Short review

Systemic immunotoxicity reactions induced by adjuvanted vaccines

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ABSTRACT

Vaccine safety is a topic of concern for the treated individual, the family, the health care personnel, and the others involved in vaccination programs as recipients or providers. Adjuvants are necessary components to warrant the efficacy of vaccines, however the overstimulation of the immune system is also associated with adverse effects. Local reactions are the most frequent manifestation of toxicity induced by adjuvanted vaccines and, with the exception of the acute phase response (APR), much less is known about the systemic reactions that follow vaccination. Their low frequency or subclinical expression meant that this matter has been neglected. In this review, various systemic reactions associated with immune stimulation will be addressed, including: APR, hypersensitivity, induction or worsening of autoimmune diseases, modification of hepatic metabolism and vascular leak syndrome (VLS), with an emphasis on the mechanism involved. Finally, the authors analyze the current focus of discussion about vaccine safety and opportunities to improve the design of new adjuvanted vaccines in the future.

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1. Introduction

Vaccination remains an important public health tool for infectious disease prevention and more recently as part of the therapeutic arsenal against chronic diseases. The safety of vaccines is a priority, particularly in the prophylactic programs [1,2]. Recent advances in vaccine research have increased our understanding of the mechanisms by which vaccine components contribute to the immune response and also to several adverse responses [3,4].

Almost all the local and systemic toxicity induced by adjuvanted vaccine is of an immunotoxic nature. The types and impact of the local reactions were highlighted in a recent review [5]. Regarding systemic toxicity, apart from fever and other minor transient symptoms sometimes often referred to as “flu-like symptoms”, the available data on the systemic side effects of vaccines are generally scarce, being regarded as rare events. Many of these are subclinical, requiring laboratory methods to be detected. However, the risk of a severe reaction in people suffering from other pathological or genetic conditions is unknown. Certainly, few studies have carefully evaluated the systemic reactions induced by adjuvants [6].

The main mechanism giving rise to toxic systemic effects after the administration of adjuvanted vaccines usually involves the hyper-activation of immunological mechanisms, often mediated by the release of proinflammatory cytokines, such as IL-1, TNF-α, IL-6 and IL-17, chemokines and other endogenous factors [7,8]. These early effects can trigger other secondary reactions in distant organs and tissues with different levels of graveness (Fig. 1). Other less well known mechanisms involving failure of a regulatory pathway, that can lead to systemic vaccine toxicity will be analyzed later.

Although it is accepted that adjuvants are the cause of many toxic reactions of vaccines due to overstimulation of the immune system, the vast majority of toxicity studies with adjuvants were performed in adjuvant + antigen formulations (experimental and clinical vaccines), with limited information regarding adjuvants alone, being a potential

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**Fig. 1.** Simplified overview of immunotoxic reactions induced by adjuvanted vaccines. After inoculation, the adjuvant can cause a local inflammatory focus initiated by danger signals contained in the vaccine itself or endogenous factors released by the local irritation. These early events promote the secretion of pro-inflammatory cytokines by the innate immune system, which consolidate the local reactions and, after subsequent transfer to the circulatory system, produce systemic manifestations of immunotoxicity in several organs or tissues. In the hypothalamic–pituitary–adrenal axis, the proinflammatory cytokines induce the influenza-like syndrome; in the liver, the release of acute phase proteins is stimulated and the activity of CYP is inhibited, affecting drug metabolism; various tissues may be affected by allergic or autoimmune reactions and the action in the endothelial cells can induce a VLS. Note: This figure depicts a theoretical extreme situation and is not representative of any particular adjuvant. Pathogen-Associated Molecular Patterns, PAMPs. *In autoimmune and allergic reactions, other mechanisms of the adaptive immune systems are involved that are not shown.

**Abbreviations:**
- C1i: C1 inhibitor
- C2,3,4,5,9: Proteins of the complement cascade
- C4 BP: C4-binding protein
- CYP: Cytochrome P 450 superfamily of monoxygenases
- CRP: C-reactive protein
- IL-: interleukins
- MBL: Mannose-binding lectin
- PAI-1: Plasminogen activator inhibitor-1
- SAA: Serum amyloid A
- TNP-α, TPA: Tissue plasminogen activator
- VLDL: Very-low-density lipoprotein
- VWF: von Willebrand Factor
problem to understand all data published. In this review, several kinds of systemic reaction will be discussed, with an emphasis on the mechanisms behind the acute phase response (APR), hypersensitivity reactions, induction or worsening of autoimmune diseases (ADs), modification of hepatic metabolism (MHM) and vascular leak syndrome (VLS). Several of these reactions have been rarely observed in prophylactic vaccine or under special conditions (therapeutic vaccines, accidentally or in experimental models). In this article, embryo–fetal immunotoxicity is not discussed as it is dealt in another study.

2. Vaccine composition

Vaccines are composed of several ingredients that can influence the safety of formulations. The more important components of vaccine are the antigen, which guarantees its specificity, and the adjuvant, that ensures the potency and quality of the immune response. The antigen can be whole-cell microorganisms (either bacteria, viruses or fungus), attenuated or killed, or their subunits purified, recombinant or synthetic (proteins, peptides, carbohydrates, genetic materials). On the other hand, adjuvants are highly variable chemically in nature and mode of action, making it hard to generalize about their toxic effects, except in some local reactions of variable magnitude that they often induce [5].

Other components normally present in vaccines are diluents, preservatives and manufacturing residues. The function of these ingredients is to dissolve all the components and to avoid any further chemical reactions and decomposition or multiplication of the biological constituents of the vaccine. They are regarded as innocuous, but sometimes trigger allergy reactions in susceptible individuals.

3. Severity of vaccine adverse events

In terms of severity, vaccine adverse events can be classified into two types [9–11]: 1) non-severe adverse events: (i) local (pain, lump at the injection site, redness >5 cm or other local events like pruritus or hema-toma) that persist at least 48 h; (ii) regional (ulcer, lymph node tenderness and/or enlargement, adenitis, abscess at the injection site); and (iii) systemic (fever ≥38 °C or any event thought to be related to vaccination, with sick leave for more than two days); and 2) severe or serious adverse events: according to the 21 Code of Federal Regulations serious cases are defined as one of the following outcomes: death, a life-threatening condition, hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity or a congenital anomaly/birth defect [12]. Other medically important conditions may be considered serious adverse events when, based upon appropriate medical judgment, they may place the subject at risk of requiring medical or surgical intervention to prevent one of the outcomes listed in this definition. Although at various points it will be necessary to refer to local reactions, this review focuses on the systemic postvaccination reactions.

4. Early events before systemic postvaccination reactions

The early events triggering systemic postvaccination reactions are initiated at the site of inoculation. These local reactions are consistently associated with severity of systemic reaction rates [13].

Naturally, invasion of a pathogen induces a rapid inflammatory response and, in the same way, immunization is followed by a local inflammation at the site of inoculation, releasing proinflammatory cytokines [5,7]. When these cytokines cross the capillary walls and enter blood circulation, they may reach distant sites, with effects on certain target cells (Fig. 1).

The stimulation of innate immunity is initiated by the interaction of highly conserved components of pathogens, called Pathogen-Associated Molecular Patterns (PAMPs) [14], or products released by damaged tissues, called Danger-Associated Molecular Patterns (DAMPs) [15,16], with receptors on immune cells named Pattern Recognition Receptors (PRRs). The recognition of these signals by PRR leads to the activation of intracellular pathways in innate immune cells. The activation of macrophages and dendritic cells (DCs) culminates in the expression of nuclear factor-κB (NF-κB) and IRF7 via MyD88/TIRAP or TRIF/TRAM, to induce inflammatory cytokines (TNF, IL-1, IL-6), chemokines (CCL2, CCL8, others), endothelial adhesion molecules (E-selectin), co-stimulatory molecules (CD80, CD86) and type I interferon [17]. Expression of these molecules creates a local pro-inflammatory environment, where phagocytes are recruited and activated, leading to the activation of the complement cascade and chemoattraction of the effector cell adaptive immune response, while the invading pathogens are contained [18].

In adjuvanted vaccines, the adjuvants mimic signals induced by pathogens, activating the innate immune cells [19]. Most of the PAMPS used as vaccine adjuvants, such as monophosphoryl lipid A (MPL); flagellin and CpG oligonucleotides, are agonists of toll-like receptors (TLRs) [20], while others such as alum are TLR-independent adjuvants [21] (Fig. 1).

Cytokines used directly as immunological adjuvants [22,23] or for treatment of several diseases [24,25] are involved in the early phase of the immune response, but are involved directly or indirectly in the pathogenesis of immune-mediated disorders described in humans and animals [24]. Therefore, it is not surprising that strong immunostimulation mediated by adjuvants, associated with subsequent release of various cytokines, is also capable of producing manifestations, depending on their concentration [3,5,7].

5. General mechanisms involved in systemic immunotoxicity induced by adjuvanted vaccines

5.1. Profuse release of inflammatory cytokine

Adjuvants can over-stimulate the inflammatory mechanisms, with high production of TNF and other mediators, such as: tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1 β), IL-2 and IL-6. They can generate a variety of symptoms, according to the quantity and the time they last in the plasma, ranging from a transitory hyperthermia to a state of shock, with danger to life, similar to that observed in sepsis [26]. In 2006, a severe reaction was reported during the Phase I clinical trial of TGN1412, a therapeutic CD28-specific monoclonal antibody (mAb), developed for the treatment of rheumatoid arthritis, leukemia and multiple sclerosis; instead of the intended pharmacologic effect, it caused near fatal reactions in all 6 healthy volunteers. Within 2 h of receiving a single intravenous dose of 0.1 mg/kg ivw, all six volunteers had high plasma levels of proinflammatory cytokines. Within 16 h, all six volunteers became critically ill with acute lung injury, multiple organ failure, and disseminated intravascular coagulation that would result in amputation of the fingers and toes for the worst affected. This newly observed phenomenon was termed ‘cytokine storm’ [27]. The mechanism of cytokine release caused by TGN1412 seems to be different from that caused by other therapeutic mAbs, because it involves a different arm of the immune system, the CD4+ effector memory T-cells, but activation of the other arms of the immune system also has the potential to cause a ‘cytokine storm’ [28]. This raised an alert about the consequences of strong stimulation of the immune system and the need of major effort to develop preclinical assays for prediction of these consequences [28]. Several consequences of high plasma levels of proinflammatory cytokines following vaccination will be analyzed in the following sections.

5.2. Off-target effect

The current data indicate that not only immunity is regulated systemically, but also each organ has distinct patterns of expression of certain immune molecules, allowing an accommodation of the immune mechanism to the characteristics of the organ [29]. This implies expression of receptor-like PRR on cells other than the classic innate immune cells, to function in regulating their microenvironment [29–32].
expression can be either constitutive or inducible. TLR4 is expressed by
human vascular smooth muscle cells [33], while various TLRs are pres-
ent in the female reproductive tract in humans [34], non-immune
liver cells [31], and intestinal epithelium [30,32], and TLR2 can be
induced in the liver after intraperitoneal injection of Freund’s adjuvant
[35].

The term “off-target effect” was used by Hauguel and Hackett to re-
fer to immunization induced by adjuvants in non-immune tissues abil-
to express innate immune receptors like TLRs. Although the TLR ex-
pression in in non-immune cells seems to play a regulatory role [29,31]
it is possible that TLR agonist adjuvants might induce undesirable inflam-
memation, even autoimmune reactions, in sensitive tissues under certain
conditions and in susceptible organisms [36,37]. More research is re-
quired to determine the possible role of these mechanisms in systemic
toxicity of adjuvanted vaccines.

5.3. Suboptimal downregulation of the innate immune system activation

The initial activation of the innate immune system constitutes the
first step toward the subsequent activation of adaptive immunity.
Once the activation of adaptive immunity begins, multiple pathways
of negative feedback inhibit innate immunity, thus avoiding an unbru-
dled activation of inflammatory cascades harmful to the organism.
This process is named transition from innate to adaptive immunity
[38]. Failure in this homeostatic mechanism is seen as a potential source
of adjuvant toxicity [37]. Although this feedback control system is not
fully understood, several of its pathways have been described. For ex-
ample, the cytokine IL-21, produced by activated T cells, may initiate
immunosuppressive activity in dendritic cells (DCs) and diminish natural
killer (NK) cell responses while stimulating adaptive immune responses
[39,40].

5.4. Failure in the contraction of adaptive immune response

Just as innate immunity is controlled to avoid an excessive inflam-
matory response, so is adaptive immunity also regulated. An effective
immune response eliminates the microbes that initiated that response.
This is followed by a contraction phase, in which the expanded lympho-
cyte clones die and homeostasis is restored, allowing the immune sys-
tem to recover from one response and return to a state of rest after
eliminating each foreign antigen, thus keeping ready to respond to
other antigens [41]. Regulatory T-cell immune responses are driven by
various types of immune cells. T regulatory cells, expressing CD25 (the
interleukin-2 (IL-2) receptor α-chain), have the ability to suppress the
activation, proliferation and effector functions of a wide range of im-
mune cells, including CD4+ and CD8+ T cells, natural killer (NK) and
NKT cells, B cells and antigen-presenting cells (APCs) [42–44]. This
unique ability to control immune responses makes FOXP3 + Treg cells
central to the prevention of autoimmunity, allergy and other immuno-
pathologies that can appear after a persistent and uncontrolled specific
immune response [44,45]. Other cells, such as plasmacytoid DCs, also
regulate B cells via interferon production and direct cell interaction
thus modulating their activation, mediated by TLR stimulation and con-
trolling possible antibody-mediated autoimmunity [46].

5.5. Loss of peripheral immunotolerance

Together with responsiveness to foreign antigens, tolerance to self-
antigens is crucial for the maintenance of the immune system.
Immunotolerance can be central, when it is developed in the primary
immune organs, bone marrow and thymus, triggering deletion of
autoreactive lymphocytes; or peripheral, occurring after the maturation
of lymphocytes away from the primary organs in various ways, includ-
ing: clonal anergy, ignorance, abortion and others [47,48]. Strong adju-
vants are able to break peripheral tolerance by several mechanisms
[49,50].

5.6. Excessive Th2-biased response

The development of Th2 responses against antigens is a malfunction
of the adaptive immune system, with excessive production of IL-4 and
several other cytokines, including IL-13, IL-18, IL-25 and IL-6 with
high production of IgE. This dysfunction of the immune system may re-
sult from false alarms generated by the innate immune system,
resulting in unexpected dendritic cell (DC) maturation after exposure
to allergens. Conditions in the local microenvironment during DC matu-
ration may also result in the preferential induction of Th2 responses
[51]. Once IgE is produced, it binds with high affinity to FcεRI on mast
cells, basophils, and other cells expressing lower levels and various
forms of FcεRI, and after subsequent encounter with the same antigen,
it activates signal transduction pathways that regulate secretion
and other functions of mast cells and basophils, inducing an allergic
inflammation [52].

5.7. Formation and deposition of immune complex

Antigen–antibody complexes are normally produced during im-
mune responses. Such complexes typically produce severe and long-
lasting tissue injury only when they are produced in excessive amounts,
are not efficiently cleared, and become deposited in tissues. The deposi-
tion of immune complexes in vessel walls leads to complement and Fc
receptor-mediated inflammation and injury to the vessels and adjacent
 tissues [53]. Two reactions mediated by immune complex are associated
to vaccination procedures. “Serum sickness” is produced by immuno-
ization with a large dose of a foreign protein antigen or multiple injections
of antigen, which lead to the formation of immune complexes, that are
deposited most often in the kidneys, arteries, and lungs. The “Arthus re-
action” is a localized form of immune complex-mediated vasculitis, in-
duced by injection of an antigen subcutaneously into a previously
immunized individual with high titers of antibodies or an animal that
has been given intravenous antibody specific for the antigen [53,54].

6. Main syndromes associated with systemic toxicity of adjuvanted vaccines

6.1. Acute phase response (APR)

APR is a transient syndrome that develops in response to an injury. It
is mediated by pro-inflammatory cytokines and is characterized by local
inflammatory response and a systemic component. This includes leuko-
cytosis, production of acute phase proteins by hepatocytes, fever and
various changes in lipid, protein and carbohydrate metabolisms that ac-
company typical side effects seen during infections, certain immuno-
therapeutic regimens, fatigue and anorexia [55,56].

Flu-like symptoms (FLSs) are observed in APR, commonly associated
with vaccination with attenuated or killed bacteria or adjuvanted vac-
cines, as an expression of hyperstimulation of the immune factors
[57]. FLS usually appears within hours of the administration of an
immunostimulating drug or vaccine, and recedes within a few hours
without complications. FLS typically consists of fever, chills, fatigue,
myalgia, headache and nausea. Fever of varying magnitudes is characteris-
tic of patients with FLS, ranging from a moderate increase in body
temperature (38–39 °C) to marked hyperpyrexia, exceeding 40 °C in
some cases [58]. IL-1, released by leukocytes, and other endogenous
factors (IL-1β, TNF-α, IFN-β, IFN-γ, IL-6, IL-8), along with macrophage
inflammatory protein-1, act as pyrogens and are the main agents
responsible for these reactions [59].

Pro-inflammatory cytokines, overflowing into the systemic circula-
tion, can gain access to the brain through saturable transport systems,
and enter the circumventricular organs through fenestrated capillaries,
where they induce the production of prostaglandins, such as PGE2, a
centrally controlled mediator of fever. Synthesis of PGE2 depends on
cyclooxygenase (COX) activity, while the induction of COX-2 in
6.2. Hypersensitivity reactions

Hypersensitivity, frequently called allergic reaction, can be the cause of certain local or systemic reactions observed following vaccination. The classic Gell and Coombs classification lists four types of hypersensitivity reactions, namely anaphylaxis (type I), antibody-mediated cytotoxic reactions (type II), immune complex-mediated reactions (type III), and delayed hypersensitivity (type IV). Although this classification was proposed more than 40 years ago, it is still widely used as an aid to understanding immune reactions, even though it does not in itself fully explain all immune-related phenomena [66].

From a clinical viewpoint, the type I hypersensitivity reaction, also known as immediate hypersensitivity and anaphylactic allergy, is the most serious vaccine-associated reaction. It is normally caused by specific IgE antibodies, resulting from a previous sensitization to one of the constituents of a vaccine: the infectious agent or one of its products; adjuvant: aluminum hydroxide; stabilizer: gelatin; preservatives: thimerosal; antibiotic: neomycin; biological culture medium: chicken embryo cells; or a latex stopper on the vial (falta uma).

Anaphylaxis is characterized by three factors: 1) the clinically apparent onset from 10 to 20 min after exposure to the agent in question; 2) the rapid sequence of symptoms that reflect a serious life-threatening condition; and 3) the reversibility of the situation resulting to appropriate treatment. The intensity of the manifestations of anaphylaxis varies from generalized urticaria with erythema and pruritis to most severe cardiovascular and respiratory symptoms with shock, bronchoconstriction and laryngeal edema. Sometimes, isolated urticaria is observed within 24 h of vaccination, which must nevertheless be considered a possible manifestation of a type I allergic reaction, even though other types of immune reaction may be the cause of this skin rash. The gastrointestinal tract may become involved, with non-specific symptoms such as incontinence, vomiting, abdominal pain and diarrhea. The central nervous system can be also affected, including a feeling of impending doom and unconsciousness, probably related directly to hypotension and hypoxia [67]. Rüeggger et al. updated the case definition of anaphylaxis and the guidelines for data collection, analysis and presentation of immunization safety data [68].

Regarding the frequency of these reactions, many studies show that it is of low frequency. For example, in New Zealand a large study over a 5-year period on 15 marketed vaccines revealed an estimated rate of 1 immediate hypersensitivity reaction per 450,000 doses of vaccine administered. Another large study, conducted within the Vaccine Safety Datalink, described a rate of reaction from 0.65 to 1.53 cases per million doses. Although these per-dose estimates suggest that true hypersensitivity reactions are quite rare, the large number of doses that are administered, especially for the commonly used vaccines, makes this a relatively common clinical problem [69].

In rare circumstances, certain vaccines may cause acute exacerba-
tion of allergic diseases, but the argument that vaccination causes allergic disease is not still substantiated by any available evidence [70]. Furthermore, there are no data supporting an association, provocative or protective, between receipt of the BCG, DTP or whole-cell pertussis vaccine and a risk of asthma in childhood and adolescence [71–73].

Even more controversy exists about the possibility that the impact of vaccination on the prevalence of atopic diseases has increased sharply in the past few decades, especially in children 0–4 years old; as hereditary factors cannot account for this increase, there is a search for environmental factors that could promote atopy, including vaccination [4, 74]. The search for the real relationship between vaccination and atopy, at the population level, will require rigorously standardized studies with larger populations, including unvaccinated subjects and a previously unrecognized sub-population genetically vulnerable to the T-helper (TH)-2 immune response [4, 75].

The question of adjuvant-induced allergy is controversial. The general ability of aluminum adjuvants to stimulate the production of IgE as part of the overall Th2 profile and increase eosinophilia is well established. However, in practical conditions, it has been difficult to demonstrate cases where vaccination with aluminum adjuvants has led to IgE-mediated allergy toward the vaccine antigen [76]. Recently, descriptions of the underlying mechanism of aluminum adjuvancy [77–79] and how aluminum adjuvants could promote and enhance non-target IgE synthesis in a genetically vulnerable person [4] have been published. Aluminum increases inflammatory activity, promoting the release of IL-1β, IL-18, and IL-33, but not IL-12, thus leading to a Th2 profile, characterized by further release of IL-3, IL-4, IL-5, IL-9, and IL-13, and IgE-potentiating factors such as SCD23. Furthermore, it stimulates macrophages to produce PGE2, which also participates in regulating immune responses. The response to aluminum-containing vaccines in children and their association with allergic reactions depend on the various genetic variants of cytokines, such as IL-4, IL-13, IL-33, and IL-18, defining a genetically vulnerable sub-population [4].

Type II hypersensitivity reactions are cytotoxic or cytolytic reactions. In these reactions, cytotoxic antibodies recognize an antigen that has attached itself to the membranes of certain cells, forming a combined antigen. These antibodies cause the destruction and lysis of the cell. These reactions are linked primarily to medication-related cytopenia and to blood transfusion injuries resulting from incompatibility. There are sporadic reports of anemia and thrombocytopenic purpura after recombinant hepatitis B vaccine, with possible involvement of type II hypersensitivity [80–82].

Type III hypersensitivity includes reactions resulting from the formation of immune complexes, by combination of an antigen and an antibody, which in turn cause an intense inflammatory reaction, leading to tissue damage. The soluble antigen–antibody complexes can be deposited either locally (to form an Arthus reaction) or be systemically distributed, causing “serum sickness”. The Arthus reaction begins from 2 to 8 h after the injection of an antigen and causes massive, painful swelling of the limb [83]. On the other hand, “serum sickness” includes symptoms such as fever, arthralgia and rashes, which are often urticarial and are observed after the administration of high levels of foreign-source antibodies (e.g., horse serum to treat tetanus) [54, 84].

Type IV reactions are not produced by antibodies, but by Th1 lymphocytes. These reactions are characterized by a delay of 24 to 72 h before the appearance of manifestations, following the introduction of the antigen into the organism, and are restricted to local inflammation at the injection site that can develop as far as a granuloma and local necrosis [83].

6.3. Induction or worsening of autoimmune diseases

Experimentally, it is easily possible to induce an autoimmune disease in genetically susceptible laboratory animals, by immunizing them with a formulation containing a strong adjuvant (e.g. FCA) and an autoantigen [85–87].

In clinical practice, there are numerous reports of suspicions of autoimmune diseases induced by vaccines [88–95]. These reactions have
been attributed principally to the presence of adjuvants [50,92,95–97] and, just recently, an autoimmune/inflammatory syndrome induced by adjuvants (ASIA) has been proposed [98] (Table 1). In addition, some animal models have been developed to seek experimental evidence of the clinical suspicions [95,99–102]. Despite the mounting evidence, there still persists a great polemic, since several epidemiological studies have not confirmed the existence of an unequivocal causal relationship [97,103–105,106,107].

For the epidemiological studies, the three following questions should be addressed when suspecting a link between vaccines and autoimmunity: is a specific vaccine associated with the development of a new unique autoimmune disease?, does a particular vaccine increase the risk of any autoimmune disease in some predisposed individuals? and can a vaccine be safely administered to a patient who is suffering from a known autoimmune disease? [103].

In theory, a classical vaccine formulation contains the necessary elements for a possible induction of autoimmune diseases in susceptible individuals [108]. The antigen may contain mimetic epitopes with self-structures, while the adjuvant stimulates the up-regulation of costimulatory molecules and other products of inflammation, leading to the polyclonal activation of autoreactive T cells [49,50,108]. This bystander activation can wake up anergic autoreactive lymphocytes, reviving their potential to trigger autoimmune reactions [3,109]. Furthermore, in the last decade, the role of innate immune activation and the importance of TLR signaling in T cell-driven autoimmunity has been investigated. It is accepted that given an inflammatory focus releasing high concentrations of proinflammatory cytokines and in the presence of TLR-agonists, T cell regulatory function may be abrogated and/or polyclonal effector T cell populations (including self-reactive T cells) might be expanded. The previous recognition of the specific antigen, with low affinity or in the absence of costimulation, could now receive alternative costimulation via TLR ligation and thus increase the strength of signal received by self-reactive T cells, unleashing their effector function against self [36,110].

Autoantibodies are a hallmark of autoimmune diseases. A transient post-vaccination elevation of autoantibodies in the serum without obvious clinical significance has been described in patients with various autoimmune diseases and in healthy animals [111–113]. However, very few data exist of an increased level of autoantibodies or the appearance of new autoantibodies in healthy persons after immunization. Recently, cases of production of autoantibodies following vaccination with recombinant hepatitis B and influenza vaccines have been reported in humans [114]. The relevance of this subclinical effect requires more detailed study.

Finally, consistent with the fact that vaccine-induced autoimmunity is indeed rare, some genetic pattern could be a predisposing factor in determined subjects, in which antigen presentation, influenced by certain HLA haplotypes such as HLA-DRB1 and DQB1, can lead to the autoimmune cascade after vaccination [98,115,116]. Thus, pre-existing risk factors, including genetic predisposition and even environmental factors, are largely accepted as being involved [117]. Fortunately, the immune system has numerous regulatory mechanisms, and naturally re-establishes the normal balance of adequate response versus over-response.

In spite of experimental and clinical reports of suspected or demonstrated association between hyperstimulation of the immune system and autoimmunity, and the elucidation of some possible mechanisms, the evidence of vaccinations inducing or worsening autoimmune disease remains unclear, highlighting the need for more research to understand the possible role of adjuvants and vaccines in triggering autoimmunity in clinical practice.

### 6.4. Modification of drug hepatic metabolism

Reports of patients developing phenytoin, warfarin or theophylline toxicity, following acute infections [118,119] and anti-influenza vaccination, have been published [119–128]. In one study, toxic elevations in the levels of the concurrent medicines were reported to occur up to 28 days after vaccination [124]. Other vaccines such as Bacillus Calmette Guerin (BCG), and immunostimulators such as interferons and various cytokines are able to produce the same effect [129–131]. The effect of immunostimulation on drug metabolism was demonstrated several decades ago in laboratory animals, using several vaccines and adjuvants, with the demonstration that cytochrome P450 hepatic enzyme inhibition as a consequence immunostimulation is involved, leading to reduced clearance of the concurrently administered medicines [132–135]. Years later, Renton reported that the release of cytokines, such as IL-1, IL-2, IL-6, TNF, TGF-β and IFNs, is involved in modulating the expression of several P450 isoforms [130]. Reversible changes in the pharmacokinetic parameters of theophylline, and decreased expression of CYP1A2, 2B1/2, and 3A subfamily, have also been reported in rats after intravenous injection of lipopolysaccharide (endotoxin) derived from Klebsiella pneumoniae [136] and a similar effect was observed after application of FCA to mice (used as a positive control), for comparison with a non-toxic intranasal adjuvant called AF01 [97].

On the other hand, Prandota have shown the rapid decrease in the total CYP450 liver content of FCA-treated rats and the selective down-regulation of specific CYP isoforms through a direct reduction in mRNA levels (CYP2B, CYP2C11, CYP3A1, and CYP2E1), protein content (CYP2B, CYP2C11, and CYP2E1) and catalytic activity (CYP2C6, CYP2C11, and CYP2E1). Thus, Prandota have highlighted that polymorphisms of drug-metabolizing enzymes and cytokines may contribute markedly to drug-induced hepatotoxicity and drug pharmacokinetic disturbances, affecting genetically predisposed subjects [137].

These reports reveal a real risk of vaccination, in older patients or susceptible people suffering from various chronic diseases, affecting drug metabolism, for example in chronic hepatitis or hepatic cirrhosis with concomitant administration of drugs such as theophylline, carbamazepine and warfarin. Fortunately, this reaction is reversible and, except in some specific serious cases, does not preclude the use of vaccination, but physicians should observe the patients closely after vaccination. Certainly more research is necessary, monitoring other drugs after vaccination, to know their specific pharmacokinetic profile under various clinical conditions.

### 6.5. Vascular leak syndrome (VLS)

Several cytokines have been tried as valuable adjuvants. They can be used either systemically or mucosally in different types of vaccines. Some of the experiments in which cytokines were used as vaccine
adjuvant include TNF-α for mucosal vaccine against HIV [138]; type I IFN as adjuvant in an anti-influenza vaccine [139,140] and hepatitis B vaccination [141]; granulocyte macrophage colony-stimulating factor (GM-CSF) as an adjuvant for hepatitis B vaccination [142], HIV-1 vaccination [143] and in anticancer vaccine [144–146]; interleukin-12 (IL-2) as a mucosal adjuvant for vaccination of humans to protect against respiratory pneumococcal infection [147–149]; interleukin-15 (IL-15) in antituberculosis vaccine [150,151], in antitumor vaccine [152] or in anti-HIV–1 [153]; and interleukin-22 (IL-22) in HBV DNA vaccination [154].

VLS is a major dose-limiting effect seen after administration of several cytokines, such as: IL-2 [24,155–157], IL-1 [158], IL-3 [159], IL-4 [160,161], GM-CSF [162,163], interferon (IFN)-α [164] and IFN-γ [165]. VLS is characterized by an increase in vascular permeability resulting in tissue edema and, ultimately, multiple organ failure. Manifestations of VLS include increased body weight, hypotension, fluid shifts, peripheral edema, pleural and pericardial effusions and ascites. In severe cases, VLS-related conditions may progress to pulmonary and cardiovascular failures [166]. The pathogenesis of vascular damage is complex and poorly understood and can involve activation of endothelial cells and leukocytes, release of cytokine and inflammatory mediators, alteration in cell–cell and cell–matrix adhesion with disturbance of vascular integrity, activation of components of the complement cascade, as well as alterations in cytoskeleton function resulting in disturbance of vascular integrity [166,167]. There have also been reports of the effect of lymphokine-activated killer (LAK) cells on vascular endothelial cells, with the development of VLS [167] involving perforin, Fas ligand and CD44, observed in gene-targeted mice [168,169]. The observation of VLS is a serious obstacle to the clinical use of cytokines as adjuvants in vaccines [3,170].

6.6. Oral immunosuppression or tolerance postvaccination

Oral administration of antigens induces systemic unresponsiveness termed oral tolerance. It is a suppressive mechanism to prevent the host immune system from overreacting to innocuous antigens, but this effect has been often described after oral administration of vaccines. Some adjuvants can induce immune deviation and immunosuppression involving down-modulating an existing immune response for therapeutic benefit [171]. This approach has been explored for the control of two noninfectious classes of disease: allergy and autoimmunity. Therapeutic vaccines against these diseases may involve switching the immune response from one type to another, for example deviation of Th2 to Th1, or suppressing an immune response by the induction of Tregs [45,171]. The development of an antigen-specific Treg inducing vaccine is a major novel goal in the field of immunotherapy against autoimmune diseases and the use of a Treg inducing adjuvant might be required to achieve an effective regulatory response [45].

Just as an adjuvant can be used for therapeutic immunosuppression, this effect can appear as an undesirable effect. Once oral tolerance is induced it is sufficiently robust that subsequent exposure to that antigen, even via a systemic route, suppresses immunity [172,173]. The mechanisms involved in oral tolerance induction and effects are complex and not fully defined, but the main mechanism involves the generation of suppressor-regulatory T cells [174].

7. Impact of doses and route of administration

The influence of the doses and the route of administration in adjuvanted vaccine toxicity has been reviewed [3,83] and the data available are principally referred to local reactions, although, as was already mentioned before, the severity of local reactions may be associated to proportional systemic manifestations [13]. With exception of undesirable tolerance frequently induced after oral vaccination, adjuvanted vaccines administered mucosally (intranasal, oral, sublingual, rectal, vaginal) or transcutaneously, seem to be less toxic for systemic reactions than parenteral vaccination [175]. Interestingly, different cytokines such as: TNF, type I interferon, IL-1, IL-12 and others that are mediators or systemic reactions, were safe and effective when administrated with antigens as mucosal adjuvants [22,138–140,148,175].

8. Non-immunological adverse reactions

Several postvaccination events are often registered but are not regarded of immunotoxic nature. They are principally of infectious, traumatic and psychological origin. Acute infections at the site of inoculation can be produced by injection of a previously contaminated product or due to lack of meticulous attention to sterile technique and disinfection before injection, local abscesses often being produced by Group A Streptococcus and Staphylococcus aureus [204,205]. On rare occasions it is reported that a vaccine caused the same infectious disease against which it was made, owing to problems of attenuation or in a patient with severe immunodeficiency. For this reason, attenuated vaccines are limited in these patients [206].

Frequently, there are reports of symptoms associated with vaccination that are of a psychological nature, with vasovagal reactions: hypotension, vertigo, syncope, vomiting and other digestive symptoms, fatigue, and cardiac arrhythmia [10,11].

9. Final considerations

Undoubtedly the future development of vaccines is dependent on the use of adjuvants. The most important attribute of any adjuvanted vaccine is that it be more efficacious than the aqueous vaccine, and that this benefit outweighs its risk. More than 50% of the adverse events reported correspond to mild or moderate local reactions and symptoms belonging to APR. Generally, grave reactions are of very low frequency or rare. This, together with the lack of understanding of the toxicity mechanisms and some difficulties with methods of assessment, stimulates several controversies over safety vaccination. Few studies have carefully evaluated systemic reactions to adjuvanted vaccines, including the role of the various cytokines [67], One reason for this is that some reactions can occur subclinically (e.g. inhibition of drug metabolism) or can appear a long time after vaccination under the influence of other factors, limiting the possibility of establishing a causal relation, as happens with delayed autoimmune reactions, so that it is hard to define if a reported effect was caused or exacerbated by vaccination or not. Many vaccine safety studies have used the temporal association of immunization as part of the case definition of the event itself. When the reaction occurs rapidly after vaccination, with a time interval of just a few minutes between onset and exposure, there is often little doubt as to the cause of the event. However, delayed anaphylactic or autoimmune events are theoretically possible. In the first cases for example, as an atypical biphasic reaction, when a certain delivery system is used, involving the release of a depot or the delayed metabolism of a vaccine, a reaction might occur late after delivery of the vaccine [207]. Consequently, the temporal relationship between vaccination and a new diagnosis on the day of vaccination can be difficult to discern [208].

Another problem in the recording of postvaccination adverse events is that the basis for the detection of these reactions is under passive vigilance, in which the actual patient or parents voluntarily report the symptoms. Frequently, these reactions are not reported and, if they occur several days later, possibly nobody will find a causal relationship, and the event goes unrecorded. Thus, reliable and well-defined incidence rates cannot be derived from passive reporting systems used in post-marketing surveillance. There is a need for large and prospective multinational studies to arrive at a better understanding of the frequency and true nature of these rare events. Only by providing robust data can we expect to reliably assess vaccine safety and maintain public confidence in our immunization programs [207].

Table 2 summarizes the different factors associated with the occurrence of adjuvanted vaccine toxicity. Undoubtedly, the type of adjuvant
used in the vaccine formulation [3,37] and individual susceptibility to adverse reactions, related to genetic differences [98,108,115,137,194–196,209], are two of the more important aspects that, at the present time, are receiving most attention with regard to vaccine safety. Recently, Poland proposed the term “adversomics” to analyze vaccine adverse side effects at the molecular/genetic/proteomics level, to identify genetic characteristics associated with definable risks for specific serious vaccine adverse events. This includes susceptibility to allergy, autoimmunity, drug metabolic capacity and other reactions.

Dependent on the vaccine formulation and procedures

- **Type of adjuvant**: The nature and mechanism of adjuvant are determinant in the balance between adjuvanticity and toxicity. E.g. oil emulsions induce local reactions, PAMP adjuvants such as LPS lead to APR and cytokines trigger VLS [5,37,176].
- **Antigen**: Antigen can favor autoimmune (molecular mimicry) and allergy [49,108,177,178].
- **Route and site of administration**: The route of administration often determines the local and systemic side effects. The subcutaneous route is more retractive than intramuscular. Correct placement of the injected vaccine into the deep muscular layers decreases the local reactions. Local reactions, notably pain and swelling, are less common when the injection is given in the buttocks than in the thigh and in the arm. No injection site is consistently associated with lower systemic reaction rates. Mucosal vaccination is regarded as safer than parenteral, though Bell paralysis (for the intranasal route) and oral tolerance have been described [6,13,179–184].
- **Doses and booster**: Low or high doses of vaccines lead to failure in the immune response. Short intervals of administration of the same or different vaccines can determine severe local and systemic toxicity. Survivability and immunity to challenge infection can be affected [13].
- **Quality during production or conservation**: Contamination of the vaccine at the time of formulation with retractive chemicals and microbial products; instability of the vaccine on storage with breakdown into retractive side products [6].

Dependent on the host

- **Age**: Vaccination at extreme age favors specific toxic manifestations, such as allergy in babies and drug toxicity by concomitant immunization with anti-influenza vaccine in older people. It is suggested that vaccines administered at birth can decrease the risk of developing diabetes mellitus, whereas primary vaccination after 2 months of age increased the risk of diabetes mellitus [184–188]. The main association might be with autoimmune diseases that are more frequent in women. More studies are necessary [189–193].
- **Gender**: Individual differences in vaccine effectiveness and susceptibility to adverse reactions are determined by HLA, TLRs and other genes. The term “adversomics” refers to vaccine adverse side effects at the molecular/genetic/proteomics level, identifying genetic characteristics associated with definable risks for specific serious vaccine adverse events. This includes susceptibility to allergy, autoimmunity, drug metabolic capacity and other reactions.
- **Genetic background**: Individual differences in vaccine effectiveness and susceptibility to adverse reactions are determined by HLA, TLRs and other genes. For instance, Bell paralysis (for the intranasal route) and oral tolerance have been described [13].
- **Personal pathologic antecedents**: Live attenuated vaccines are contraindicated in immunodeficient patients or those under immunosuppressive treatments. Vaccination of patients with chronic diseases can lead to toxic manifestation such as local infection (site of injection) in diabetics, allergic manifestation in atopic individuals, disturbances of drug metabolism and chronic hepatitis [197–200].

Dependent on external factors

- **Environmental factors**: Several reports warn that environmental factors such as contamination might facilitate the induction of autoimmune diseases postvaccination. More research is necessary. The full content of this review was presented as part of the First Course of Immunotoxicity of Biological Products, for the Program of Biosciences and Biotechnology Applied to Pharmacy, held in February of 2013, in the Faculty of Pharmaceutical Sciences, Unesp Araraquara, SP, Brazil. The authors are grateful for the support granted by the Unesp and CAPES.

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