (live, attenuated), mumps virus, Jeryl Lynn® (B level) strain (live, attenuated), rubella virus, Wistar RA 27/3 strain (live, attenuated); Varivax, varicella virus, Oka/Merck strain (live, attenuated); Havrix, inactivated hepatitis A virus (HM175 strain), Al hydroxide; Fluviral, inactivated influenza strains A/California/7/2009 (H1N1)-like strain, A/Perth/16/2009 (H3N2)-like strain, B/Brisbane/60/2008-like strain; Daptacel, pertussis toxoid, FHA, pertactin, fimbriae types 2 and 3, diphtheria toxoid, tetanus toxoid, Al adjuvant. Abbreviations: HBsAg, hepatitis B surface antigen; IPV, inactivated poliomyelitis vaccine; Hib, *Haemophilus influenza type b*; PRP, polyribosylribitol phosphate; OMPC, outer membrane protein complex; FHA, filamentous hemagglutinin.

adjuvants have been historically known as potent and specific stimulators of Th2 immunity and presumably could not activate cytotoxic T cells (CTL),[41,42] current evidence suggests that the classical Al-induced Th2 responses can be shifted towards Th1 polarization in the presence of other Th1-inducing compounds such as lipopolysaccharide (LPS) or recombinant influenza protein antigen (Table 1). Routine contamination of vaccine formulations with residual compounds from the production process, including LPS and various peptidoglycans,[4] could thus account for different adjuvant properties of individual batches. Furthermore, it is also possible for Al adjuvants to trigger autoimmunity through a bystander effect by activating dormant autoreactive T cells in certain individuals.[43,44]

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 for the upregulation of immune cytokines, which in turn are able to trigger polyclonal activation of autoreactive T cells.[4,44] Consistent with these observations, the immunotoxic effects of vaccine adjuvants are generally recognized to be a consequence of hyperstimulation of immunological responses and are known to be mediated by pro-inflammatory cytokines.[4]

It is perhaps not surprising then to find that simultaneous administration of as little as two to three immune adjuvants, or repeated stimulation of the immune system by the same antigen, can overcome genetic resistance to autoimmunity.[26,45] These facts are often overlooked in the design of routine vaccination schedules. For example, as shown in Table 2, according to the US vaccination schedule currently recommended for preschool children, 2-month-old infants receive a total of 22 viral/bacterial antigens and 4 attenuated viruses along with high amounts of Al adjuvants. Such a potent immune challenge is then more or less repeated at 4, 6, and 12 months of age (Table 2). Hence, by the time children are 4 to 6 years of age, they will have received a total of 126 antigenic compounds (90 viral/bacterial antigens, 36 attenuated viruses) following the current US vaccination guidelines.

Vaccine safety: how reassuring is the evidence?

In spite of the widespread agreement that vaccines are largely safe and serious adverse complications are extremely rare, a close scrutiny of the scientific literature does not support this view. For example, to date, the clinical trials that could adequately address vaccine safety issues have not been conducted (i.e., comparing health outcomes in vaccinated versus non-vaccinated children). The lack of such controlled trials may be because historically, vaccines have not been viewed as inherently toxic by regulatory agencies (as documented in the 2002 publication by the US Food and Drug Administration).[46]

Although the temporal association between vaccinations and serious adverse reactions (ADRs) is clear, causality is rarely established.[47] Thus, it is often concluded that, (i) the majority of serious ADRs that do occur are coincidental[48] and (ii) true serious ADRs following vaccinations (i.e., permanent disability and death) are extremely rare.[49] However, the lack of evidence of causality between serious ADRs and vaccinations may simply be due to methodological inadequacy of vaccine trials (Table 3). In addition, the fact that a large number of vaccine safety trials use an Al adjuvant-containing placebo or another Al-containing vaccine as a "control"[50] precludes correct calculations of vaccine-related ADRs. In addition, historically, vaccine trials have routinely excluded vulnerable individuals with a variety of pre-existing conditions (i.e., premature birth, personal or immediate family history of developmental delay, or neurologic disorders including convulsive disorders of any origin, hypersensitivity to vaccine constituents including Al, etc.).[51–53] Because of such selection bias, the occurrence of serious ADRs resulting from vaccinations may be considerably underestimated. All this should be of concern given that the conditions named above are precisely those which are under current immunization guidelines considered as "false-contraindications" to vaccinations.[54] For all these reasons, the true health risks from vaccinations remain unknown.

Conclusions and future goals

Infants and young children should not be viewed as "small adults." Their unique physiology makes them much more vulnerable to noxious environmental insults in comparison with the adult population. In spite of this, children are routinely exposed to much higher levels of Al vaccine adjuvants than adults, even though adequate safety data on these compounds are lacking. That Al vaccine adjuvants can induce significant autoimmune conditions in humans can hardly be disputed,

Table 3 Sample of vaccine safety study designs

Study	Methods	Comments
Miller et al.[81]	For safety assessment, children were observed for 7 days post- vaccination for local reactions such as erythema, swelling, or tenderness at site of injection, or fever	The follow-up of study participants was too short and hence detected only the most immediate minor adverse reactions
GlaxoSmithKline[82]	Study subjects were monitored for only 4 days post-hepatitis B vaccination	As above. Given that hepatitis B is the only vaccine mandated to newborn babies and to prevent a disease to which an infant is extremely unlikely to be exposed (i.e., hepatitis virus is transmissible through sexual contact or injection with contaminated material),[83] a more rigorous safety assessment would appear to have been warranted
Verstraeten et al.[84]	Authors state that the safety study on new ASO-4 adjuvanted vaccines (including the human papilloma virus [HPV] vaccine) was not set up primarily to study autoimmune disorders	If the purpose of the study was to assess ADRs of autoimmune etiology, as the title itself clearly states,[84] then the study should have been designed to detect these. An increasing number of reports of previously unrecognized severe autoimmune conditions in HPV vaccine recipients have emerged in recent years[78,79,85,86]
Phillips et al.[87]	In exploring the potential association between Gulf War syndrome and anthrax vaccination, potential subjects were excluded if they reported bad reactions to immunizations or injections	It should be obvious that subjects who reported adverse reactions to immunizations should have been included in the study

although still debatable is how common such side effects are. However, the existing data (or lack thereof) raise questions on whether the current vaccines aimed at pediatric populations can be accepted as having adequate safety profiles. Because infants and children represent those who may be most at risk for complications following vaccination, a more rigorous evaluation of potential vaccine-related adverse health impacts in pediatric populations than what has been provided to date is urgently needed.

Conflict of interest

CAS is a founder and shareholder of Neurodyn Corporation, Inc. The company investigates early-state adult neurological disease mechanisms and biomarkers. This work and any views expressed within it are solely those of the authors and not of any affiliated bodies or organizations.

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