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Conference report

Workshop summary
Aluminum in vaccines

On May 11–12 in San Juan, Puerto Rico the National Vaccine Program Office (NVPO) sponsored a workshop on aluminum in vaccines. The meeting was attended by a diverse group of vaccinologists, immunologists, experts on metals, pathologists, rheumatologists, and other interested parties. The objectives of this meeting were to: (1) establish a better understanding of the role and need of aluminum as an adjuvant in vaccines; (2) explore the possibility of adverse events due to the use of aluminum in vaccines; and (3) develop a research agenda to expand existing knowledge of the impact of aluminum on the human body. From the Metal Ions in Biology and Medicine International Symposium held immediately prior to the aluminum workshop, we learned about "pervasive uncertainty", a phrase used in this workshop to denote missing data on pharmocokinetics and toxicities of aluminum injected into humans. Even with identification of areas needing further study, it was apparent that aluminum which has been used as a vaccine adjuvant for more than 70 years, has an established safety record with low incidence of reported adverse events.

The first session of the workshop was devoted to important background about immunologic adjuvants in general and aluminum adjuvants in particular. Dr. Robert Hunter, University of Texas, provided a broad overview of the history and development of adjuvants, and the conventional views of their mechanism of action and uses. Aluminum adjuvants have been thought to form a repository of antigen in tissue, to produce particulate antigen for presentation to immune cells, and perhaps to activate complement and other immune enhancers. The immune response to some, but not all, protein antigens is enhanced by aluminum salts, however, these salts have little effect on peptide and polysaccharide antigens. Aluminum adjuvants enhance the primary immunization series, reducing the amount of antigen needed per dose and the number of required doses. They increase the proportion of responders, however, there appears to be little effect of adjuvant in subsequent booster doses.

Dr. Norman Baylor, US Food and Drug Administration, provided a detailed analysis of aluminum adjuvants, as well as regulatory perspectives. The three general types of aluminum-containing adjuvants are: (1) aluminum hydroxide, (2) aluminum phosphate, and (3) alum, or potassium aluminum sulfate. Each of these types of formulations has different isoelectric points, and properties; they are not simply interchangeable. The efficacy of each salt as an adjuvant depends also on the characteristics of the antigens in the vaccine. FDA regulations limit the aluminum content of an individual dose of a vaccine to 0.85 mg. of elemental aluminum. This is equivalent to 15 mg. of alum per dose.

The immunologic advantage conferred by these adjuvants has been well documented, although most of this documentation is found in studies published before 1970. In general, these studies showed that aluminum-adjuvanted vaccines resulted in higher and more prolonged antibody responses than did comparable aqueous vaccines. This advantage was most apparent during primary immunization; there seemed to be little advantage to incorporating adjuvant in booster doses.

The US licensed products that contain aluminum adjuvants include DTP, DTaP, some but not all HIB vaccines, hepatitis B vaccine, and all combination DTaP, HIB, or HB vaccines. Others containing aluminum include hepatitis A vaccine, lyme disease vaccine, anthrax vaccine, and rabies vaccine. Inactivated vaccines that do not contain aluminum salts include IPV and influenza vaccines. Of interest was the fact that there are substantial differences among manufacturers both in the specific aluminum adjuvant used, as well as the amount of that adjuvant, in vaccines such as DTaP and in combination vaccines made by several manufacturers. Dr. Baylor also pointed out that any alteration of a vaccine, such as removal of aluminum in booster doses, would necessitate treating the altered vaccine as a new product requiring the collection of additional clinical data.

Adverse reactions that have been reported with aluminum-containing vaccines are generally local reactions including sterile abscesses, erythema, subcutaneous (SC) nodules, granulomatous inflammation, and contact hypersensitivity. None of these reactions, however, has been sufficiently frequent to arouse concern.

Dr. John Clements, World Health Organization, provided a global perspective, pointing out the extensive global record of safety for aluminum-containing vaccines. For example, three of the six antigens used in the Global Programme on

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Immunization (diphtheria, tetanus and pertussis) contain aluminum. He pointed out that the global DTP supply is quite fragile, much production being local, for example in India and China. While it may be desirable to identify new adjuvants, he emphasized that the message from this conference for the global public should stress the safety of both these adjuvants and these vaccines.

An exploration of adjuvant immunology was presented by Dr. Carl Alving, Walter Reed Army Institute of Research. He outlined that a good adjuvant should do four things: enhance immune contact, increase the height of the antibody response, prolong the immune response, and influence the type of immune response. These need to be accomplished with no increase in adverse effects. A variety of non-aluminum-based adjuvants were discussed, including Freund's incomplete adjuvant, mineral oil/arlacel A, liposomes, and others. He expressed a personal preference for reconsideration of Freund's incomplete adjuvant, since safety concerns are reduced. Particularly appealing was a method of cutaneous immunization devised in his laboratory, which may ultimately prove to be widely applicable in a variety of settings.

Dr. HogenEsch, Purdue University, presented data that suggests that it is most likely that aluminum compounds directly stimulate the immune response by activation of antigen-presenting cells, complement activation, and the induction of chemokines. He described the adjuvant properties of aluminum compounds as type II immune responses, with production of humoral immunoglobulins, especially IgG1, and IgE. These responses appear to be driven largely by interleukins 4, 5, and 13 (IL-4, -5, and -13). Overall, however, the adjuvant effect of aluminum salts are relatively weak in comparison to other adjuvants. He also discussed preliminary animal experiments using aluminum 26 to track aluminum from the local injection site.

Dr. Bruce Fowler, University of Maryland, discussed the toxicities of binary metal mixtures, and how mammalian cells protect themselves against toxic challenges. He pointed out that individuals are exposed to mixtures, not just one thing at a time. There are four types of interactions: no interaction; additivity; synergistic; and antagonistic. In the population at risk, each individual is unique and differs according to pharmacology (dose, time, etc.) and individual characteristics (race, age, gender, etc.). Each individual has a molecular level of protective response to toxins. One of the cellular level protective mechanism is the formation of metallo-thionine complexes, capable of binding many metals, such as mercury but not aluminum. There is no data on the potential toxicitiy of the mixture of mercury and aluminum. Discussion focused on the desirability of identifying possible biomarkers for toxicity.

Session II was led off by Dr. Stanley Hem, of Purdue University. He discussed the pharmacology of aluminum salts, and introduced the workshop participants to just how much aluminum we are exposed to, how much is in us, and how it is handled within the body. An average daily exposure is about 10–15 mg, most of which comes from foods. While aluminum adjuvants have been used in vaccines for many years, their disposition following intramuscular (IM) administration has not been studied because the low dose did not cause detectable changes in the normal plasma concentration (5 μg aluminum/l). Now, accelerator mass spectrometry (AMS) can accurately measure very small concentrations (10^{-17} g) of aluminum 26 which has no measurable radiation and is considered safe. Preliminary animal experiments have shown that the aluminum adjuvants are dissolved by citrate in the interstitial fluid, leaving the body rapidly. The ability of the body to eliminate aluminum-containing adjuvants may be partly responsible for the excellent safety record of these adjuvants.

Drs. Sam Keith and John Wheeler, both of the Agency for Toxic Substances and Disease Registry (ASTDR), discussed aluminum toxicology and minimum risk levels. These were particularly helpful presentations both in understanding the ubiquity of aluminum in our environment (aluminum is our third most abundant element behind oxygen and silicon), and understanding the uncertainties incorporated into establishing guidance levels. There seems to be abundant data concerning risk levels for ingested aluminum, but scant data about risk levels for injected aluminum. The oral minimum risk level, for example, appears to be in the range of 2–60 mg/kg of aluminum per day but there are no comparable data for injected aluminum. The uncertainties notwithstanding, there appeared to be a large margin of safety for aluminum adjuvants.

Dr. Margaret Rennels, University of Maryland, next presented the results of her studies on the extensive limb swelling occasionally seen with booster doses of DTaP vaccines. This was seen following dose four or higher in from 5–27% of vaccinees; it is a self-limited reaction, usually not accompanied by significant pain or fever. There was a trend toward an increasing frequency of swelling with higher concentration of antigens, particularly pertussis, in the vaccine, however there was a lack of a consistent relationship between the quantity of aluminum in the vaccines and rates of extensive limb swelling.

The last presentation of session II was by Dr. Phillip Pittman, US Army, Fort Detrick, who reported on a pilot study of adverse reactions to anthrax vaccine. Although there was no effect of route of administration IM versus SC on systemic reactions, there was clearly a higher rate of local reactions in those given vaccine by the SC route. Regardless of route of administration, however, there was marked increased frequency of extensive limb swelling reactions in women.

Discussion sessions brought out a number of questions. Although it was tempting to recommend using the IM route only for anthrax vaccine, for example, it became clear that a larger trial still needed to be performed to be able to establish this indication. There was discussion by manufactures that aluminum salts are also important during the formulation of vaccines. Thus in addition to the adjuvant effects

of aluminum salts, they may absorb toxins such as endotoxin, reduce the reactogenecity of antigens like diphtheria and tetanus, and solublize some antigens. We know the advantages provided by use of aluminum in vaccines; we do not know how vaccine efficacy and immune system response would be affected by removal of the aluminum salts used in some vaccines. Therefore, to eliminate aluminum from the vaccines in which it is contained, careful sound scientific research would be required to address the questions raised. Noting that many of the symptoms of aluminum overdose are classical symptoms of complement activation, it was suggested that research agendas include studies of complement activation with aluminum adjuvants in animal models to attempt to identify a potential biomarker.

The final session of the workshop was divided into two sections. The first half was utilized to discuss the histologic entity macrophagic myofasciitis (MMF), and possible clinical associations described by Dr. Romain Gherardi, of the Universtaire Henri Mondor, France. The second half of the session was a discussion of "what we know and what we don't know" concerning aluminum and other vaccine adjuvants toward the development of a prioritized research agenda.

The newly recognized histologic entity, MMF, was originally described in France in 1993, and first published in 1998 (Lancet 352 (1998) 347–352). In his presentation, Dr. Gherardi stated that there have been 100 cases collected so far, 92 of which are from France. The observations that he presented were derived from the first 50 such patients. Deltoid muscle biopsies in those individuals revealed an unusual pattern of extensive infiltration of macrophages around, but not inside, muscle fibers. There were also a few CD8+ T cells. Many of the macrophages were noted to contain PAS-positive crystalline structures, which subsequently were identified as aluminum salts. Notably there was not muscle fiber damage, necrosis, giant cells, nor mitotic figures.

Dr. Gherardi also noted among these patients a cluster of symptoms consisting of diffuse myalgia, arthralgia, and fatigue. Laboratory evidence of inflammation was variable; most patients had a normal white blood count, but about half had some serum autoantibodies present. In addition, serum levels of certain cytokines seemed to be significantly increased, particularly IL-1 receptor antagonist and IL-6.

These patients were mostly middle-age adults with males and females about equally represented. All of them had received aluminum-containing vaccines, mostly hepatitis B vaccine, presumably in the biopsied deltoid muscle. A mean of 36 months had elapsed between vaccination and muscle biopsy. A high proportion of patients were health care workers, had a sport affiliation, or had traveled extensively. There was a seemingly higher than expected proportion of patients with concurrent autoimmune disease, 34%. In fact 6 of his 50 patients had multiple sclerosis. He reported that most patients responded to treatment with steroids and/or antibiotics.

Dr. Gherardi stated his belief that the clinical symptoms in these patients were caused by the aluminum adjuvant present in the vaccines the patients had received. He suggested these findings develop only in a few patients who are otherwise "primed" or predisposed in some way to aberrant immunologic effects. Although Dr. Gherardi referred to the cluster of symptoms as MMF, the other participants felt that that term should be reserved for the histologic lesion originally described in his 1998 paper. No normal control patients have similarly been examined because of the invasiveness of the procedure.

Discussion by the workshop participants was spirited, and clarified several important points. First, MMF has appeared thus far primarily in France; Dr. Gherardi believed there were two reasons for this: first, an aggressive campaign to immunize adults with hepatitis B vaccine had recently been carried out there; second, the French typically do a deltoid biopsy whenever muscle biopsy is indicated. Other investigators stated that a muscle biopsy would not be considered warranted for the described symptoms and that deltoid biopsy would not be utilized because of potential artifact; most would utilize the gastrocnemius whenever muscle biopsy were needed.

The fact that all the patients with the cluster of clinical symptoms had all undergone muscle biopsy, may in fact explain the apparent over-representation of people with autoimmune disorders. Further, the fact that so many patients had received hepatitis B vaccine may explain the unusual frequency of health care workers and travelers in the patient population. Thus, there were felt to be many potential problems with patient selection biases and lack of asymptomatic controls.

The major criticism centered about the causation issues and the lack of suitable controls. Dr. Gherardi concurred that his causation thesis had yet to be proven. Some participants thought that the lesions of MMF may simply represent the normal immune response; that is an epiphenomenon, with little pathologic significance of its own. Some wondered if the steadily rising number of cases in France may reflect an epidemic of recognition. Most were skeptical that the histological findings were pathologic and feel that the "lesion" may be common to many of our own deltoids. It was agreed, however, that it was critical to try to identify a control population to study, such as, trauma victims.

Dr. Francoise Verdier, Aventis Pasteur, France, next described a large number of animal studies, both planned and already in progress, to examine the evolution of aluminum adjuvant—associated histology. In addition, a variety of in vitro studies of human macrophages exposed to various aluminum salts will be undertaken. Thus, a broad array of studies to further define this histologic entity is being undertaken. Autopsy studies of humans were suggested as another approach to generating population control data.

Next, two panel discussions, aptly entitled "what we know" and "what we don't know: establishing a research agenda" served to summarize the deliberations of the

workshop. The first panel identified the following facts that are known about aluminum in vaccines.

1. We want vaccines to be safe and effective.
2. There is a 70-year history of safe and effective use of aluminum salts in vaccines which continue to save millions of lives annually.
3. Minor reactions have occurred but there have been few serious reactions.
4. Aluminum-containing vaccines injected SQ appear to produce more severe local reactions than after IM injection.
5. There is not a consistent relationship between aluminum content and the rate of severe local reactions following IM injection.
6. More data is needed on the toxicopharmacology of aluminum exposures by the IM route; however, there appears to be little potential for toxicity with vaccine-level exposures to aluminum.
7. There is no obvious substitute for aluminum as an adjuvant in many vaccines.
8. Roles of aluminum adjuvants:
   - to bring the antigen into contact with the immune system and influence the type of immunity produced, as well as the quality of the immune response (magnitude or duration), the affinity, isotype and the specificity;
   - to decrease the toxicity of certain antigens such as pertussis; and
   - to provide solubility to some vaccines components.
9. The term MMF should be reserved to identify the recently recognized histologic entity found primarily in France.
10. The MMF lesions contain aluminum hydroxide crystals; the histologic entity is likely caused by aluminum-containing vaccines.
11. The relationship between the focal MMF lesions in the injected muscle and the systemic symptoms of some patients who have the lesion remains to be established.
12. Causality has not been established for Dr. Gherardi's claim that MMF, the histologic entity, is associated with a "symptom complex" of fatigue and ascending myalgias.

The second panel discussed "what we don't know" about aluminum-containing adjuvants and identified the following areas to be more thoroughly studied.

1. Toxicology and pharmacokinetics of aluminum adjuvants. Specifically, the processing of aluminum by infants and children.
2. Mechanisms by which aluminum adjuvants interact with the immune system.
3. Necessity of adjuvants in booster doses.
4. Definition of frequency and duration of the MMF lesion in normal people.
5. Role of aluminum in the pathophysiology of the MMF lesion.
6. Human control studies to assess the relationship between the "symptom complex" identified by Dr. Gherardi in patients who have the MMF lesion and the MMF lesion.
7. New adjuvant development.
8. Expanded trials of IM rather than the SQ route of injection for anthrax vaccine and non-needle vaccine administration technologies.

Mr. Max Lum, ATSDR, who emphasized issues in risk communication, delivered the closing formal presentation of the workshop. Historically, the vaccine "establishment" has done well in communicating the enormous benefits of vaccines, but less well in communicating vaccine risks.

In summary, a variety of aluminum salts have useful physicochemical and immunogenic properties that lend these minerals to use in vaccines. Based on 70 years of experience, the use of salts of aluminum as adjuvants in vaccines has proven to be safe and effective. Aluminum as an adjuvant enhances antigen presentation and stimulates a type II immune response. It has been possible, using aluminum adjuvants, to reduce the number of injections and the amount of antigen per dose, and thereby decrease the toxicity of some antigens. Without extensive research, it is impossible to know how removal of aluminum from vaccines would affect the known benefits of vaccines in which it is contained. More pharmacokinetic data are needed but there is an apparent wide margin of safety with the use of aluminum adjuvants and reported adverse events have been mostly minor and of low incidence. MMF histologic lesions may be a consequence of the normal immune response and may, in fact, be a wholly serendipitous finding in patients with ascending myalgias and fatigue. Some identified areas of research include: expanding the aluminum pharmacokinetic database, especially following IM injection in young children, conducting bimetal (mercury and aluminum) toxicological studies in animals, identifying biomarkers of toxicity, defining the frequency and duration of MMF in normal controls, determining the role of aluminum in the pathophysiology of the MMF lesion, developing new adjuvants, and establishing new methods for administering immunizations.

Theodore C. Eickhoff
Division of Infectious Disease
University of Colorado Health Sciences
4200 East 9th Avenue
Denver, CO 80262, USA
Corresponding author. Tel.: +1-303-315-3052
E-mail address: theodore.eickhoff@uchsc.edu (T.C. Eickhoff)

Martin Myers
National Vaccine Program Office
1600 Cligton Road
MS 0-66, Atlanta, GA 30333, USA
E-mail address: mmyers@osophs.dhhs.gov (M. Myers)