ADVERSE EFFECTS OF ADJUVANTS IN VACCINES

Adjuvants are chemical substances which are added to vaccines to boost immune response, but many of them are known to cause a range of serious side-effects.

Part 1 of 2

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[Editor's Note: This article refers to research studies involving animals. We wish to advise readers that we at NEXUS do not condone or support the validity, efficacy or morality of animal experimentation or vivisection.]

ADJUVANTS, PRESERVATIVES AND TISSUE FIXATIVES IN VACCINES

Vaccines contain a number of substances which can be divided into the following groups:

1. Micro-organisms, either bacteria or viruses, thought to be causing certain infectious diseases and which the vaccine is supposed to prevent. These are whole-cell proteins or just the broken-cell protein envelopes, and are called *antigens.*
2. Chemical substances which are supposed to enhance the immune response to the vaccine, called *adjuvants*.
3. Chemical substances which act as *preservatives* and *tissue fixatives*, which are supposed to halt any further chemical reactions and putrefaction (decomposition or multiplication) of the live or attenuated (or killed) biological constituents of the vaccine.

All these constituents of vaccines are toxic, and their toxicity may vary, as a rule, from one batch of vaccine to another.

In this article, the first of a two-part series, we shall deal with adjuvants, their expected role and the reactions (side effects).

ADJUVANTS

The desired immune response to vaccines is the production of antibodies, and this is enhanced by adding certain substances to the vaccines. These are called *adjuvants* (from the Latin *adjuvare*, meaning "to help").

The chemical nature of adjuvants, their mode of action and their reactions (side effects) are highly variable. According to Gupta et al. (1993), some of the side effects can be ascribed to an unintentional stimulation of different mechanisms of the immune system, whereas others may reflect general adverse pharmacological reactions which are more or less expected.

There are several types of adjuvants. Today the most common adjuvants for human use are aluminium hydroxide, aluminium phosphate and calcium phosphate. However, there are a number of other adjuvants based on oil emulsions, products from bacteria (their synthetic derivatives as well as liposomes) or gram-negative bacteria, endotoxins, cholesterol, fatty acids, aliphatic amines, paraffinic and vegetable oils. Recently, monophosphoryl lipid A, ISCOMs with Quil-A, and Syntex adjuvant formulations (SAFs) containing the threonyl derivative or muramyl dipeptide have been under consideration for use in human vaccines.

Chemically, the adjuvants are a highly heterogenous group of compounds with only one thing in common: their ability to enhance the immune response—their adjuvanticity. They are highly variable in terms of how they affect the immune system and how serious their adverse effects are due to the resultant hyperactivation of the immune system.

The mode of action of adjuvants was described by Chedid (1985) as: the formation of a depot of antigen at the site of inoculation, with slow release; the presentation of antigen to immunocompetent cells; and the production of various and different lymphokines (interleukins and tumour necrosis factor).

The choice of any of these adjuvants reflects a compromise between a requirement for adjuvanticity and an acceptable low level of adverse reactions.

The discovery of adjuvants dates back to 1925 and 1926, when Ramon (quoted by Gupta et al., 1993) showed that the antitoxin response to tetanus and diphtheria was increased by injection of these vaccines, together with other compounds such as agar, tapioca, lecithin, starch oil, saponin or even breadcrumbs.

The term *adjuvant* has been used for any material that can increase the humoral or cellular immune response to an antigen. In the conventional vaccines, adjuvants are used to elicit an early, high and long-lasting immune response. The newly developed purified subunit or synthetic vaccines using biosynthetic, recombinant and other modern technology are poor immunogens and require adjuvants to evoke the immune response.

The use of adjuvants enables the use of less antigen to achieve the desired immune response, and this reduces vaccine production costs. With a few exceptions, adjuvants are foreign to the body and cause adverse reactions.

Part 1 deals with the following types of adjuvants (after Gupta et al., 1993):

- Oil emulsions
  - Freund's emulsified oil adjuvants (complete and incomplete)
    - Arlacel A
    - Mineral oil
    - Emulsified peanut oil adjuvant (adjuvant 65)
- Mineral compounds
- Bacterial products
  - *Bordetella pertussis*
  - *Corynebacterium granulosum*-derived P40 component
  - Lipopolysaccharide
  - *Mycobacterium* and its components
  - Cholera toxin
- Liposomes
- Immunostimulating complexes (ISCOMs)
- Other adjuvants
  - Squalene

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Oil Emulsions

In the 1960s, emulsified water-in-oil and water-in-vegetable-oil adjuvant preparations used experimentally showed special promise in providing exalted "immunity" of long duration (Hilleman, 1966). The development of Freund's adjuvants emerged from studies of tuberculosis. Several researchers noticed that immunological responses in animals to various antigens were enhanced by introduction into the animal of living *Mycobacterium tuberculosis*. In the presence of *Mycobacterium*, the reaction obtained was of the delayed type, transferrable with leukocytes. Freund measured the effect of mineral oil in causing delayed-type hypersensitivity to killed mycobacteria. There was a remarkable increase in complement-fixing antibody response as well as in delayed hypersensitivity reaction.

Freund's adjuvant consists of a water-in-oil emulsion of aqueous antigen in paraffin (mineral) oil of low specific gravity and low viscosity. Drakeol 6VR and Arlacel A (mannide monooleate) are commonly used as emulsifiers.

There are two Freund's adjuvants: *incomplete* and *complete*. The *incomplete* Freund's adjuvant consists of water-in-oil emulsion without added mycobacteria; the *complete* Freund's adjuvant consists of the same components but with 5 mg of dried, heat-killed *Mycobacterium tuberculosis* or butyricum added.

The mechanism of action of Freund's adjuvants is associated with the following three phenomena:

1. The establishment of a portion of the antigen in a persistent form at the injection site, enabling a gradual and continuous release of antigen for stimulating the antibody;
2. The provision of a vehicle for transport of emulsified antigen throughout the lymphatic system to distant places, such as lymph nodes and spleen, where new foci of antibody formation can be established; and,
3. Formation and accumulation of cells of the mononuclear series which are appropriate to the production of antibody at the local and distal sites.

The pathologic reaction to the Freund's adjuvants starts at the injection site with mild erythema and swelling followed by tissue necrosis, intense inflammation and the usual progression to the formation of a granulomatous lesion. Scar and abscess formation may occur. The reactions observed following the administration of the *complete* adjuvant are generally far more extensive than with the *incomplete* adjuvant. The earliest cellular response is polymorphonuclear, then it changes into mononuclear and later includes plasmocytes. The adjuvant emulsion may be widely disseminated in various organs, depending on the route of inoculation, with the development of focal granulomatous lesions at distal places. Various gram-negative organisms may show a potentiating effect of the adjuvant, similar to that displayed by mycobacteria.

The earliest use of oil emulsion adjuvants was made with the influenza vaccine by Friedwald (1944) and by Henle and Henle (1945). Following their promising results on animals, Salk (1951) experimented with such adjuvants on soldiers under the auspices of the US Armed Forces Epidemiological Board. He used a highly refined mineral oil, and developed a purified Arlacel A emulsifier which was free of toxic substances, such as oleic acid which had caused sterile abscesses at the injection site, and he administered the vaccine by intramuscular route.

Subsequently, Miller et al. (1965) reported their failure to enhance the antibody and protective response to types 3, 4 and 7 adenovirus vaccines in mineral oil adjuvant compared with aqueous vaccine. Unpublished studies have revealed the need for an adequate minimal amount of antigen to trigger an antibody response to the emulsified preparations.

Salk et al. (1953) applied Freund's adjuvant to poliomyelitis vaccine, and later followed with extensive testing of killed crude as well as purified polio virus vaccine in animals and humans, where the reactions in humans were considered inconsequential.

Grayston et al. (1964) reported highly promising results with the trachoma vaccine using an oil adjuvant. However, the trachoma vaccine lost its relevance because, as demonstrated by Dolin et al. (1997) in their 37 years of research in a sub-Saharan village, the dramatic fall in the disease occurrence was closely connected with improvements in sanitation, water supply, education and access to health care. According to Dolin et al. (1997), the decline in trachoma occurred without any trachoma-specific intervention.

Allergens in Freund's adjuvant deserve special attention because they can be dangerous. These dangers include an overdose, i.e.,

the immediate release of more than the tolerated amount of properly emulsified vaccine in sensitive persons, or the breaking of the emulsion with the release of all or part of the full content of the allergen within a brief period of time. Long-term delayed reactions include the development of nodules, cysts or sterile abscesses requiring surgical incision. It is also likely that some allergens used, such as house dust or mould, might have acted like mycobacteria to potentiate the inflammatory response. Such reactions have been reduced with the use of properly tested and standardised reagins.

One must also consider that the first application of Freund's adjuvants was made at a time when modern concepts of safety were non-existent. Indeed, mineral oil adjuvants have not been approved for human use in some countries, including the USA.

Mineral Compounds

Aluminium phosphate or aluminium hydroxide (alum) are the mineral compounds most commonly used as adjuvants in human vaccines. Calcium phosphate is another adjuvant that is used in many vaccines. Mineral salts of metals such as cerium nitrate, zinc sulphate, colloidal iron hydroxide and calcium chloride were observed to increase the antigenicity of the toxoids, but alum gave the best results.

The use of alum was applied more than 70 years ago by Glenny et al. (1926), who discovered that a suspension of alum-precipitated diphtheria toxoid had a much higher immunogenicity than the fluid toxoid. Even though a number of reports stated that alum-adjuvanted vaccines were no better than plain vaccines (Aprile and Wardlaw, 1966), the use of alum as an adjuvant is now well established. The most widely used is the antigen solution mixed with pre-formed aluminium hydroxide or aluminium phosphate under controlled conditions. Such vaccines are now called *aluminium-adsorbed* or *aluminium-adjuvanted*. However, they are difficult to manufacture in a physico-chemically reproducible way, which results in a batch-to-batch variation of the same vaccine. Also, the degree of antigen absorption to the gels of aluminium phosphate and aluminium hydroxide varies. To minimise the variation and avoid the non-reproducibility, a specific preparation of aluminium hydroxide (Alhydrogel) was chosen as the standard in 1988 (Gupta et al., 1993).

Despite these conflicting results, aluminium compounds are universally used as adjuvants for the DPT (diphtheria-pertussis-tetanus) vaccine.

The aluminium adjuvants allow the slow release of antigen, prolonging the time for interaction between antigen and antigen-presenting cells and lymphocytes. However, in some studies, the potency of adjuvanted pertussis vaccines was more than that of the plain pertussis vaccines, while in others no effect was noted. The serum agglutinin titres, after vaccination with adjuvanted pertussis vaccines, were higher than those of the plain vaccines, with no difference in regard to protection against the disease (Butler et al., 1962). Despite these conflicting results, aluminium compounds are universally used as adjuvants for the DPT (diphtheria-pertussis-tetanus) vaccine. Hypersensitivity reactions following their administration have been reported which could be attributed to a number of factors, one of which is the production of IgE along with IgG antibodies.

It was suggested that polymerased toxoids, such as the so-called glutaraldehyde-detoxified purified tetanus and diphtheria toxins, should be used instead of aluminium compounds. They are used combined with glutaraldehyde-inactivated pertussis vaccine.

Calcium phosphate adjuvant has been used for simultaneous vaccination with diphtheria, pertussis, tetanus, polio, BCG, yellow fever, measles and hepatitis B vaccines and with allergens (Coursaget et al., 1986). The advantage of this adjuvant has been seen to be that it is a normal constituent of the body and is better tolerated and absorbed than other adjuvants. It entraps antigens very efficiently and allows slow release of the antigen. Additionally, it elicits high amounts of IgG-type antibodies and much less of IgE-type (reaginic) antibodies.

Bacterial Products

Micro-organisms in bacterial infections and the administration of vaccines containing whole killed bacteria and some metabolic products and components of various micro-organisms have been known to elicit antibody response and act as immunostimulants. The addition of such micro-organisms and substances into vaccines augments the immune response to other antigens in such vaccines.

The most commonly used micro-organisms, whole or their parts, are *Bordetella pertussis* components, *Corenybacterium*-derived P40 component, cholera toxin and mycobacteria.

• B. pertussis components

The killed *Bordetella pertussis* has a strong adjuvant effect on the diphtheria and tetanus toxoids in the DPT vaccines. However, there are a number of admitted and well-described reactions to it, such as convulsions, infantile spasms, epilepsy, sudden infant death syndrome (SIDS), Reye's syndrome, Guillain-Barre syndrome, transverse myelitis and cerebral ataxia. Needless to say, the causal link to it is often (even though not always) vehemently disputed and generally considered "coincidental".

Paradoxically, in one case of shaken baby syndrome in which the baby developed subdural and retinal haemorrhages from the disease whooping cough, doctors accused the father of causing these injuries and strenuously denied that the disease pertussis can and does cause such haemorrhages—forgetting that this is the very reason why pertussis vaccine was developed against such a potentially devastating disease in the first place. Such devastating effects are caused by the pertussis toxin, the causative agent of the disease (pertussis is a toxin-mediated disease), employed as the active ingredient in all pertussis vaccines whether whole-cell or acellular (Pittman, 1984).

Gupta et al. (1993) concluded that PT is too toxic to be administered to humans, but chemically detoxified or genetically inactivated PT may not exhibit the adjuvant effects comparable to the native PT.

• Corynebacterium-derived P40

P40 is a particulate fraction isolated from *Corynebacterium granulosum*, composed of the cell wall peptidoglycan associated with a glycoprotein. In animals, it displays a number of activities such as stimulation of the reticulo-endothelial system, enhancement of phagocytosis and activation of macrophages.

P40 abolishes drug-induced immunosuppression and increases non-specific resistance to bacterial, viral, fungal and parasitic

infections. It induces the formation of IL-2, tumour necrosis factor, and interferon alpha and gamma (Bizzini et al., 1992). In clinical trials, P40 was claimed to be efficacious in the treatment of recurrent infections of the respiratory and genito-urinary tracts. Allergens coupled to P40 have been said to be instrumental in desensitising allergic patients without any side effects.

• Lipopolysaccharide (LPS)

LPS is an adjuvant for both humoral and cell-mediated immunity. It augments the immune response to both protein and polysaccharide antigens. It is too toxic and pyrogenic, even in minute doses, to be used as an adjuvant in humans.

• Mycobacterium and its components

Interestingly, *Mycobacterium* and its components, as originally formulated, were too toxic to be used as adjuvants in humans. However, the efforts to detoxify them resulted in the development of N-acetyl muramyl-L-alanyl-D-isoglutamine, or muramyl dipeptide (MDP). When given without antigen, it increased non-specific resistance against infections with bacteria, fungi, parasites, viruses, and even against certain tumours (McLaughlin et al., 1980). However, MDPs are potent pyrogens (maybe that's why they may be effective against certain tumours—my comment) and their action is not completely understood; hence they are not acceptable for use in humans.

• Cholera Toxin

A major drawback with cholera toxin as a mucosal adjuvant is its intrinsic toxicity.

Liposomes

Liposomes are particles made up of concentric lipid membranes containing phospholipids and other lipids in a bilayer configuration separated by aqueous compartments. They have been used parenterally in people as carriers of biologically active substances (Gregoriadis, 1976) and considered safe.

Immunostimulating complexes (ISCOMs)

ISCOMs (DeVries et al., 1988; Morein et al., 1990; Lovgren et al., 1991) represent an interesting approach to stimulation of the humoral and cell-mediated immune response towards amphipathic antigens. It is a relatively stable but non-covalently-bound complex of saponin adjuvant Quil-A, cholesterol and amphipathic antigen in a molar ratio of approximately 1:1:1. The spectrum of viral capsid antigens and non-viral amphipathic antigens of relevance for human vaccination, incorporated into ISCOMs, comprises influenza, measles, rabies, gp340 from EB-virus, gp120 from HIV, *Plasmodium falciparum* and *Trypanosoma cruzi*.

ISCOMs have been shown to induce cytotoxic T-lymphocytes (CTL). Following oral administration, some types of CTLs were found in mesenteric lymph nodes and in the spleen, and specific IgA response could be induced.

ISCOMs have only been used in veterinary vaccines, partly due to their haemolytic activity and some local reactions all reflecting the detergent activity of the Quil-A molecule.

Other Adjuvants: Squalene

Squalene is an organic polymer with some antigenic epitopes which might be shared with other organic polymers acting as immunostimulators. It has been used in experimental vaccines since 1987 (Asa et al., 2000) and it was used in the experimental vaccines given to a great number of the participants in the Gulf War. These included those who were *not* deployed but received the same vaccines as those who *were* deployed.

The adjuvant activity of non-ionic block copolymer surfactants was demonstrated when given with 2% squalene-in-water emulsion. However, this adjuvant contributed to the cascade of reactions called "Gulf War syndrome", documented in the soldiers involved in the Gulf War. The symptoms they developed included arthritis, fibromyalgia, lymphadenopathy, rashes, photosensitive rashes, malar rashes, chronic fatigue, chronic headaches, abnormal body hair loss, non-healing skin lesions, aphthous ulcers, dizziness, weakness, memory loss, seizures, mood changes, neuropsychiatric problems, anti-thyroid effects, anaemia, elevated ESR (erythrocyte sedimentation rate), systemic lupus erythematosus, multiple sclerosis, ALS (amyotrophic lateral sclerosis), Raynaud's phenomenon, Sjörgren's syndrome, chronic diarrhoea, night sweats and low-grade fevers.

This long list of reactions shows just how much damage is done by vaccines, particularly when potentiated by powerful "immuno-enhancers" such as squalene and other adjuvants. Interestingly, vaccinators as a rule consider such problems as mysterious and/or coincidental with vaccines. Since the administration of a multitude of vaccines to the participants (and prospective participants) in the Gulf War is well-documented (in fact, veterans claim they were given many more than were even recorded), this list of observed reactions further incriminates the vaccines as causing such problems.

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Editor's Note:

Due to space constraints, we are unable to publish the references for Part 1 of Dr Scheibner's article until next issue. If any readers urgently require a copy of these references, please contact your nearest NEXUS office.

About the Author:

Viera Scheibner, PhD, is a retired principal research scientist with a doctorate in natural sciences. During her distinguished career, she published three books and some 90 scientific papers in refereed scientific journals.

Since the mid-1980s when she helped develop the Cotwatch breathing monitor for babies at risk of cot death (sudden infant death syndrome, or SIDS), Dr Scheibner has done extensive research into vaccines and vaccinations. In 1993 she published *Vaccination: The Medical Assault on the Immune System*, and in mid-2000 followed up with *Behavioural Problems in Childhood: Link to Vaccination* (see review, 7/05).

Dr Scheibner regularly conducts lectures, attends conferences and debates, is often asked by lawyers to provide expert reports for vaccine-damage court cases. Her previous articles for NEXUS covered the SIDS/vaccines link (2/05), the brain-eating bugs/vaccines connection (3/03), and the shaken baby syndrome/vaccination controversy (5/05).