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# Assessing the Safety of Adjuvanted Vaccines

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Despite the very low risk-to-benefit ratio of vaccines, fear of negative side effects has discouraged many people from getting vaccinated, resulting in reemergence of previously controlled diseases such as measles, pertussis, and diphtheria. Part of this fear stems from the lack of public awareness of the many preclinical and clinical safety evaluations that vaccines must undergo before they are available to the general public, as well as from misperceptions of what adjuvants are or why they are used in vaccines. The resultant “black box” leads to a preoccupation with rare side effects (such as autoimmune diseases) that are speculated, but not proven, to be linked to some vaccinations. The focus of this review article is to open this black box and provide a conceptual framework for how vaccine safety is traditionally assessed. We discuss the strengths and shortcomings of tools that can be and are used preclinically (in animal studies), translationally (in biomarker studies with human sera or cells), statistically (for disease epidemiology), and clinically (in the design of human trials) to help ascertain the risk of the infrequent and delayed adverse events that arise in relation to adjuvanted vaccine administration.

## INTRODUCTION

Although vaccines have been responsible for preventing more deaths than virtually any other medicinal product, the administration of vaccines to otherwise healthy subjects compels a high level of scrutiny for toxicity. Vaccines are composed of diverse components: attenuated or live organisms (viruses, bacteria, or parasites), living irradiated cells, virus-like particles (VLPs), recombinant viruses, plasmid DNA, synthetic peptides, polysaccharides, or purified/recombinant proteins. Furthermore, to enhance the immune response, some vaccines are formulated with adjuvants, which include compounds such as inorganic salts, oil emulsions, oligonucleotides, and modified lipopolysaccharides (alum, MF59, CpG DNA sequences, and monophosphoryl lipid A, respectively). This diversity of ingredients calls for a careful evaluation for safety similar to that for any new chemical entity.

Preclinical toxicology studies (Table 1) provide an important set of signals that may indicate a risk for human toxicity. There are clear and defined guidelines governing these studies from the U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), and the World Health Organization (WHO), which are nicely summarized in a recent review (1). The strength of these studies lies in the dual assessment of inherent toxicity of vaccine formulations (including various excipients and added adjuvants) as well as from the induced immune response.

However, these current preclinical toxicology studies have limitations and may not be adequate for identifying an increased risk for certain types of clinically relevant vaccine-associated serious adverse events (such as febrile seizures, anaphylaxis, immediate hypersensitivity, and intussusception). Indeed, in a few dramatic cases, such as the cytokine storm produced by anti-CD28 therapeutic antibody in humans, which was not observed in preclinical animal studies (2), such routine studies can fall short in predicting acute toxicities in humans because of the lack of relevant species for toxicity evalua-

tion. For adjuvanted vaccines, in particular, species-specific differences in innate cells, receptors, and tissue distribution of common components of the immune system may lead to inappropriate conclusions regarding human safety. This issue becomes even more complex when trying to predict toxicities that are rare and that might occur acutely (such as viscerotropic disease and neurotropic disease after yellow fever vaccination) or arise either months or years after vaccination (such as autoimmune disease). Thus, large randomized clinical trials are critical to compare the frequency of side effects in a vaccinated population and in a control population.

Both in the previous decades for nonadjuvanted vaccines and more recently for adjuvanted vaccines, a major vaccine safety concern has centered on the possibility of potent stimulators of the immune response increasing the development of autoreactivity. However, the events surrounding the false association between the MMR (measles, mumps, and rubella) vaccine and autism (3) also illustrate the danger to the public welfare when an effective and generally safe vaccine is incorrectly associated with a rare and delayed adverse event (decreased vaccination followed by increased morbidity and mortality in subjects not protected from natural infection). Thus, this review will focus on vaccines containing adjuvants and the rare and delayed adverse event of autoimmune diseases.

## WHY USE ADJUVANTS?

Although adjuvants, particularly alum, have been used for many decades in vaccine formulations, they have become increasingly important for several reasons. First, although the use of more highly purified vaccine antigens (subunit and recombinant antigens) has led to vaccines that are better defined, these antigens lack some of the immunogenicity provided by contaminants in vaccines developed in the first half of the 20th century. For example, the immunogenicity of earlier vaccines, like whole-cell diphtheria, pertussis, and tetanus (DPT) vaccine (4), was enhanced by the presence of low levels of ill-defined immunostimulatory components (5), perhaps ligands for Toll-like receptors (TLRs) and other innate immune receptors (6, 7). The addition of adjuvants can compensate for the reduced immunogenicity of purer antigen preparations and provide a vaccine better mimicking natural infection. Second, adjuvants are becoming increasingly important for enhancing

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the immune response of individuals who, for reasons of health or age, do not respond as well as healthy young adults to current vaccines. Finally, several adjuvants currently being evaluated by vaccine manufacturers are designed to generate immune responses that are not possible with vaccine antigens alone—for example, stimulating CD8<sup>+</sup> T cell responses to purified protein antigens (8).

Vaccine adjuvants were traditionally considered to be either immunostimulatory agents or carriers for antigen delivery. However, recent studies have blurred this distinction: Two widely used adjuvants in the latter category, alum and MF59, have demonstrated potent immunostimulatory activities that may account for much of their effectiveness (9–11). Indeed, advances in our understanding of how pathogens are sensed by the innate immune system (12, 13) have focused the effort in new adjuvant development on ways to mimic specific necessary elements of innate immune responses, such as TLR ligands or VLPs, while avoiding unwanted secondary effects, such as reactogenicity. Paradoxically, as the ligands, receptors, and signaling pathways for innate recognition of pathogens become better characterized, the potential concern for triggering inappropriate immune responses with adjuvanted vaccines, particularly autoimmunity and inflammation, has increased.

**PUBLIC PERCEPTION OF VACCINE DANGERS:  
WHAT IS THE CAUSE FOR CONCERN?**

In recent years, the risk of developing autoimmune diseases from newly developed vaccines has been the focus of much media attention and has resulted in low public acceptance of new vaccines, such as the pandemic influenza vaccine, in many countries, including Greece (14), Hong Kong (15), Spain (16), Germany (17), UK (18), France (19), and Italy (20). However, there are actually very few documented cases of vaccines inducing autoimmunity. The 1920s rabies vaccine made from phenolized sheep brain induced an acute disseminated encephalomyelitis—a demyelinating disease—in 0.1% of vaccine recipients because of the development of myelin basic protein antibodies (21). The 1970s influenza vaccine, which contained the high-yielding

influenza recombinant X-53 (22, 23), had an estimated attributable risk for Guillain-Barré neuritis (24) of about 1 case per 100,000 vaccine recipients, although newer influenza vaccines have a substantially lower risk of 1 in 1 million (25, 26). The measles and MMR vaccines, which are live, attenuated vaccines, are associated with a risk of autoimmune thrombocytopenia in 1 of 30,000 vaccinees. This rate is lower than the rate of autoimmune thrombocytopenia (1 in 6000 or 1 in 3000) that is associated with actually contracting measles or rubella, respectively (24, 27). Notably, none of these vaccines contained adjuvants. More recently, an intranasal inactivated virosomal subunit influenza vaccine containing *Escherichia coli* heat-labile toxin adjuvant was developed and licensed in Switzerland, but is no longer in clinical use because of a strong association with the development of Bell’s palsy (28). Although high profile, these four cases stand in stark contrast to the safety and efficacy of most vaccines currently on the market.

Although actual adverse events resulting from vaccination are rare, natural infection has been shown to influence the development and severity of autoimmune diseases. For instance, infections can trigger a “flare” in patients with previously diagnosed autoimmune diseases. In a study with 69 patients with systemic lupus erythematosus (SLE) and 54 patients with rheumatoid arthritis, influenza-vaccinated and nonvaccinated cohorts were followed for occurrence of acute bacterial bronchitis and viral respiratory infections (29). Every viral and bacterial infection resulted in the worsening of the main disease. Similarly, in another study, a patient with SLE developed a severe flare after infection with parvovirus B19 (30). Because a vaccine stimulates some of the same host responses (such as innate immune activation by pattern recognition receptors and T and B cell activation through specific antigen receptors) as an infection (12, 31), vaccines might similarly exacerbate a preexisting autoimmune condition or trigger a clinical manifestation of autoimmune disease in healthy subjects that are genetically predisposed to developing autoimmune disease. However, Stojanovich reported that preexisting autoimmune disease was exacerbated by infection but not influenza vaccination (29). Indeed, a recent meta-analysis indicated that several vaccine-preventable infections occurred more often in patients with autoimmune disease, that vaccines were efficacious in these patients, and that there did not appear to be an increase in vaccination-related harm compared to nonvaccinated patients (32). Therefore, several new recommendations for the vaccination of adults with autoimmune disease based on this meta-analysis have recently been published (33).

A second connection between vaccination and autoimmune disease is the existence of animal models in which autoimmune disease is triggered using autoantigen preparations that contain adjuvants. The adjuvants used in these studies are typically strong adjuvants [such as complete Freund’s adjuvant (CFA)], which are being used with self or self-mimetic antigen to intentionally break immunological tolerance in animals. Indeed, CFA, which is a solution containing antigen and inactivated and dried mycobacteria emulsified in mineral oil, is not approved for use in people. In the case of vaccine preparations that prevent infectious diseases in humans, adjuvants are formulated to promote immune responsiveness, not break immunological tolerance. Moreover, vaccine antigens are screened for molecular mimicry to exclude autoantigens, using *in silico* and *in vitro* studies.

The perceived division between adjuvanted and non-adjuvanted vaccines is quite artificial; live vaccines, for example, frequently contain endogenous immune stimulatory components that function as adjuvants despite their classification as nonadjuvanted vaccines. Still,

**Table 1.** Summary of preclinical vaccine toxicology studies: defining vaccine toxicity.

| Type of study                                               | Toxicity assessed                                                                                                           |
|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| Single dose                                                 | Mortality, changes in physiology (fever), body weight, and food consumption                                                 |
| Repeat dose                                                 | Similar to single dose but expanded to include ophthalmic exam, urine/blood studies, and histopathology of all major organs |
| Local tolerance                                             | Macroscopic and histopathologic irritation at injection site                                                                |
| Safety pharmacology                                         | Undesirable effects on specific organ systems                                                                               |
| Development and reproductive                                | Fertility, fetal development, and postnatal development of offspring                                                        |
| Specialized toxicity                                        | Theoretical/potential concerns (for example, virulence/neurovirulence studies for live, attenuated virus vaccines)          |
| Toxicology studies for new chemically synthesized adjuvants | Systemic hypersensitivity and genotoxicity                                                                                  |

any evidence for an association between adjuvanted vaccines and an increased risk of autoimmunity in humans is a cause for concern. Thus, there is a need for more effective means of assessing the risk of vaccine-induced autoimmunity, whether at the preclinical, translational, or clinical stage of vaccine development, and of relaying these findings to the broader public community.

### PRECLINICAL SAFETY STUDIES: CAN ANIMAL MODELS PREDICT RARE ADVERSE EVENTS?

Preclinical studies in animal models are an essential precursor to safety testing of vaccines in man. Currently accepted strategies for preclinical identification of potential adjuvant toxicity have proven valuable for identifying, before human testing, common and immediate adverse reactions to a vaccine, such as fever, injection-site rash or induration, and musculoskeletal manifestations (Fig. 1). However, suitable preclinical models for predicting relatively rare and often delayed adverse events, such as an increase in the incidence of an autoimmune disease, have proven elusive. Human autoimmune diseases are diverse and complex, and the nature of the trigger cannot be simplified to the point that one “test” can be reliably expected to predict disease induction. Evaluating the effects of a vaccine or adjuvant on one or two disease models within this category would provide no information about the effects on other diseases within the category. Difficulties and limitations with these tests include the differences between animal disease models and real human disease as well as fundamental genetic and physiological differences between humans and commonly used laboratory animals.

One such limitation is that although many animal models exist for autoimmune disease that mimic specific features of a human disease, most of these models have been developed on the basis of similarities to end-stage pathology of the human disease rather than similarities in the etiology or pathogenic mechanisms. For example, several widely used mouse models of lupus, such as the (NZBxNZW)F1 and MRL-*Fas*<sup>lpr</sup> mouse strains (34), approximate the high levels of autoantibody and consequent lupus nephritis but do not reflect many other features of this complex disease, such as musculoskeletal or neurological symp-

toms. In addition, factors that initiate disease in animal models might not necessarily be relevant to human disease development. Models in which disease is induced by immunization with high doses of auto-antigens and adjuvants might reflect the effector stage of organ pathology, but the induction phase in these models has little, if any, relevance to disease development in humans. In one example, a widely used rodent model for multiple sclerosis, experimental autoimmune encephalomyelitis, is induced by immunizing animals with a homogenate of spinal cords emulsified in CFA (35). Such models are unlikely to provide insight into the potential of a candidate vaccine or adjuvant to influence onset or severity of autoimmune diseases in humans. Both the incomplete correlation with human disease and the divergent mechanisms of disease initiation limit the relevance of even the “best” animal models to specific human diseases.

Spontaneous models of autoimmunity may be more relevant to human disease development, but these models also have significant limitations. Rodent models in which disease develops spontaneously reflect a combination of genetic and environmental factors analogous to those thought to determine human autoimmune disease. For example, autoimmune diabetes in nonobese diabetic (NOD) mice might be the most similar to its human counterpart in both antigenic and genetic components as well as in its pathological features (36). Although it may be possible to use NOD mice to screen agents for their potential to hasten disease development or increase the severity of autoimmune diabetes, CFA, which is widely used in induced autoimmune models, paradoxically confers protection against diabetes development in NOD mice (37, 38) as do a number of TLR ligands under evaluation as human vaccine adjuvants (39). Other widely used spontaneous models develop autoimmune diseases that have pathological similarities to the counterpart in humans; however, the genetic basis and mechanism of pathogenesis of the diseases are quite different. A good example is the MRL-*Fas*<sup>lpr</sup> mouse model which spontaneously develops lupus nephritis. The key genetic element in this model is a null mutation in the *Fas* gene that leads to defective apoptosis in lymphocytes (34). However, polymorphisms in *Fas* have not been linked to lupus susceptibility in any of the large genome-wide association studies of this disease in humans (40, 41).

An additional limitation to using animal models to evaluate delayed vaccine adverse events is that animal models that develop autoimmune disease at high frequency (50 to 90% incidence within the animal population) are not designed to evaluate factors that increase the incidence of rare events in humans. In well-characterized animal models of autoimmunity, the genetic background and immunization conditions are optimized to reproducibly generate disease at a high frequency that in human populations may occur at a frequency of only 1 in 10,000 to 1 in 100,000 per year. A vaccine that increases the annual incidence of a disease in humans from 1 in 100,000 to 10 in 100,000 (a 10-fold increase) might be considered an unacceptable risk; however, studies in current animal models cannot predict such an increased incidence.

Moreover, species-specific differences between rodents and humans complicate

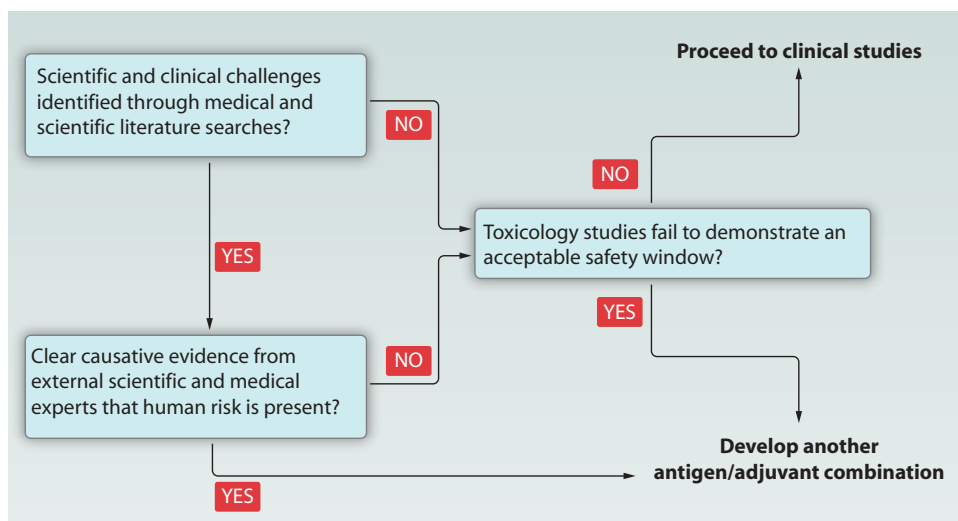


**Fig. 1.** Preclinical advancement of candidate vaccines. Flowchart outlining preclinical safety assessment using evidence-based medicine and toxicology in animal models to guide reformulation of vaccines or translation into human studies.

translation of preclinical observations to human disease. In humans, TLR9 is expressed in B cells and plasmacytoid dendritic cells (42–44), whereas in mice, TLR9 expression has been demonstrated in macrophages, myeloid dendritic cells, and activated T cells in addition to plasmacytoid dendritic cells and B cells (45–48). In rodents, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), a major contributor to many autoimmune diseases, is strongly induced in monocytes and macrophages by CpG-containing immunostimulatory oligonucleotides (CpG-ODNs). Treatment of mice or rats with CpG-ODN at doses only 5- to 10-fold higher by body weight than therapeutic doses in humans results in lung inflammation, increased plasma levels of inflammatory cytokines, including TNF- $\alpha$  and interleukin-6 (IL-6), and weight loss (49–51). However, because monocytes and macrophages do not express TLR9 in humans, TNF- $\alpha$  induction by CpG-ODN is negligible, both in vivo and in vitro. This difference is reflected by the safety and tolerability of inhaled or injected CpG-ODN in human and nonhuman primates (52–57). The difference in cellular distribution of TLR9 between rodents and humans influences the interpretation of preclinical toxicology studies of CpG-ODN-containing vaccines and is one of several examples of the limitations of extrapolating animal studies to humans. Clearly, the development of more appropriate animal models (such as transgenic, humanized, and nonrodent animal models) is warranted but will need to be carefully considered in light of the animal welfare act [National Institutes of Health (NIH) Revitalization Act of 1993 and Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Authorization Act of 2000], which aims to “reduce, refine, or replace animal tests” and may limit preclinical studies on rare serious adverse events such as autoimmune disease.

## CAN UNDERSTANDING HOW THE ADJUVANT WORKS IMPROVE SAFETY?

Recent progress in molecular biology, genetics, and immunology has shown that adjuvants are not just “dirty” ingredients capable of irritating the immune system, but, in many cases, induce specific pathways of innate immunity. For example, a recent report succinctly summarizes the studies that have collectively demonstrated that the adjuvant activity of alum is due to induction of members of the IL-1 family through NLRP3-mediated caspase-1 activity (58). In the case of MF59, a recent study has demonstrated the role of apoptosis-associated speck-like protein in the adjuvant effect of MF59 when combined with H5N1 subunit vaccines (59). Elucidation of the mechanism of action of adjuvants is important not only as a necessary step for developing better adjuvants but also to predict unwanted side effects of vaccination, and animal studies are required to advance this knowledge.

However, care must be taken in extending these new mechanistic insights into human patients. Because of differences between animal models and humans, preclinical studies on the mechanism of action of adjuvants might generate data that can lead to skewed conclusions about safety in humans. For example, a recent study examined gene expression and cell migration patterns in mice after injection of MF59 in comparison to other adjuvants, including alum (9). MF59 induced stronger gene expression of many genes involved in immunity such as cytokines, cytokine receptors, adhesion molecules involved in leukocyte migration, and genes related to antigen presentation. However, the stronger induction of genes was not predictive for an increased risk of adverse events in humans as evidenced by the well-documented safety record of MF59 in 45 million human recipients (60). This ex-

ample illustrates the potential pitfalls of trying to translate preclinical mechanistic studies to the prediction of long-term adjuvant safety in humans. Another example is the yellow fever vaccine, for which preclinical studies have generated profiles that, without context, could suggest potential concerns of autoimmune disease development in humans. In vivo mouse (61) and human (62, 63) studies showed that the yellow fever vaccine stimulates a strong “inflammatory” response: an increased expression of IL-12p40, IL-6, and interferon- $\gamma$  (IFN- $\gamma$ ), induced through at least four TLRs (2, 7, 8, and 9). Yet, this vaccine has not been shown unequivocally to increase the incidence of autoimmune disease (64), despite many decades of use. Indeed, even if preclinical studies were predictive of human adverse events, the utility of preclinical mechanistic studies for predicting vaccine-induced autoimmune disease remains uncertain given the incomplete knowledge on the cause of human autoimmune diseases. Thus, studying the mechanism of adjuvant action in animal models represents an important starting point for formulating hypotheses and planning safety testing in humans, but offers few answers about the risk of autoimmune disease in humans.

## TRANSLATIONAL STUDIES: CAN BIOMARKERS ASSESS RISK?

The discovery of serum biomarkers obtained from human patients experiencing adverse events (such as autoimmune disease) during vaccine clinical trials could potentially identify risks associated with such vaccines (Fig. 2). However, such an effort will need to take into account the limitations in drawing conclusions from clinical studies that are retrospective versus prospective, and the statistical requirements for the large sample sizes required to detect rare adverse events. One study that illustrates the potential of such an effort is seeking to predict the risk of fever after the smallpox vaccine on the basis of genetic predisposition (65). This retrospective study, which was conducted 4 years after receipt of the Dryvax smallpox vaccine (66, 67), genotyped and sequenced DNA obtained from whole blood of 346 subjects. Certain haplotypes in the *IL-1* gene complex and in *IL-18* were predictive of fever after vaccination, whereas a haplotype in the *IL-4* gene was associated with reduced development of fever. One could speculate that these same haplotypes could identify individuals at risk of fever after receipt of other live virus vaccines; however, it should be kept in mind that such genetic analyses are unlikely to allow for more than an estimation of different degrees of risk for reactions among a population rather than delineating those with no risk or those with near 100% risk. Furthermore, a study like this one would have been more informative if designed as a prospective study—collecting baseline data including cells, serum, and RNA at the time of immunization (day 0) and shortly afterward (for example, days 1, 3, 7, and 21, and day of adverse event)—to tighten the correlation between downstream adverse events and the status of the subject’s immune system at the time of immunization. The study of Querec *et al.* (62) elegantly illustrates how both genome and nongenome factors can be used to analyze the innate immune responses in humans after vaccination (YF-17D), and such an approach could readily be extended to identify signatures of vaccine toxicity. However, the major hurdle facing the development of predictive biomarkers for vaccine-related autoimmune disease is the absence of predictive markers for spontaneous human autoimmune disease.

Although risk-related biomarker development is a promising area, at present it must be considered a goal rather than a reality because

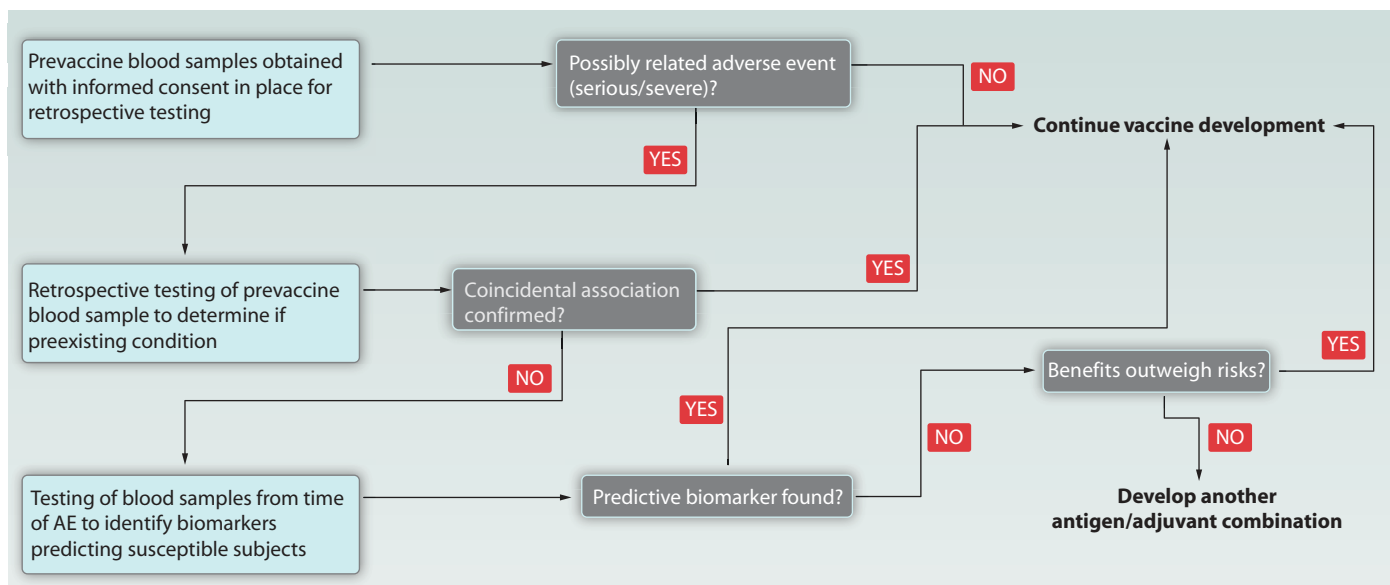


**Fig. 2.** Clinical advancement of candidate vaccines. Flowchart outlining the potential for biomarkers identified from clinical samples to guide continued refinement of vaccines during clinical studies.

much information is lacking. First, and perhaps most important, it is not known how identified biomarkers would behave in the context of immunization with licensed vaccines and adjuvants with established safety records. These data would provide a benchmark needed to evaluate the specificity of a given biomarker for identifying risk. Second, the identification of human risk-related biomarkers relies heavily on the compliance on the part of physicians and vaccinated subjects in reporting postvaccination events. For consistency and reliability, such biomarker data should be collected in the context of a clinical trial or a study with similar infrastructure. A recent study exploring the potential immunological and genetic abnormalities underlying the rare adverse event of viscerotropic disease shortly after yellow fever vaccination highlights the need for a clinical infrastructure that enables timely access to patient material (68). Finally, for rare adverse events that are temporally delayed from time of vaccination, like autoimmune disease or even autoimmune-related symptoms, a large-scale biomarker study would require collection and storage of blood samples from tens of thousands of subjects to attain adequate statistical power. A more efficient way to accumulate the numbers needed could include banking and pooling of appropriate specimens from all clinical trials to attain adequate statistical power. Thus, although information regarding baseline data and the benchmark profiles of current vaccines is sure to become available in the future, few data are currently available. The short-term solution to this dilemma will be the application of epidemiological studies to understand the background of adverse events and indirectly discern association with vaccination.

## WHAT CAN WE LEARN FROM EPIDEMIOLOGICAL STUDIES?

With the introduction of any vaccine, there is the risk for coincidental association with naturally occurring autoimmune disease. That is, in any population, there is a background rate of these events that occur

despite vaccination and a concomitant risk of such event occurring at the same time as the vaccine, by chance alone. These “temporal” associations can confound the interpretation of vaccine safety. Therefore, autoimmune disease prevalence and incidence data in a given population should be collected before vaccination to illustrate these coincidental associations with adjuvanted vaccines. As an example of this method, a cohort study ( $n = 214,896$  female adolescents;  $n = 221,472$  young adults) was carried out to monitor the prevalence of autoimmune disease in girls and young women before introducing the human papillomavirus (HPV) vaccine (69). Here, data on the frequency of immune-mediated conditions leading to outpatient visits, the number of adolescent and adult women hospitalized, and the most frequently diagnosed autoimmune disease were used to model temporal associations that would have occurred theoretically had the vaccine been used with 80% coverage. Such population-based efforts enable one to identify, in advance, confounding issues affecting safety perception, which can then be rapidly applied to avoid a negative impression of an inherently safe vaccine. The advantage of this approach becomes more evident when considering the recent reports (70, 71) of an association between vaccination with an adjuvanted A(H1N1) pandemic vaccine (Pandemrix) and narcolepsy in Finland and the combined counties of Sweden, which was determined by observational studies. The lack of increased reporting of narcolepsy in Norway, UK, Germany, and Canada, where 3.5 million children/adolescents were vaccinated with Pandemrix, and a recent analysis showing no case of narcolepsy in recipients of an A(H1N1) pandemic vaccine using a different oil-in-water adjuvant (MF59) (72) illustrate the need for caution in using single observational studies to establish causal relationship.

It is important to emphasize that without better incidence data on autoimmune diseases, the increased large-scale surveillance in vaccine studies may make it appear that all vaccines increase the risk of autoimmune diseases. To help illustrate this concept, Table 2 has been adapted primarily from autoimmune disease epidemiological data

**Table 2.** Estimated new cases in 2009 of autoimmune disease in the United States based on mean weighted incidence rates reported in a systematic meta-analysis (71). Expected new diagnoses were extrapolated using the 2009 U.S. Census data for population >18 years of age (73).

| Autoimmune disease               | Incidence<br>(per 100,000 persons per year) | Expected new diagnoses<br>(persons >18 years of age) |
|----------------------------------|---------------------------------------------|------------------------------------------------------|
| Adult rheumatoid arthritis       | 23.7                                        | 55,092                                               |
| Thyroiditis (hypothyroidism)     | 21.8                                        | 50,675                                               |
| Graves disease (hyperthyroidism) | 13.9                                        | 32,311                                               |
| Type 1 diabetes (age >20 years)* | 8.1                                         | 18,829                                               |
| Systemic lupus erythematosus     | 7.3                                         | 16,969                                               |
| Sjogren disease*                 | 3.9                                         | 9,065                                                |
| Multiple sclerosis               | 3.2                                         | 7,438                                                |
| Primary systemic vasculitis*     | 2.0                                         | 4,649                                                |
| Polymyositis/dermatomyositis     | 1.8                                         | 4,184                                                |
| Systemic sclerosis*              | 1.4                                         | 3,254                                                |
| Addison disease*                 | 0.6                                         | 1,394                                                |
| Myasthenia gravis                | 0.4                                         | 929                                                  |
| Total                            |                                             | 204,789                                              |

\*Additional categories of autoimmune diseases with an age distribution older than 18 years for which missing or updated incidence data were available in a subsequent publication (72).

reported in a systematic review (73); four additional categories were added from a study published in 2003 (74)—to our knowledge, these combined data represent the most comprehensive and conservative estimates to date. Table 2 intentionally focuses on the incidence of autoimmune diseases more commonly occurring in adults because the adult population is traditionally enrolled in first-in-human clinical trials with vaccines. Information on the rates of autoimmunity in pediatric patients, though limited, suggests that they are quite different and reflect the contributions of time and environmental exposure to disease development. The 12 autoimmune diseases, based on previously reported estimates of incidence (73, 74), would have been responsible for 204,789 new cases of autoimmune disease in a population >18 years of age in the United States based on the 2009 U.S. Census (75). These numbers provide a valuable perspective on the risk of coincidental association that can occur when an autoimmune disease is diagnosed in a subject immunized during a vaccine clinical trial (independent of the vaccine’s effect on the subject). Table 3 uses the incidence data from these 12 autoimmune diseases and calculates the probability of observing at least one case of autoimmune disease in clinical trials ranging from 200 to 10,000 subjects. As expected, there is an increase in the probability of observing one patient with autoimmune disease when looking at 200 subjects (15%) versus 3000 subjects (91%), which helps to explain why coincidental association during phase III vaccine studies can, and does, occur. The value and need for obtaining better epidemiological data on the background of autoimmune diseases that is stratified by age and geography are clear.

However, the possibility that autoimmune diseases may begin months or years before clinical diagnosis poses logistical challenges, emphasizing the importance of discovering biomarkers predictive for autoimmune disease. Perhaps an approach could be applied similar to that for autoantibody development in SLE (76), which tested the prospectively and sequentially collected serum specimens contained in the Department of Defense Serum Repository (from more than

5 million U.S. Armed Forces personnel) before the development of autoimmune disease to define predictive biomarkers.

HOW CAN SAFETY BE IMPROVED USING CLINICAL STUDIES?

Safety concerns will continue to loom large as novel adjuvant candidates advance through clinical development. The limitations that exist in translating preclinical findings to responses in humans highlight the importance of developing better strategies for the early identification of increased risk for autoimmune disease during the clinical development of a new adjuvanted vaccine. Although the inclusion of subjects with autoimmune disease in vaccine clinical trials raises potential ethical issues, an alternative may be to define predictive “bioprocess” markers in normal subjects. These bioprocess markers would provide a transcriptional signature of a biological process in a normal subject after vaccination that is similar to a signature associated with autoimmune disease. For example, the “IFN signature” of genes regulated by type I IFNs is highly associated with lupus and several other autoimmune syndromes (77). Although this is one of the clearest examples of an autoimmune disease signature, it also illustrates the challenges in using transcriptional profiling to evaluate disease risk because elevated expression of type I IFN-regulated genes accompanies most viral infections and constitutes a prominent part of the changes in gene expression induced by live, attenuated influenza (78) and yellow fever vaccines (62, 63), which are both regarded as safe vaccines for humans. Therefore, the value of this bioprocess marker becomes clearer when viewed in the context of the duration of such a signature—a transcriptional signature expressed transiently (and safely) in response to vaccination or normal infectious challenges should be distinguished from that elevated chronically, as has been clearly associated with autoimmune diseases. As transcriptional signatures for autoimmune diseases become better defined and the roles of the corresponding gene

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**Table 3.** Probability of observing autoimmune diseases owing to coincidental temporal association during clinical trials of various sizes (n). The probability of observing at least one subject with each of the listed autoimmune diseases was calculated using a Poisson probability distribution.

| Autoimmune disease               | Study sample size                        |          |          |          |            |
|----------------------------------|------------------------------------------|----------|----------|----------|------------|
|                                  | n = 200                                  | n = 1000 | n = 2000 | n = 3000 | n = 10,000 |
|                                  | Probability to observe at least one case |          |          |          |            |
| Adult rheumatoid arthritis       | 4.6%                                     | 21.1%    | 37.8%    | 50.9%    | 90.7%      |
| Thyroiditis (hypothyroidism)     | 4.3%                                     | 19.6%    | 35.3%    | 48.0%    | 88.7%      |
| Graves disease (hyperthyroidism) | 2.7%                                     | 13.0%    | 24.3%    | 34.1%    | 75.1%      |
| Type 1 diabetes (age >20 years)  | 1.6%                                     | 7.8%     | 15.0%    | 21.6%    | 55.5%      |
| Systemic lupus erythematosus     | 1.4%                                     | 7.0%     | 13.6%    | 19.7%    | 51.8%      |
| Sjogren disease                  | 0.8%                                     | 3.8%     | 7.5%     | 11.0%    | 32.3%      |
| Multiple sclerosis               | 0.6%                                     | 3.1%     | 6.2%     | 9.2%     | 27.4%      |
| Primary systemic vasculitis      | 0.4%                                     | 2.0%     | 3.9%     | 5.8%     | 18.1%      |
| Polymyositis/dermatomyositis     | 0.4%                                     | 1.8%     | 3.5%     | 5.3%     | 16.5%      |
| Systemic sclerosis               | 0.3%                                     | 1.4%     | 2.8%     | 4.1%     | 13.1%      |
| Addison disease                  | 0.1%                                     | 0.6%     | 1.2%     | 1.8%     | 5.8%       |
| Myasthenia gravis                | 0.1%                                     | 0.4%     | 0.8%     | 1.2%     | 3.9%       |
| Total                            | 15.0%                                    | 55.5%    | 80.2%    | 91.2%    | 100.0%     |

products in pathogenesis become clearer, it will be important to evaluate these as “bioprocess” markers in studies with new vaccines.

CAN STANDARDIZED REPOSITORIES OF CLINICAL STUDY DATA HELP?

The value of preclinical studies for novel vaccines would be greatly increased if comparable data from different vaccine developers were combined in a standardized format and made widely available for query. A significant challenge in this process will be developing structures that preserves legitimate proprietary interests while making available such data to the entire field. Such repositories would likely require public funding and agreement on standardization of the studies, but could advance the field of mechanism of action studies on early-stage vaccines and adjuvants. Still missing from a well-stocked repository of “omics” data about novel adjuvants and vaccines under development is a frame of reference for interpreting the data and generating hypotheses about factors that predict safety. To provide this framework, comparison studies will need to be undertaken to examine human and animal model responses to approved vaccines for which the safety profiles and the adverse events are well known. Induction of a set of potent cytokines, for example, by a new vaccine or adjuvanted vaccine candidate would be less concerning if a similar pattern were known to be induced by a safe and widely used vaccine, reiterating the need for such benchmark data sets.

The regulatory considerations for first-in-human clinical trials with vaccines are elegantly presented in a recent publication by Goetz *et al.* (79). These considerations could be used as a frame of reference for designing clinical studies to better understand risks factors related to autoimmune disease. As more is learned about the human response to licensed vaccines and adjuvants with established safety records, the creation of a set of guidelines on how to use that information to evaluate

novel adjuvants and vaccines will become critical. As one example, the U.S. Food and Drug Administration has formed a Genomics Evaluation Team for Safety to develop approaches for applying novel technologies to biologics safety. Using the Voluntary Exploratory Data Submission (VXDS) process (80), voluntary submission of biomarker data from vaccines with known benefit-risk profiles could be a crucial step for biomarker development, clinical translation, and regulatory qualification.

The limited access to information about why clinical studies are delayed or terminated is itself problematic. Although protecting proprietary information is essential, the establishment of a repository of preclinical or clinical data with open access to all regulatory agencies and all vaccine developers for safety-related information could be envisioned. Such a repository could guide manufacturers to rethink their preclinical and clinical research in the event that an adjuvant with a similar mode of action belonging to a competitor was clearly found to have unacceptable toxicities. The end result of such a repository would be elimination of unnecessary duplication of human studies, increased transparency on adjuvant safety, and reduced likelihood of a problematic vaccine or adjuvant entering widespread circulation.

HOW CAN WE BALANCE RISK AND BENEFIT?

Vaccines are an essential component of preventive medicine, and delays in vaccine development because of rare adverse events need to be balanced with the larger infection-associated morbidity and mortality in the general population not receiving the vaccine. When focusing specifically on the safety of adjuvanted vaccines, there is a need for carefully planned preclinical animal studies that decipher their mechanism of action (using standards developed in conjunction with regulatory agencies) and also large-scale epidemiological studies in human populations to confirm adjuvant safety with regard to rare or delayed adverse events, such as development of autoimmune disease. Autoimmune

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diseases are not rare; as such, their occurrence should be anticipated in clinical trials and not used as reasons for stopping clinical trials, unless of course the rates exceed those expected by temporal coincidence. It should also be appreciated that coincidental associations will occur with longer follow-up periods of vaccinated subjects because of factors unrelated to or distant in time from vaccination. These factors include environment, diet, age-related changes in the immune system, and conditions associated with triggering autoimmune disease in susceptible subjects (such as pregnancy or exposure to natural infections).

How long should we follow vaccinated subjects to reliably detect autoimmune adverse events? Current thinking regarding length of follow-up in vaccine trials has concluded that 6 months is adequate; this time frame reflects our current understanding of autoimmune disease pathogenesis (79). It is reassuring to know that in the case of influenza, older epidemiological studies with a follow-up of 16 to 18 years in 18,000 subjects receiving the influenza virus vaccine (adjuvanted with light mineral oil) demonstrated no increased risk of allergic and autoimmune diseases (81).

How many vaccinated subjects should be followed to reliably detect autoimmune adverse events? The recent widespread introduction of adjuvanted H1N1 influenza vaccines in response to a pandemic has provided the necessary number of subjects to enable statistical analysis powered adequately to detect rare adverse events. For example, a two-fold increase in relative risk of an autoimmune disease with an incidence of about 1 in 100,000 requires about 5 million subjects (82). A report from the European Medicines Agency for the European Economic Area (EEA) indicates that at least 36.6 million people in the EEA alone have been vaccinated with adjuvanted pandemic vaccines (83). Although there has been no increased risk of autoimmune disease in the tens of millions of recipients receiving MF59-adjuvanted influenza vaccines since its licensure in 1997 (60), one can hope that the additional safety demonstrated with adjuvanted influenza vaccines used globally in the context of the H1N1 pandemic and the ongoing collaborative efforts of academic researchers, vaccine manufacturers, and regulatory agencies will rebuild the public trust in the safety of adjuvanted vaccines.

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