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Abstract

A workshop entitled "Challenges and Opportunities in Evaluating Protein Allergenicity across Biotechnology Industries" was held at the 51st Annual Meeting of the Society of Toxicology (SOT) in San Francisco, California. The workshop was sponsored by the Biotechnology Specialty Section of SOT and was designed to present the science-based approaches used in biotechnology industries to evaluate and regulate protein allergenicity. A panel of experts from industry and government highlighted the allergenicity testing requirements and research in the agricultural, pharmaceutical/biopharma, and vaccine biotechnology industries and addressed challenges and opportunities for advancing the science of protein allergenicity. The main learning from the workshop was that immunoglobulin E-mediated allergenicity of biotechnology-derived products is difficult to assess without human data. The approaches currently being used to evaluate potential for allergenicity across biotechnology industries are very different and range from bioinformatics, in vitro serology, in vivo animal testing, in vitro and in vivo functional assays, and "biosimilar" assessments (ie, biotherapeutic equivalents to innovator products). The challenge remains with regard to the different or lack of regulatory requirements for allergenicity testing across industries, but the novel approaches being used with bioinformatics and biosimilars may lead to opportunities in the future to collaborate across biotechnology industries.

Keywords

biotechnology, allergenicity, immunotoxicity, protein

Introduction

Biotechnology is a field at the cutting edge of science, using living cells and materials produced by the cells to prevent and fight disease, improve food production, and benefit other industries as well. There are more than 250 biotechnology health care products and vaccines that people are benefiting from today for diseases that were previously untreatable, and agricultural biotechnology is being used by more than 13.3 million farmers around the world to increase yields, prevent damage from insects and pests, and reduce environmental impact.¹ Concerns over the allergenicity of biotechnology products continue to receive attention in the public and regulatory domains. These concerns range from the transfer of an existing allergen or cross-reactive protein into a food source where it is not normally present or increasing the levels of allergens that are naturally present in certain crops to accidental modification of therapeutic proteins resulting in allergic reactions.

Allergenicity is a hypersensitivity disorder of the immune system. There are 4 types of hypersensitivity reactions, and allergy is known as type I (immediate) hypersensitivity that

is immunoglobulin E (IgE) mediated.² Upon initial exposure to the allergen (protein in the case of biotechnology products), IgE is produced and binds to the surface of the mast cells and basophils. Reexposure results in an allergic response, whereby IgE cross-linking on the surface of mast cells and basophils causes the release of histamine and other inflammatory mediators. This reaction results in symptoms that can range from mild symptoms of red eyes, itchiness, running nose, and eczema to severe asthma or anaphylaxis leading to death.³

Biotechnology-derived drugs, vaccines, and foods are regulated by different agencies globally or different centers within

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the agencies. As a consequence, the types of protein allergenicity testing requirements may differ. The concept of the workshop for evaluating protein allergenicity across biotechnology industries evolved from 2 questions:

1. What are the science-based approaches used in the different areas of biotechnology to evaluate and regulate protein allergenicity?
2. Are there opportunities to advance the science more by understanding approaches used across different areas?

Drs Nicola Stagg and Hanan Ghantous chaired this workshop. Dr Stagg opened the workshop with an overview of the advancements in the biotechnology field, allergenicity concerns with biotechnology products, and the different types of biotechnology protein allergenicity research that would be covered at the workshop. Dr Greg Ladics provided the agricultural biotechnology perspective. Dr Robert House provided the vaccine perspective. Dr Kenneth Hastings provided the pharmaceutical/biopharma perspective. Dr Steven Gendel provided a regulatory perspective, primarily focused on food allergenicity. The panel of experts highlighted the allergenicity testing requirements and research in the different areas of biotechnology, and identified the challenges and opportunities for advancing the science of protein allergenicity.

Protein Allergenicity: Perspective From the Agricultural Biotechnology Sector (Gregory S. Ladics)

Plant breeding is the science of improving the yield and quality of cultivated plants. The objective of the plant breeder is to identify the gene/genes of interest and transfer it into the elite germplasm of the cultivated variety. The genetic manipulation of plants has been going on since the dawn of agriculture, but until recently has required the slow process (8-10 years) of cross-breeding plant varieties through selection. Agricultural biotechnology can speed the process (down to 3-4 years) in a more precise manner and broaden the scope of what can be done. Transgenesis is the process of inserting a gene from one source into a living organism that would not normally contain it using recombinant DNA. Transgenic or genetically engineered (GE) crops are crops that contain a gene/genes that has been artificially inserted from species within the same kingdom (plant to plant) or between kingdoms (bacteria to plant) into that crop, instead of being inserted through pollination. Currently, agricultural biotechnology involves the discovery and development of genes for agricultural traits such as herbicide tolerance, insect resistance, disease resistance, and water and nutrient use efficiency.

Unlike conventionally bred crops, an extensive safety assessment process exists for GE crops. The goal of this process is to demonstrate that the GE crop is "as-safe-as" non-GE crops in the food supply. One of the major issues for GE crops is the assessment of the expressed protein for allergenic potential. The purpose of this evaluation is (1) to protect allergic consumers from exposure to known allergenic or

cross-reactive proteins and (2) to protect the general population from risks associated with the introduction of genes encoding proteins that are likely to become food allergens.

A food allergy is a reaction of the immune system to an otherwise harmless protein in food. Importantly, allergic reactions to food are relatively rare. The incidence of food allergy in the United States ranges from approximately 1% to 2% in adults and 6% to 8% in young children.^{4,5} Relatively, few foods (eg, peanuts, cow's milk, hen's egg, fish, crustacean, wheat, tree nuts) are responsible for the vast majority of significant food-induced allergic reactions. The prevalence of food allergy to specific foods, however, can also vary geographically (eg, increased buckwheat allergy in Asia; celery allergy in Europe).⁶

Currently, no single end point or response is recognized as a predictor of protein allergenicity. Consequently, a weight-of-evidence approach, which takes into account a variety of factors and approaches for an overall assessment of allergenic potential, is conducted.^{5,7} This assessment is based on what is known about allergens, including the history of exposure and safety of the gene/genes source (ie, whether the gene source for the new protein is known to induce allergy); similarity to known allergens (in silico amino acid sequence identity bioinformatic comparisons to a database [www.allergenonline.org] of known human allergens); stability to pepsin digestion in vitro⁸; an estimate of exposure of the novel protein/protein to the gastrointestinal tract where absorption occurs (eg, protein abundance in the crop, processing effects [heat stability]); and when appropriate (ie, gene derived from a known allergenic source or amino acid sequence identity to a known allergen is observed) specific IgE binding studies. The IgE binding studies require the use of well-characterized human sera from individuals known to be allergic to the identified allergen source and present ongoing challenges in terms of standardization of test materials, shortage of available well-characterized sera from a sufficient number of clinically allergic patients, and validation of procedures. The bioinformatic search strategy involves the evaluation of sequence for amino acid identity using local alignment programs, such as FASTA or BLAST.⁹ A potential match to an allergen is indicated when there is $\geq 35\%$ identity observed over an 80 or greater amino acid window. The use of a second criteria, the evaluation of amino acid sequence for potential (theoretical) IgE epitope matches (ie, short, contiguous, identical amino acid matches), is not recommended due to the large amount of false positive findings observed.^{10,11}

Some regulatory authorities also require the evaluation of GE crops (ie, soybean) for changes in endogenous allergen levels compared to non-GE varieties.¹⁰ Quantifying levels of endogenous allergens in GE crops is of negligible value in the safety assessment. Most importantly, allergic individuals will try to avoid offending foods such as soybean which would be labeled as allergenic regardless of whether it is GE or non-GE derived. Across non-GE crop cultivars, endogenous allergen levels have been found to vary considerably due to genotype, environmental conditions, harvest timing, or storage conditions.¹²⁻¹⁸ Furthermore, it is currently not possible to

correlate protein expression with biological relevance because of limited data on quantitative thresholds for sensitizing individuals to most allergens. There is a lack of identification of safe consumption levels, as the quantity of food consumed is not subject to regulation—further introducing sources of exposure variability. Interestingly, a recent literature review found that transgenesis had less impact on genome expression and concentrations of proteins or metabolites compared to conventional breeding or plant nondirected mutagenesis.¹⁹

A number of challenges were discussed in the workshop. As agricultural biotechnology evolves beyond the first generation of crops (ie, insect-resistant *Bacillus thuringiensis* (Bt) and herbicide tolerance), more complex modifications to plant metabolic pathways and/or membrane-bound proteins will be employed. Additional tools will be needed to help predict the potential allergenicity of the next generation of GE crops. Moreover, with the first generation of GE crops, a hazard assessment approach has been used to ensure safety. The testing strategy for the next generation of GE crops, however, will need to be integrated into a risk assessment paradigm (ie, hazard assessment, dose–response evaluation, exposure assessment, and risk characterization). In the future, advanced analytical profiling techniques (eg, proteomics) may be used to complement the compositional analysis for detection of unintended effects. However, such profiling techniques require further standardization and validation. Importantly, such techniques need to be utilized in a more targeted, hypothesis-based fashion. Over the last 12 years, there have also been multiple documents that have provided differing recommendations for assessing the potential protein allergenicity of GE crops.^{7,20,21} As such, safety assessment requirements have not always been consistent across geographies and have often resulted in the arbitrary inclusion of nonvalidated or refuted tests. Ideally, in the future, there needs to be better harmonization of testing guidelines for assessing potential protein allergenicity across geographies and inclusion of only those end points that have been “validated” based on “sound science” (ie, peer-reviewed published data).

Evaluating the Potential Allergenicity of Vaccines (Robert V. House)

Probably, more than any other medical product, vaccines face a high standard of safety testing; this is due to their large-scale and widespread use in otherwise healthy individuals, including special populations such as children and the elderly individuals. Vaccines undergo extensive safety testing before, during, and after human exposure, particularly regarding purity and composition. However, unlike nearly every other medical product, vaccines are designed to cause a specific and essentially permanent modification to the immune system. Because of this feature, it is particularly important to understand the potential these products have to exert immunotoxic effects.

Potential safety issues associated with vaccines may fall into at least 3 different categories:

Unwanted effects related to the process of vaccination itself.

These reactions are generally nonspecific and most commonly include fever, injection site reaction, injection site edema, rash, and (perhaps surprisingly) syncope. Localized inflammatory reactions are also a frequently reported adverse event, occurring most often with adjuvanted vaccines. These inflammatory reactions may be painful or not, with painful reactions generally occurring when an adjuvanted vaccine induces a local reaction via the inflammasome. Pain may also result from a localized Arthus reaction, as with booster vaccination with some vaccines. Nonpainful reactions also occur and can include (sometimes significant) edema.

Unwanted and unanticipated immune-related responses secondary to the action of the vaccine.

Vaccines achieve their intended effect through immune response to specific antigens. It is rare for allergic (or other immune) reactions to occur following exposure to vaccine antigens themselves. However, vaccines may be complex mixtures including the antigen of choice along with animal proteins (ovalbumin, chicken proteins, gelatin, calf lymph, etc), manufacturing expression system proteins (recombinant products), preservatives such as thimerosal, adjuvant components such as aluminum, and antibiotics. Any of these components may elicit an unwanted immune reaction, including allergenicity, so it is important that the potential for any vaccine component to induce allergenicity be understood before they are used.²² Notably, such allergic reactions are still extremely rare.

Unwanted immune reactions related but secondary to the antigenic basis of vaccination.

Until recent decades, vaccines were derived from living organisms and effectively mimicked the response seen with exposure to the pathogen the vaccine was designed to protect against. These vaccines worked well, but adverse reactions called for the development of highly purified antigenic vaccines, now most often in the form of recombinant proteins or peptides. Unfortunately, these modern vaccines are generally not as robust as their predecessors, necessitating the use of agents known as adjuvants. Adjuvants are substances used to enhance the immunological response to a concomitantly administered antigen, and may be used when the antigen itself does not induce a robust immunogenic response. The first vaccine adjuvant was alum (~1925), and it is still widely used today. However, the mechanism of action of alum has never been totally elucidated, so improvements to its utility have been very limited. With recent advances in our understanding of the role of innate immunity in the development of effective adaptive immunity, we are now witnessing the development of newer, more chemically diverse adjuvants with highly defined mechanisms, often based on the engagement of pathogen-associated molecular patterns and related receptors of the innate immune system.

The new “rational” adjuvants tend to work via 2 primary mechanisms. The first is as vehicles to deliver antigens to the antigen-presenting cells (eg, MF 59, AS03, iscoms, virus-like particles, and nanoparticles, among others). The second mechanism is as immunopotentiators which activate antigen-presenting cells via Toll-like receptors (TLR; eg, TLR agonists, certain cytokines, and small molecule immunomodulators).²³

This intentional immunomodulation can be extremely useful for vaccine development, but carries the potential for inducing unintentional immune reactions.^{24,25} Potential reactions can include:

- inflammatory reactions (fairly common);
- IgE-mediated reactions (immediate hypersensitivity);
- immune complex-mediated reactions, such as localized Arthus reactions;
- non-IgE-mediated reactions (pseudoallergic reactions, which are extremely rare);
- unintended immunostimulation reactions;
- Autoimmune reactions.

The testing paradigm for vaccine safety assessment is now fairly well established. Generally, preclinical safety assessment takes the form of a repeat-dose toxicology study (including a recovery group) using the intended dose formulation and regimen, “plus one” (that is, one more administration than would be planned for human) and the full intended dose volume. Assessments include routine end points (general toxicity, clinical toxicology including immunogenicity) and pathology with emphasis on certain tissues (lymphoid, administration site). End points that are more characteristic of vaccine studies would include reactogenicity (local tolerance, since this is a common reaction to immunization) and demonstration that the vaccine is having its intended effect (usually this would be assessment of immunogenicity of the vaccine). Safety pharmacology is generally not required. However, certain additional safety testing might be appropriate based on the nature of the vaccine itself; as an example, specific studies would be required when testing live attenuated vaccines. Reproductive toxicology studies are required for vaccines, whereas genotoxicity studies are not required except for DNA vaccines. Additional testing for potential hypersensitivity reactions is generally not warranted unless any of the vaccine components are known to be allergenic.

Animal studies are imperfect in predicting human responses, whether safety or efficacy; this is true enough for antigens alone, but becomes doubly true with the newer adjuvants. This is complicated by continued improvements in vaccination technology including the use of devices and alternative routes of administration that introduce further nuances in how the immune responses are initiated and maintained. Although immunotoxicology testing per se is not required for vaccine development, given the variety of immune reactions described above, the potential for unintended immunomodulation (negative or positive) should always be taken into consideration.

Allergenicity Research in the Pharmaceutical/Biopharma Sector (Kenneth L. Hastings)

The term “drug allergy” is used to describe adverse reactions that appear to have an immunological basis. However, many adverse drug reactions described as “drug allergy” likely do not involve a specific immune response to a drug. Small-molecular-weight drugs must form haptens, seen as harmful, in order to induce a true allergic reaction. However, protein drugs—referred to here as biotherapeutics—do not need to be biotransformed into hapten–protein complexes in order to induce drug allergy.

Unintended immune responses to biotherapeutics include anaphylaxis, organ-specific immunopathy, autoimmune reactions, and systemic hypersensitivity. Unintended immunogenicity can result in altered pharmacodynamics (PD), pharmacokinetics (PK), and various immunopathies.

Drug allergy associated with biotherapeutics can be divided into 2 general classes: a true immune-mediated drug hypersensitivity reaction or a pseudo-allergic reaction. Many apparent drug allergic reactions are “anaphylactoid,” not mediated by drug-specific IgE. A key difference in IgE-mediated anaphylaxis and anaphylactoid reactions is that the latter can often be modeled in animals. A practical example is the recent issue of adulterated heparin. In 2008, there were reports from renal dialysis units in the United States of apparent anaphylaxis associated with administration of heparin, in some cases resulting in death.²⁶ Batches of suspect heparin were found adulterated with over-sulfated chondroitin sulfate (OSCS), which has potent anticoagulant activity. It quickly became apparent that OSCS was an intentional adulterant, added to increase the apparent potency of heparin. The proof that OSCS was the cause of this apparent increase in heparin-associated anaphylaxis came from the studies conducted in Yorkshire domestic farm pigs at Virginia Tech: OSCS and samples of suspect heparin were demonstrated to directly activate complement (generating potent anaphylatoxins C3a and C5a). In addition, both the test articles activated the kinin–kallikrein system with generation of vasoactive bradykinin (demonstrated using human plasma). Both complement products and bradykinin activate coagulation factor XII, a known anaphylactoid mechanism. Thus, the connection between intentional adulteration of heparin with OSCS and what appeared to be anaphylaxis was established.

There is an important implication of the heparin contamination tragedy; many anaphylactic-like reactions seen with biotherapeutics are likely pseudoallergic. The recent clinical trial disaster with a humanized agonist anti-CD28 monoclonal antibody TGN1412, which produced a severe sepsis-like reaction in volunteers, demonstrates the importance of these anaphylactoid reactions.²⁷ Many biotherapeutics are intrinsically immunomodulatory. It is likely that most adverse reactions to biotherapeutics described as allergy are, in fact, due to activation/release of proinflammatory mediators; this phenomenon has been called “cytokine release syndrome,” “cytokine storm,” and “sterile sepsis.”

Finally, there are examples of apparent drug allergy that may, in fact, be due to other immunomodulatory mechanisms. In 2006, a review of adverse events associated with drug-eluting coronary stents (late restenosis and thrombus formation, with fatalities) concluded these were hypersensitivity reactions.²⁸ Histologic examination of coronary arteries obtained at autopsy demonstrated marked stent-associated inflammatory reactions characterized as granulomatous with macrophages, eosinophils, and giant cells. Although certainly suggestive of a hypersensitivity reaction, these findings could also be related to infection. In fact, the drugs used in these stents, paclitaxel and sirolimus, are known immunomodulatory compounds. Allergic contact dermatitis is a well-understood phenomenon and is associated with dermal, not systemic exposure. Skin rash associated with systemic exposure to drugs might actually be due to viral activation.²⁹ Skin rash is a common adverse reaction associated with biotherapeutics, many of which are immunomodulatory. Thus, the issue of biotherapeutic-associated allergy is more complex than generally appreciated.

Immunogenicity is an especially important issue in the development of “biosimilars,” biotherapeutic equivalents to innovator products. Adverse immune responses can be produced by contaminants from the production of biotherapeutics and by altered protein structures. In either case, if the immune response observed is significantly different from what was observed with an innovator product, this could indicate a cause for concern. Unlike a generic low-molecular-weight drug, which is identical to an innovator product—and is therefore considered to be interchangeable and therapeutically equivalent—a biosimilar cannot be assumed to have identical properties.

In February, 2012 the Food and Drug Administration (FDA) issued 3 guidance documents providing a methodology enabling marketing of biosimilar equivalents of marketed innovator biotherapeutics.³⁰⁻³² A biosimilar is defined as highly similar to the reference [innovator] product *notwithstanding minor differences in clinically inactive components* ... [italics added] and demonstrates “... no clinically meaningful differences [compared to] the reference product ...” There are 2 important points to consider in this definition (1) a product is *deemed* by FDA to be biosimilar if shown to have sufficient structural and biologic activity compared to an innovator product and (2) a determination of biosimilarity does not mean that the 2 products are *interchangeable*.

Why is a biosimilar inherently not interchangeable with an innovator product, as is the case with small molecular weight drugs? Simply put, any change in a biotherapeutic can result in characteristics that alter clinical efficacy and safety. Of the various alterations that can change clinical response to a biosimilar versus an innovator product, the most difficult to predict is unintended immunogenicity.

The implications of unintended immunogenicity can be best illustrated by a disaster between 1988 and 2004; at least 500 cases of pure red-cell aplasia (PRCA, clinically equivalent to aplastic anemia) were reported to be associated with a

recombinant erythropoietin (rEPO) product (Eprex).³³ The rEPO products were among the first biotherapeutics to be marketed and had, prior to Eprex, demonstrated impressive efficacy and safety. However, in the late 1980s, the maker of Eprex removed human serum albumin due to concern over possible transmission of Creutzfeldt-Jacob disease. It was soon discovered that patients given Eprex and developing PRCA had circulating antibodies that inhibited bone marrow erythroid-colony formation. Essentially, Eprex had induced an autoimmune reaction. When albumin was added back to the product, the incidence of PRCA decreased dramatically. Although other changes were made to manufacturing the product, it is likely that removal of albumin from the rEPO resulted in unintended immunogenicity. Thus, the FDA guidances on biosimilars convey significant and understandable concern that a biosimilar could produce a similar pattern of unintended immunogenicity with adverse consequences.

The FDA guidances propose a totality-of-evidence approach to evaluating a biosimilar product; this reflects an understanding that physicochemical and pharmacodynamic similarities are not sufficient to assure interchangeability. The guidance on biosimilar quality recognizes the unique issue of immunogenicity with the following statement (footnote 14 of the guidance): “In some cases, in vivo immunogenicity studies may be able to detect subtle differences in structure or impurities not detected by other methods.”³⁰ The problem is that animal immunogenicity studies are not predictive of human immune responses to biotherapeutics. Thus, although animal studies may be useful in the totality-of-evidence approach to demonstrating biosimilarity, immunogenicity cannot be addressed in this way. Recognition of this problem has led to reliance on clinical immunogenicity studies. Although both PK and PD data would be compared as well, it is likely that comparative immunogenicity would be the pivotal end point, especially with respect to interchangeability. In the worst case scenario, a patient being treated with an innovator product is given a biosimilar product and develops an immune response that renders both the innovator and the biosimilar ineffective, or worse (such as an autoimmune-type reaction).

The approach advocated by FDA is thus (1) conduct a premarket clinical trial powered to detect major differences in immunogenicity followed by (2) a postmarket clinical study to detect subtle differences in immunogenicity. Immunogenicity would be compared to the innovator product in a head-to-head design. Since the emphasis is on increased immunogenicity, a relatively small study would likely be sufficient. However, the number of participants needed for the postmarketing study would likely be much greater. Hence, the FDA proposes that a population pharmacokinetic study might be needed; sparse sampling of large numbers of participants would detect clinically significant differences in product attributes. For example, if a significant difference is observed in product C_{min} compared to the innovator values, this might be due to altered immunogenicity. If there is a valid PD marker (or markers), combining this with population PK could be useful.

Evaluation of Allergenicity: A Food Regulatory Perspective (Steven M. Gendel)

Food allergy is an important public health issue in the United States and internationally. Because there are no treatments or cures for food allergy, the only risk management tool available to allergic consumers is to practice avoidance.

Allergenicity assessment impacts a number of food safety areas in FDA. These include programs as diverse as the evaluation of food additives and generally recognized as safe substances, food labeling, and good manufacturing practices. This wide range presents unique challenges because legal authorities and standards differ for each. The use of risk and safety assessment approaches provides a common framework for approaching food allergen safety in each context.

It is important to realize that doing risk assessments in a regulatory agency is not the same as doing risk assessments in a clinical or academic organization. There are a number of legal and policy constraints, technical requirements, and reporting requirements that need to be considered in conducting a regulatory risk assessment and in reporting the results. It is also important to recognize that the scope of a regulatory risk assessment is defined by the charge developed by agency risk managers. This charge needs to consider the practical aspects of developing and implementing agency responses to risk.

Food safety allergenicity is also technically challenging. There are significant uncertainties associated with the available clinical data on allergy sensitivity, the limited understanding of the underlying biological processes that affect reaction severity and individual sensitivity, and the effects of food processing. It is also not clear which of the several assessment models (eg, microbial, chemical, or mixed) is the most appropriate approach to use for food allergens because the hazard being considered is something which is nutritious and desirable for most of the population and not something which is added to, or occurs in, foods as a separate substance.

Protein Allergenicity Workshop Outcome and Future Outlook

The goal of this workshop was to raise awareness to the types of protein allergenicity research taking place across the different biotechnology industries and address the challenges and opportunities for advancing the science of protein allergenicity. It was apparent from the workshop that IgE-mediated allergenicity of biotechnology-derived products is difficult to assess without human data. Currently, very different approaches are being taken, ranging from bioinformatics and in vitro serology testing in the agricultural sector, in vivo animal testing, and in vitro and in vivo functional assays with vaccines and "biosimilar" assessments (ie, biotherapeutic equivalents to innovator products) in the Pharmaceutical/Biopharma Sector. The challenge also remains with regard to the different or lack of regulatory requirements for allergenicity testing across industries. However, some of the novel approaches being used, particularly bioinformatics and biosimilarity, may lead to

opportunities in the future to collaborate across biotechnology industries in order to advance the science and align the regulatory requirements.

Comments and questions were entertained from the audience at the end of the talks. These questions were about terminology used, relevance of findings in animals to humans, utility of certain assays for assessing allergenicity, and specifics on how certain assays are conducted. A question was raised about macrophage myofasciitis and vaccines to which Dr House responded that there has been evidence in animal studies but not good correlation between animals and humans, and it is also very localized in animals and reversible as demonstrated with recovery groups. Dr House was also asked about the relationship of the influenza vaccine and cases of narcolepsy being reported in Scandinavia. He answered that the association appears to be real, but that he was unaware of the etiology and did not know how this could have ever been anticipated from animal studies.

Authors' Note

The views expressed are those of the authors. The content and opinions in this article are those of the author and do not represent Sanofi SA. No official support or endorsements by the US Food and Drug Administration is provided.

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