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Adjuvants, their Role, and Safety Profile in Vaccines

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Abstract

The vaccines in their content have some ingredients called excipients added for a specific purpose. Excipients added in vaccines are used:

- Preservatives to prevent contamination - thimerosal.
- Adjuvants to help stimulate a stronger immune response - aluminum salts.
- Stabilizers to keep the vaccine potent during the transport and storage - sugar or gelatin.

Some excipients are residual trace amounts used during the manufacturing process and removed as the case for cell culture materials used to grow the vaccines antigens- egg protein, various culture media, and inactivating ingredients used to kill viruses or inactivate toxins such as formaldehyde. The purpose of antibiotics using in vaccines like neomycin is to prevent contamination by bacteria [1].

***Keywords**: Vaccine; Adjuvants; Immune Response; Excipient; Aluminum Salt*

Introduction

Additives and stabilizers that are added to vaccines have the role of improving the effectiveness of the vaccines and are present in routine vaccines used for young children. It is very important that this excipient not only increase the immune response but be safe and does not cause an adverse reaction. The amount of Aluminum (Al) taken during the 6 months of the first year of life in young children is 1,5 mg, 3 mg and up to 3,5 mg. Like preservatives, adjuvants are used in the vaccine production process and are removed at the end of the process. The adjuvants are chemical substances that are used in vaccines to increase the immunization response and are called additives. More used are compounds of aluminum like aluminum salts Al $(OH)_3$, the mixture of Al $(OH)_3$, and $AlPO_4$ -salts derived from sodium aluminum phosphate. Aluminum is neurotoxic and it is not known if any immediate biological interaction has between Al and Hg [2]. The production of antibodies is the desired immune response of vaccines. The increase in antibodies can be achieved by adding certain substances to vaccines. These substances added for increasing the antibodies are called adjuvants (from the Latin adjuvare means "help"). The nature of adjuvants is very different depending on the chemical nature of adjuvants, their form of action, and their reactions (side effects).

Materials and Methods

The manuscript is based on the study performed on the role of adjuvants used in human vaccines and their impact on immune response and side effects in the clinical trial and Adverse Events Following Immunization (AEFI). In 1993 Gupta and others studied the side effects of adjuvants caused by unwanted stimulation of different mechanisms of the immune system and rarer adverse pharmacological reactions [3].

Results and Discussion

Types of adjuvants used in vaccines production

Adjuvants are added during the production of vaccines to have an immune response and durability as high as possible. The production of synthetic vaccines with modern and recombinant technologies is immunogenically poor and requires adjuvants as additives to enhance the immune response. The use of adjuvants reduces the cost of vaccines as during the added the adjuvants need less antigen to achieve the desired immune response.

Adjuvants are foreign to the body and we have some adverse reactions caused by them. The most common adjuvants are aluminum salts Al $(OH)_3$, $AlPO_4$, and $Ca_3(PO_4)_2$. Other adjuvants which are used today are based on oil emulsions, bacterial products their synthetic derivatives as well as liposomes or gram-negative bacteria, endotoxins, cholesterol, fatty acids, aliphatic amines, paraffinic and vegetable oils. ISCOMs with Quil-A, Monophosphoryl lipid A, and adjuvants formulations SAFs containing threonyl derivatives or muramyl dipeptides are adjuvants which recently been considered for use in human vaccines.

As mentioned above, adjuvants are chemical compounds, generally heterogeneous groups with only one thing in common, which is the increase of the immune response. The adjuvants are different in terms of how they affect the immune system and what serious adverse effects they cause, due to the resulting overreaction of the immune system.

Table 1 lists the types of adjuvants according to their classification and composition.

Squalene is another adjuvant organic polymer with several epitopes that can be shared with other organic polymers acting as an immunostimulator.

Glenny., *et al*. were the first they found that a precipitated suspension of diphtheria toxoid alum had a much higher immunogenicity than volatile toxoid.

The efficiency of vaccines is based on the ability to form antibodies and the action of adjuvants is based on the immunological action of the vaccine. The efficiency of vaccines depends on the nature of the antigen and the amount of antigenic substance and the mechanism for the diversity of immune actions is complex and not yet very well-known and understood.

N0	Type of adjuvants	Name of compounds
1	Emulsions	Freund's complete adjuvant Freund's incomplete adjuvant Montanide ISA 720 adjuvant Ribi vaccine adjuvant [4].
2	Lipopolym-saccharide (LPS)	Monophosphoryl lipid A (MPL).

3	Mineral components	Ca phosphate [$Ca_3(PO_4)_2$], Colloidal iron hydroxide, Calcium chloride, Zinc sulfate, and cerium nitrate Adjumer Aluminum hydroxide [$(AlOH)_3$], Aluminum phosphate [$Al(PO_4)$] Alhydrogel Aluminum vaccine adjuvant Aluminum potassium sulfate adjuvant 6 Amorphous aluminum hydroxyl phosphate sulfate adjuvant (AAHSA) 2 Adjumer Calcium phosphate gel 1 Calcium phosphate vaccine adjuvant DOC/Alum complex Rehydragel HPA
4	Mycobacterial carbohydrate adjuvants and its components	Lipoarabinomannans Muramyl dipeptide Trehalose-6, 6′-dimycolate (TDM or cord factor) [5].
5	Bacterial products	Cholera toxin Cholera toxin B subunit CpG DNA vaccine adjuvant LTR192G vaccine adjuvant MPL adjuvant [6,7].
6	Corynebacterium - derived P40 O	Porin [8].
7	Immunostimulating complexes (ISCOMs)	CSL Ltd (Australia) [9].
8	Liposomes	AS01E (or AS01B) [10].

Table 1: *Types of adjuvants for human vaccines.*

Today exist many theories about the response of antibodies as evidence of immunization and some way of the classification of adjuvants. The classification of adjuvants is based on:

a) Source origin of adjuvants if is natural, synthetic, or endogenous.

b) Mechanism of action.

c) Physical and chemical properties.

The activity of the adjuvants depends on some factors and this explains the fact why the enhanced immune response obtained with one antigen cannot as a rule be extrapolated to another antigen. The needs of antigens are different help from adjuvants and this depends on the physical, biological, and immunogenic properties of antigens.

The criteria for an adjuvant to use in a human vaccine is to be very effective for increasing immune response and have no side effects.

Theory for the explanation of action and mechanism of adjuvant

The vaccine must have more than one adjuvants which must be in the final products of vaccines and can be combined with one particular antigen.

Chedid in 1985 described the way of action of adjuvants. According to Chedid the action of adjuvants happen in 3 stages:

1. The first stage is the formation of an antigen depot at the inoculation site, with slow passage.
2. The second stage is the presentation of immunocompetent antigen cells.
3. The third stage is the production of different types of lymphokines, interleukins, and death factor, tumor cells.

One adjuvant to use for vaccines should reflect a compromise between requirements to increase the immune response and low levels of adverse reactions as low as possible. During 1925 - 1926 Ramon discovered adjuvants and showed that the antitoxin response to tetanus and diphtheria was enhanced by the injection of these vaccines, along with other compounds such as agar, tapioca, lecithin, oil, and starch. According to the study performed by the Mbow., *et al*. the activities of adjuvants are:

1. Presentation of the antigen, defined by the physical appearance of the antigen in the vaccine;
2. Antigen/adjuvant uptake;
3. Distribution (during this stage happen to target specific cells);
4. Immune potentiation/modulation included activities for the regulation of qualitative and quantitate aspects of immune responses;
5. The protection of the antigen from degradation and elimination.

Adjuvants added to vaccines performed the presentation of the antigen to the immune system, where the microbial synthetic and endogenous adjuvants act by direct stimulation or modulation of the immune system. The role of adjuvants in the presentation of the antigen to the immune response the mode of action of the emulsions is to promote slow antigen release and protection from rapid elimination. The adjuvants in the content of vaccines create the repository of adjuvants at the side of injection and it will lead to the synthesis of pro-inflammatory cytokines and stimulation of innate immunity important for the initial steps of the immune response [11,12].

Below is figure 1 which shows the action of adjuvants for an enhanced immune response [13].

On the surface and inside of Al $(OH)_3$ adsorbed particles of antigens aggregate. The adsorption of antigens helps to save the chemical and physical characteristics of the antigens. The adjuvant particles submit deposited antigens to the immune cells and promote interactions between antigens and immune cells for a long duration to induce immune responses called repository effect or “repository effect”. The factors that influenced the “repository effect” is the physical properties of Al $(OH)_3$ such as surface area, electric charge, morphological

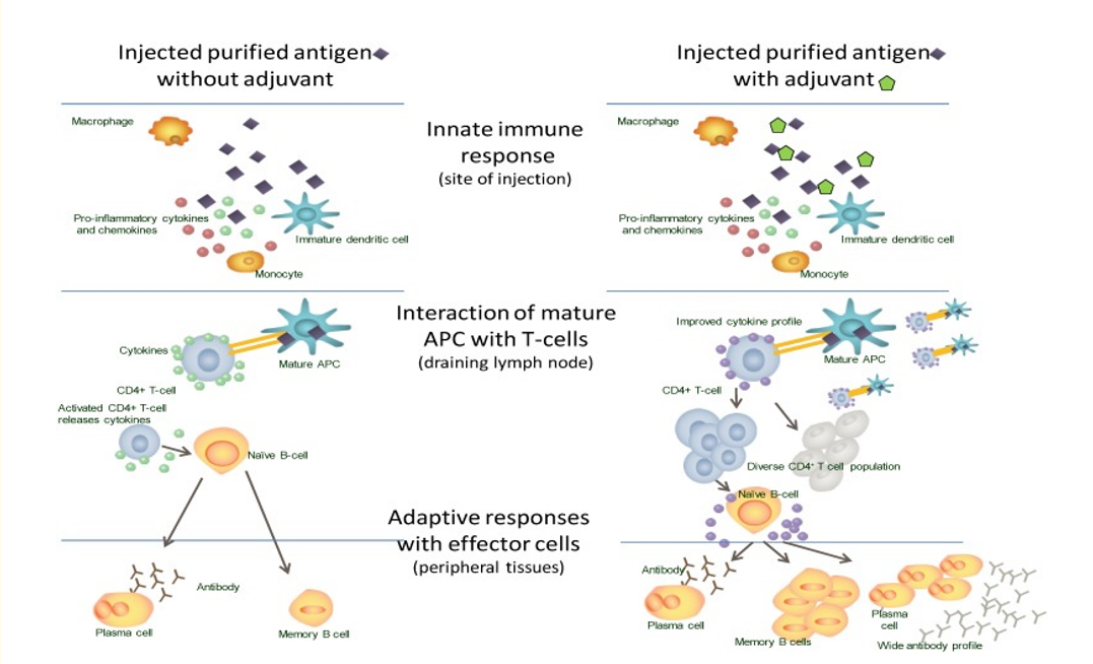

Figure 1: *The role of adjuvants in immune response in vaccines [13].*

structure, etc. According to the study temperature 25^{0}C and pH 7,4 increased the adsorption capacity that promoted antigen storage interaction with antigen-presenting cells (APCs) and an overall stronger immune response. After injection of the vaccine into the organism the antigen adsorbed on aluminum interacts with APCs which primarily evokes an immune response. The antigen inside Al $(OH)_3$ during the decomposition of Al $(OH)_3$ is released gradually, delaying the consumption of antigen and prolonging the duration of stimulation of the immune system. If the interval of interaction between APCs and antigens is prolonged, the immune response will result better. The repository effect has been accepted as one of the mechanisms of Al $(OH)_3$ to stimulate immune responses for a long time.

The study was performed for the Al $(OH)_3$ adjuvant effect in vaccines tetanus toxoid administered with and without adjuvant using radiolabeled tracer experiments. The labeled toxoid was incubated with macrophages *in vitro* and 10 minutes to 6 hours post incubation. Five folds increase the speed of uptake of tetanus toxoid by macrophages in presence of Al $(OH)_3$. The speed of uptake of the antigens by the macrophages will increase 10 folds, 3 hours post-injection compared to the group without adjuvant. Al $(OH)_3$ adjuvant help increases the immune response in vaccines against antigens Adjuvant Al $(OH)_3$ is used in DTP and Hep. B vaccine is safe [11].

Some of the adjuvants used in vaccines are licensed in USA or Europe. Below is the list of adjuvants used in human vaccines licensed in the USA in table 2 [14], and table 3 are adjuvants licensed in Europe [4]. The adjuvants used in vaccines licensed for prophylaxis in humans are very few from those mentioned Alum, and aluminum salts used for more than 70 years and until recently represented the only adjuvant approved in the USA.

Adjuvants	Composition	Vaccines
Aluminum	One or more of the following: amorphous aluminum hydroxyphosphate sulfate (AAHS), aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate (Alum)	Anthrax, DT, DTaP (Daptacel), DTaP (Infanrix), DTaP-HepB-IPV (Pediarix), DTaP-IPV (Kinrix), DTaP-IPV (Quadracel), DTaP -IPV/Hib (Pentacel), DTaP-IPV-Hib-HepB (VAXELIS), HepA (Havrix), HepA (Vaqta), HepB (Engerix-B), HepB (PREHEVBRIO), HepB (Recombivax), HepA/HepB (Twinrix), HIB (PedvaxHIB), HPV (Gardasil 9), Japanese encephalitis (Ixiaro), MenB (Bexsero, Trumenba), Pneumococcal (Prevnar 13, Prevnar 20, VAXNEUVANCE), Td (Tenivac), Td (Mass Biologics), Td (no trade name), Tdap (Adacel), Tdap (Boostrix), Tick-Borne Encephalitis (TICOVAC)
ASO1B	Monophosphoryl lipid A (MPL) and QS-21, a natural compound extracted from the Chilean soapbark tree, combined in a liposomal formulation	Zoster vaccine (Shingrix)
ASO_4	Monophosphoryl lipid A (MPL) + aluminum salt	Human papillomavirus, or HPV (Cervarix)
CpG1018	Cytosine phosphoguanine (CpG), a synthetic form of DNA that mimics bacterial and viral genetic material	Hep B
Matrix M™	Saponins derived from soapbark tree (*Quillaja saponaria* Molina)	COVID-19 vaccine (Novavax COVID-19 Vaccine, Adjuvanted)
MF59	Oil in water emulsion composed of squalene	Influenza (Fluad and Fluad Quadrivalent)
No adjuvants		Chickenpox, cholera, COVID-19 (includes mRNA Pfizer-BioNTech, mRNA Moderna and adenoviral Johnson & Johnson/Janssen), dengue, Ebola, Hib (ActHIB, HIBERIX), measles, mumps & rubella (MMR), meningococcal (Menactra, Menveo, MenQuadfi), polio (IPOL), rabies, rotavirus, seasonal influenza (except Fluad and Fluad quadrivalent), smallpox and monkeypox (ACAM2000, JYNNEOS), Typhoid, yellow fever, zoster live (Zostavax)

Table 2: *Licensed vaccine adjuvants in USA [14].*

Adjuvants	Composition	Vaccines
MF59	Oil in water emulsions	Influenza vaccines
$AS0_3$	Oil in water emulsions	Influenza vaccines
ASO_4	Combination of monophosphoryl lipid A9MPL adsorbed to alum	HBV and HPV vaccines
Mineral compound	Calcium phosphate gel Calcium phosphate vaccine adjuvant	

Table 3: *Adjuvants licensed in Europe [4].*

Adjuvants use for vaccines production

Alum especially Aluminum salt increases the stability and immunogenicity of antigens and is in the formulation as adjuvants in many vaccines including HAV, HBV, DT, Hib (Haemophilus influenza type B), and pneumococcal conjugate vaccines. The action of adjuvants is based on adsorption onto alum particulates increasing antigen.

MF59 Oil in water emulsions like the squalene-based oil-water emulsion MF59 are used as an adjuvant in flu vaccines and is licensed in Europe. This adjuvant was used in 1997 for increasing the immunogenicity of flu vaccines in the elderly. According to the data, MF59 enhances hemagglutination inhibition (HI) titers and cross-protection also in young children.

ASO_3 is similar to MF59 and is licensed in Europe for pandemic flu vaccines, and was used widely in the production of flu vaccines during the H1N1 pandemic flu. MPL is a very important element for effective and safe vaccine adjuvants and this new class of adjuvants is based on targeting the TLR pathways to identify potential vaccine adjuvants and therapeutic agents.

Novel adjuvants and their combination

Agonist (a substance that initiates a physiological response when combined with a receptor).

TLR9 agonists are the most favorite candidate as an adjuvant in vaccines.

Cytosine phosphor guanosine (CpG) are repeating sequences of the immunostimulatory sequences (ISS). CpG dinucleotides targeting TLR9 are being evaluated as vaccine adjuvants. Adjuvant 1018 ISS unmethylated cytosine phosphor guanosine ODN (oligodeoxynucleotides) [15] which use for the recombinant hepatitis B surface.

In some cases, for some difficult vaccines, the single adjuvants maybe are not sufficient to achieve a protective immune response and this is the reason explains why is necessary the combination of TLR Agonists with other classes of adjuvants. This combination is tested in preclinical and clinical trials. The results of the clinical trial show that the reduction of malaria infection achieved was 37% using *Plasmodium falciparum*.

Future adjuvant targets

As we mention all efforts are to find the most potential and safe adjuvants vaccine.

The next generation of vaccine adjuvants based on clinical trial data for effective subunit vaccines is the combination of existing adjuvants. It is the experience with oil in water emulsions in pandemic flu and of MPL with HPV (human papillomavirus) and HBV (hepatitis B vaccine) vaccines that will be the reference for the clinical development of next generation adjuvants [10].

Quality control of adjuvants used for human vaccines

The quality of adjuvants is related to the immune response of the vaccine and for this should be considered some aspects for the evaluation of the quality of a vaccine/adjuvant formulation like:

a) Demonstration of the compatibility of the adjuvant(s) with the antigenic component(s) present in the vaccine,

b) Proof of an adequate and consistent association of the antigen with the adjuvant,

c) Demonstration that no significant de-association takes place in the course of the shelf-life,

d) Degree of association throughout the shelf life,

e) Effect of the adjuvant on the ability to assay components,

f) Biochemical purity and pyrogenicity,

g) Important in the case of aluminum hydroxide gels, aluminum phosphate gels, calcium phosphate gels, and ISCOMS is the adsorption because ionic interaction occurs with charged dimethyl dioctadecyl ammonium (DDA) micelles.

In the case of the emulsions and liposomes, we have another mechanism encapsulation, saponin derivatives, or other extracts, and interactions with antigens are lipophilic/ hydrophilic or ionic.

The adjuvants used in the manufacturing process of vaccines today have different origins and natures. Aluminum is a simple inorganic compound, and PLG (Poly-α-L-Glutamine) is statistically comparable to that of CFA and better than alum in the context of H_1 antigen (Ag85B and ESAT-6 fusion). Using of the adjuvants PLG in 1 mg/dose causes the increasing of immune response of Ag85B, BP26, and protective antigen (PA) by increasing serum antibodies and cytokines in the culture supernatant of antigen-stimulated splenocytes.

Virosomes can be derived from disparate viral particles, and MDP is derived from bacterial cell walls.

Saponins are of plant origin, squalene is derived from shark liver while recombinant endogenous immunomodulators are derived from recombinant bacterial, yeast, or mammalian cells [15].

The testing of adjuvants is performed based on guidelines that should be in conjunction with the Guidelines on pharmaceutical and biological aspects of combined vaccines (CPMP/BWP/477/97). Also, Ph.Eur should consult for different cases and aspects of adjuvants.

For manufacturers of recombinant protein adjuvants, it is useful to consult relevant CHMP and ICH guidelines, for instance, cell substrates (CPMP/ICH/294/95), viral safety (CPMP/ICH/295/95), rDNA proteins (CPMP/ICH/139/95).

Where the adjuvant is a nucleic acid, reference should be made to the CPMP Note for Guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99).

Quality control of adjuvants is based in identifying which are critical parameters and which parameters characterize them and the performed testing for vaccines/adjuvants. Also and other parameters should include in routine testing because the safety and efficiency of vaccines are very important for preventing of infection and for the life of the population.

The characteristics of adjuvants depend from the nature of adjuvants but are not limited necessary as:

- Chemical composition (qualitative and quantitative).
- Physical characteristics (e.g. visual appearance, density, viscosity, pH, size and size distribution, surface charge).
- Biochemical characteristics.
- Purity (e.g. endotoxin content, bio burden, manufacturing residuals).

The studies performed for adjuvants resulted in some adjuvants were never accepted for routine vaccination because had safety concerns such as acute toxicity. The decision for using the adjuvant depends on the analyses of the benefit-risk of any adverse reaction. If we are in a case where the vaccines will inject into healthy people the ratio benefit-risk of vaccination the safety is over the efficacy, but in the case of people with high risk as patients with cancer and AIDS or other "therapeutic vaccines", an increased level of toxicity may be acceptable if the benefit of vaccines is substantial.

The surveillance of AEFI (adverse events following immunization) is necessary for the safety of vaccines/adjuvants and if no serious adverse effect is observed in non – clinical toxicological and safety study, it cannot be guaranteed that the new vaccine/adjuvant formulation presents no risks to vaccines and unexpected events may occur. For this reason, a final safety evaluation of the newly developed vaccine formulation can only be conducted on the basis of clinical trials.

Adjuvants and future perspectives

The combination of adjuvants was the future perspective, but this has critical aspects as depends on the biological properties of antigen/adjuvants e.g. adsorption, and binding characteristics, and should be identified and monitored. It is not easy the combination more than one adjuvant because should perform a study and get the appropriate information for each adjuvant and antigen in detail. The combination of adjuvants and antigens can happen during the producing intermediate bulk or final bulk depending on the vaccines and adjuvants. If the combination of adjuvants will perform during the final bulk should add any excipients or diluent for the combination of adjuvants and antigens and these excipients should not affect the potency of vaccines, should not affect the combination of adjuvants and antigens. The manufacturer performed routine testing of vaccines/adjuvants for the intermediate bulk, final bulk, and final production and documented detailed information about the results of testing and evaluation.

Impact of the adjuvant on the immunological response of vaccines

The immune response in the vaccine involved the administration of each antigen anticipated in the final product alone and with the adjuvants. It is an important issue determination of the humoral immune response and this determination and titration of functional antibodies using the international standard for example the standard of WHO or any other international standard.

Also other properties of antibody response should estimate immunoglobulin subclass responses and will be investigated. The investigation should be explained with results of testing performed for antigen specific T-cell responses (including Th1, Th2, and T regulator cells, and/or relevant cytokines). This information should be in the dossier of vaccines. The information about the new adjuvants for their safety profile, data from the pre-clinical studies, and every change in dose and route of administration should be declared. All information about the content of vaccines is listed in Package. In case the suspicion for an adjuvant will perform the evaluation in humans of a pharmacokinetic study. During the clinical study administrate the information for adjuvants alone and if necessary can obtain further scientific/regulatory advice from EU Regulators [17].

Side effects of adjuvants

Local toxicity is a side effect of adjuvant-associated range from mild injection site pain, tenderness, redness, inflammation, and swelling at one end of the spectrum, to the formation of granulomas, sterile abscesses, lymphadenopathy and chronic skin ulceration at the other end.

If this happens local toxicity will have chemical irritation due to a non-physiological pH, osmolality, salt concentrations, or direct cell toxicity associated with immediate and severe injection site pain, followed by an inflammatory response triggered by the tissue damage. The local reactogenicity caused by adjuvants saponins (e.g. Quil A, QS21, immune-stimulatory complexes [ISCOMs], Iscomatrix and oil emulsions (e.g. complete Freund's adjuvant [CFA], incomplete Freund's adjuvant [IFA], Montanide, MF59, $AS0_3$), which it is not life-threatening. It is not life-threatening but must be careful as lead the significant morbidity as at worst, a sterile abscess needing surgical drainage or skin ulceration requiring skin grafting [18].

Systemic toxicity is a side effect caused by vaccines and adjuvants and the symptoms are fever, headache, malaise, nausea, diarrhea, arthralgia, myalgia, and lethargy. This side effect causes local tissue damage. Systemic toxicity is a rare side effect and happens from abnormal immune activation, promote by the adjuvants and consider the problem is immune bias like eosinophilia, allergic reactions, and

anaphylaxis caused by Th2 bias imparted by aluminum adjuvants. The syndrome MMF was thought to be caused by systemic toxicity, but it was questioned by the World Health Organization (WHO) Global Advisory Committee on Vaccine Safety (GACVS). The hypothesis that adjuvants can cause autoimmune disease cannot be proven.

A study needs to perform for the chronic toxicity caused by the adjuvants as until now cannot explain the doubt about the adjuvants of aluminum or oil emulsions that form long-term tissue depots. The side effect caused by the adjuvants requires new research methods because the studies performed on animal do not guarantee the accuracy of reflecting the human context.

Aluminum adjuvants and side effects

Aluminum is safe and all new adjuvants compare it to the gold standard. Aluminum adjuvants cause local chronic granulomatous lesions in cats, dogs, and ferrets which can progress to malignant fibro sarcomas, but this did not happen in humans immunized with aluminum adjuvants vaccines, and the reason why cannot explain.

The data reported show that aluminum adjuvants in humans cause MMF with symptoms of myalgia, arthralgia, marked asthenia, muscle weakness, and fever, increasing creatine kinase levels, and increase of erythrocyte sedimentation rate plus a myopathic electromyograph. This hypothesis is an area for debate and studies in the future. In 1993 more than 600 cases have been diagnosed in France with MMF syndrome and with sporadic case reports from other countries. Interestingly, the symptoms of MMF closely resemble those of Muckle-Wells Syndrome (MWS), which is caused by inherited mutations that result in constitutive inflammasome activation. As aluminum adjuvants are now known to also induce inflammasome activation, it is possible to speculate that MMF might occur in individuals who are also susceptible to chronic inflammasome activation. The GACVS recommended that to further understand MMF, additional research studies need to be undertaken to evaluate the clinical, epidemiological, immunological, and basic science aspects of this disease.

Tetanus toxoid hyper-immunization is able to reproduce APS (Antiphospholipid syndrome) in mice, which correlates with the induction of cross-reactive low-affinity anti-β (2) glycoprotein I [anti-β (2)GPI] antibodies. APS in humans is not known. If the amount of aluminum is high it affects brain and bone tissues and causes fatal neurological syndrome and dialysis-associated dementia. Cerebral aluminum accumulation has also been observed in Alzheimer's disease. Low doses of aluminum are renally excreted, and under conditions of reduced renal function, aluminum can accumulate in the body and become toxic. None of the studies so far have failed to prove the negative effects of aluminum adjuvant and aluminum adjuvant remains a safe and potential adjuvant. All hypotheses for toxicity, and Alzheimer remain simple hypotheses and have not been proven so far.

Oil emulsion adjuvants and side effects

Oil emulsions adjuvants reflect their ability to induce a strong inflammatory reaction at the injection site, with local cell death leading to the production of DAMPs and inflammasome activation. The oil component also forms a potential long-term depot, which entraps the antigen and slows down its systemic release. Local toxicities of oil emulsions include severe injection site pain due to local tissue damage followed by severe inflammatory reactions, which, in some cases, may progress to the formation of a sterile granuloma or ulceration at the injection site. Emulsion adjuvants, since they have a high degree of reactogenicity, are not ideal adjuvants for use in vaccines. Squalene emulsions in single intradermal injection induce adjuvants arthritis in susceptible murine and rats models using T cells which are inhibited from anti-T- cell antibodies. This action increases pro-inflammatory cytokines, including IL-1 and IFN-γ, in the draining lymph nodes. Oil emulsion adjuvants activate auto-reactive arthritogenic T cells. Really has no information for experimental data about the risk for humans who share genetic susceptibility features with these models could similarly be prone to developing adjuvant arthritis, lupus, autoimmune hepatitis, uveitis or some other form of autoimmune disease after exposure to oil emulsion adjuvants alone or combined with other potent innate immune activators, such as MPL, but have only a theoretical risk.

It might be relevant to the $AS0_3$ adjuvant containing squalene and tocopherol included in the narcolepsy-associated pandemic influenza vaccine. Unfortunately, it is not known what causative factor triggered the narcolepsy, but the $AS0_3$ adjuvant could have played a major role, as no increase in narcolepsy was seen in children who received alternative unadjuvanted vaccines. It is only hypothesized that inflammation induced by the $AS0_3$ adjuvant could have contributed to the breaking of self-tolerance.

IL-17 is thought to play a major role in autoimmune disorders, including multiple sclerosis, rheumatoid arthritis, psoriasis, and experimental allergic encephalitis (EAE). Oil emulsions are potent at inducing inflammatory cytokines, including IL-1 and IL-17. Given the importance of IL-17 for breaking self-tolerance and allowing T cells to cross the blood–brain barrier, this could explain why inflammatory oil emulsion adjuvants are so important to autoimmune disease induction in animal models, and it could also potentially explain the mechanism whereby the $AS0_3$-adjuvanted pandemic influenza vaccine caused narcolepsy in susceptible *HLA-DQB1**0602 (DR2-positive) children.

Saponins adjuvants and side effects

One saponin adjuvant is Quil A which is an extract derived from the bark of *Quillaja saponaria*. This fraction purified from the extract by reverse-phase chromatography, such as QS-21, induces strong humoral and T-cell responses. These saponin adjuvants have been extensively utilized in experimental therapeutic cancer vaccines. Saponin has the effect of detergent and this effect of saponin disrupts cell membranes causing moderate to severe injection site pain and muscle cell damage and death, causing local redness, swelling, and granuloma formation.

Saponin adjuvants also cause red blood cell hemolysis, reflecting the affinity of saponins for cholesterol present in erythrocyte membranes. Reducing of toxic effects of saponin adjuvants can make from the mixing of QS21 can be mixed with cholesterol to form ISCOMs and ISCOM particles induce less hemolysis but still induce systemic side effects, including flu-like symptoms, fever, and malaise. The potential of saponin adjuvants to trigger autoimmunity in humans is not known. Some elderly human subjects in a clinical trial of a QS21-adjuvanted experimental Alzheimer's disease vaccine did develop meningoencephalitis, although the role, if any, of the QS21 adjuvant in these adverse reactions is not known.

TLR agonist adjuvants and side effects

These types of compounds are likely to have very different toxicities and their efforts for reducing toxicity decrease the activity of TLR Agonist adjuvants. This action is case of conversion of the highly toxic TLR4 ligand lipopolysaccharide to the less toxic.

Adjuvants of TLR Agonist activate the nuclear factor of transcription inflammation factor (NF)-κB through proper proteins TLR MYD88 and TRIF.

MPL (Monophosphoryl Lipid gives modest potency and with a combination of aluminum or other adjuvants give the best effect. An example of this combination is ASO_4 adjuvant (a combination of MPL and aluminum). ASO_4 is approved for the HBV (Hepatitis B Virus) vaccine used for prophylaxis of patients with low responder renal dialysis and a prophylactic human papillomavirus vaccine. HBV with ASO_4 adjuvant was more locally reactogenic than a standard aluminum adjuvanted vaccine, with pain at the injection site occurring with 41% of HBV-$AS0_4$ doses, versus 19% of standard vaccine doses, consistent with increased vaccine reactogenicity due to the MPL component.

TLR 4 adjuvants in susceptible animal models cause autoimmunity diseases from the inflammatory agents trehalose dimycolate β-glucan, pristane and squalene oil-are potent inducers of inflammatory arthritis in susceptible animal. It is not known this side effect in human as the amount of adjuvants in human was lower than in animal models. TLR4 agonist adjuvant used in an intranasal influenza vaccine during the immunization of mice caused worsening illness and death when immunized animals were challenged with influenza, with the worsening lung pathology, which was later found to be due to TLR4 agonist as caused an excessive IL-17 response.

Enterotoxin adjuvants and side effects

This type of adjuvant includes cholera toxin (CT) and *Escherichia coli* heat-labile toxin (LT), and mutated variants thereof. CT has a complex range of adjuvant activities which are the promotion of CD40, CD80, and CD86 co-stimulation molecule exponent and IL-4 exponent, influencing the increase of the response of Th2 and a B-cell isotype making possible the production of IgA and IgG, while erasing IFN regulatory factor-8, IL-12 production, and T-cell CD40 ligand exponent, thereby erasing Th1 responses. Severe diarrhea is the main toxicity that depends on the dose of CT unmodified adjuvant and it happens from the increase of secretion of electrolytes and water in the gut lumen. Also are developed and detoxified versions of CT and LT, mucosal adjuvants based in enterotoxin which during the clinical trial of intranasal inactivated influenza vaccine caused facial nerve palsy in a small number of vaccination.

Polysaccharide adjuvants and side effects

The classification of polysaccharide adjuvants based on the activation of NF-kB and in this case are pro- inflammatory include dextran, zymosan, β-glucan, mannan or when NF NF-κB are inactivated and this case are non-inflammatory (delta inulin).

During the injection, the adjuvant of delta inulin has been safe for pregnant rats and 7 days old rat pups. This injection influenced and gave protection with the single dose of the influenza vaccine.

Activation of the complement of polysaccharides cause anaphylatoxin release and if happen the activation of basophil and mast cell will have possible symptoms of anaphylactic shock which appear only after intravenous injection and did not happen during the intramuscular or subcutaneous injection. The complement activation is negative from dextran and delta inulin, binds plasma lipoproteins adjuvants, and is not possible to cause in anaphylactic shock.

Glycolipid adjuvants and side effects

This type of adjuvant has no data for the use as adjuvants in human vaccines, just only animal testing [3,10,19].

Conclusion

1. Adjuvants used in vaccines enhanced immune response and decrease the cost of vaccines.
2. The best adjuvants will be a compound with a very high immune response and without or very less side reactions and this is challenge for the future for vaccines that actually have a low immune response.
3. Adjuvants used in human vaccines are safe.

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