

Task Force Report: Future research needs for the prevention and management of immune-mediated drug hypersensitivity reactions

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Abstract

Immune-mediated drug hypersensitivity reactions (IDHR) have a significant impact on clinical practice, drug development, and public health. However, research to understand IDHR mechanisms and to develop diagnostic and predictive tests has been limited. To stimulate more research, a task force with representatives from the key stakeholders (research clinicians, regulatory scientists, and immunotoxicologists from the pharmaceutical industry) was assembled to identify critical data gaps and opportunities and to make recommendations on how to overcome some of the barriers to IDHR research and address research needs. It is hoped that this report will act as a springboard for future discussions and progress toward increased funding and development of organizational structures for IDHR research. (J Allergy Clin Immunol 2002;109:S461-78.)

Key words: Adverse drug reactions, drug allergy, drug development, drug hypersensitivity, immunotoxicology

Abbreviations used

ADR: Adverse drug reaction
AERS: US FDA's adverse event reporting system
AIDS: Acquired immunodeficiency syndrome
CFA: Subcutaneously with complete Freund's adjuvant
FDA: US Food and Drug Administration
HMOs: Health maintenance organizations
IDHR: Immune-mediated drug hypersensitivity reaction
ILSI: International Life Sciences Institute
ITC: Immunotoxicology Technical Committee
LLNA: Local lymph node assay
LMW: Low molecular weight
MHC: Major histocompatibility complex
NIH: National Institutes of Health
PLNA: Popliteal lymph node assay
RAST: Radioallergosorbent test
SAR: Structure-activity relationship
SNP: Single nucleotide polymorphisms

EXECUTIVE SUMMARY

Background

Immune-mediated drug hypersensitivity reactions (IDHR) are uncommon adverse events in clinical medicine that are restricted to a subset of vulnerable patients and pharmaceutical products. Nevertheless, such reactions, and the fear of them as serious and potentially life-threatening consequences of therapy, have a huge impact on clinical practice, drug development, and the public health. IDHRs accounted for 137,000 to 230,000 hospital admissions in the United States in 1998, with estimated attendant costs of \$275 to \$600 million annually. IDHRs are estimated to account for 6% to 10% of all adverse drug reactions, most of which occur in nonhospitalized patients with unknown direct costs for the healthcare delivery system. The concern for IDHR, which are currently unpredictable, and the compromises in optimal medical care undertaken in efforts to avoid recurrence of such reactions in patients with prior histories are substantial impediments to delivery of best-available medical care. For example, many of the estimated 25 million Americans who have had some adverse experience with beta-lactam antibiotics (most of which are IDHRs) receive alternative antibiotics that are sometimes less effective, often more toxic, and usually more expensive.

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A better understanding of predisposing factors and pathophysiology, with attendant progress in diagnostic and predictive tests, could be expected to reap substantial benefits for afflicted patients in the provision of optimal medical care as well as substantial savings in healthcare costs. The lack of an adequate knowledge base about IDHR similarly afflicts drug development programs because it is currently difficult to predict which new therapeutic compounds may elicit IDHRs before clinical trials and marketing.

Task force goals

This report was prepared by a task force assembled by the Immunotoxicology Technical Committee (ITC). The ITC is a part of the nonprofit Health and Environmental Sciences Institute, a branch of the International Life Sciences Institute (ILSI). This task force comprised immunotoxicologists from pharmaceutical companies, regulatory scientists from the US Food and Drug Administration (FDA), and clinical investigators from universities with expertise in IDHR. The goals of the task force report are (1) to bring attention to the seriousness and scope of the IDHR problem, (2) to review the state of the science for IDHR, (3) to identify critical data gaps and research needs, and (4) to make recommendations on how to overcome some of the barriers to IDHR research and address the critical data gaps. The report focuses on potential risk factors as well as clinical and preclinical issues related to IDHR when low molecular weight (LMW) drugs are given by oral or parenteral routes.

Opportunities

Accumulating evidence indicates that some IDHRs may aggregate within families, suggesting genetic predispositions. There are examples of risk factors that are caused by polymorphisms in drug metabolism and possibly to differences in the genetic control of the immune recognition of foreign chemicals (drugs). Recent advances in the genomics of drug metabolism, antigen processing, and immune regulation will create unprecedented opportunities for understanding and profiling of individual risk factors for a wide variety of IDHRs. This information coupled with the dramatically increasing knowledge base in immunology and drug metabolism could result in significant strides in basic IDHR research. Information gained from these studies would provide the basis for better clinical diagnostic methods and preclinical *in vitro* tests and animal models to assess the potential of drugs to produce IDHR. The use of databases from Health Maintenance Organizations (HMOs), the FDA, and pharmaceutical companies on drugs that produced IDHR could be very helpful in understanding and predicting which drugs may produce IDHR and in which individuals in the population. Novel mechanisms for sharing information from the databases without compromising confidentiality should be developed. An increasing public awareness of adverse reactions is helping to create a climate to encourage the development of new and improved surveillance systems for adverse drug

reactions (including IDHRs), uniform classification schemes, and analysis of existing datasets within HMOs and pharmaceutical postmarketing studies.

Status quo

Although IDHRs have an adverse impact on public health and the development of safe new therapeutic products, there has been limited progress in IDHR research in the United States in the past 25 years. In part this is attributable to structural impediments that have not facilitated the type of multidisciplinary research that this scientific problem requires nor the formation of clinical networks needed to document and share access to cases of IDHRs. In the past decade, the majority of high-quality investigations of IDHR have come from Europe, where IDHR working groups and networks are common, and interdisciplinary funding mechanisms have been developed. Another contributing factor to the current state of inadequate IDHR research progress in the United States is the lack of focus on this research area by the stakeholders involved, either individually or in concert. Neither the pharmaceutical industry nor the US FDA has targeted significant resources toward the study of IDHR. Affected patients have no organized advocacy group, and university research efforts, largely funded by the National Institutes of Health, have not focused on facilitating the multidisciplinary teams needed for research and training in this area.

Recommendations

Additional investments in understanding IDHRs are clearly warranted to investigate further the specific risk factors and ultimately the biological bases of IDHRs. Such information will have important practical applications for the surveillance and management of IDHRs for marketed pharmaceuticals as well as for new drug development. The research agenda needs to be multifaceted and collaborative. Efforts toward novel approaches to use confidential information from HMOs, the FDA, and pharmaceutical companies on drugs that produce IDHR need to be initiated. A systematic approach to prospectively assessing the potential for immunogenicity is crucially important for both IDHR management and for drug development. More fundamental studies on the genetic factors at work in IDHR and on defining the biological basis for known increased risks in special populations should pay large dividends in defining subpopulations at risk so that those not at risk can enjoy the benefits of receiving potentially sensitizing drugs. For example, investigators need to take advantage of the single nucleotide polymorphisms (SNP) database and research opportunities from the National Human Genome Research Institute as well as new opportunities in the area of pharmacogenetics funded by the National Institute of General Medicine Sciences. Tables I and II outline priorities for IDHR research and recommended organizational needs. Without organizational initiatives to stimulate and support fundamental and clinical research in IDHR, this proposed portfolio of IDHR research is unlikely to progress. This would be most unfortunate,

since new advances in immunology, genetics, and pharmacogenetics have created new research opportunities that can be expected to advance the field rapidly, given adequate resources and the facilitation of multidisciplinary and multi-institutional research and training.

INTRODUCTION

As physicians, clinical investigators, regulatory scientists, and toxicologists, we have seen first-hand the impact on public health and drug development of immune-mediated drug hypersensitivity reactions (IDHRs). We continue to be frustrated by the barriers that inhibit basic research efforts needed to develop approaches for the prevention and management of IDHRs. Those of us working for pharmaceutical companies look upon the Immunotoxicology Technical Committee (ITC) as a means to address these issues. The ITC is composed of representatives from pharmaceutical, chemical, and consumer product companies who work together to address common immunotoxicology problems in industry. Over the past years, the ITC has worked on other immunotoxicology issues involving testing methods or approaches to risk assessment. This approach, however, is not feasible for the area of IDHRs since accurate methods for preclinical testing and risk assessment have not yet been validated. Thus, in hopes of initiating efforts to break down barriers to IDHR research, the members of the ITC decided to prepare this report. To achieve this goal, a task force was formed comprising immunotoxicologists from pharmaceutical companies of the ITC, regulatory scientists from the US FDA, and university-based clinical investigators with expertise in IDHR.

The ITC is a part of the nonprofit Health and Environmental Sciences Institute, a branch of the International Life Sciences Institute (ILSI).

Purpose of the Task Force report

The report has four major goals: (1) to bring attention to the seriousness and scope of the IDHR problem, (2) to briefly review the state of the science for IDHR, (3) to identify critical data gaps and research required to address the IDHR prevention and management needs, and (4) to make recommendations on how to overcome some of the barriers to IDHR research and address the critical data gaps. The report focuses on potential risk factors as well as clinical and preclinical issues related to IDHR when LMW drugs are given by oral or parenteral routes. Issues dealing with chemically induced contact reactions or respiratory hypersensitivity reactions have been comprehensively reviewed elsewhere and are not included here.¹⁻⁴ Issues relating IDHR to recombinant proteins and monoclonal antibodies (biologics) differ in some respects from those pertaining to LMW compounds and are thus beyond the scope of this report.

Working definitions

A hypersensitivity reaction is a type of adverse drug reaction (ADR restricted to a small subset of the general population may be either immune-mediated or non-immune-

TABLE I. Priorities for IDHR research

- Identify host risk factors (metabolism, infection, genetic background) and drug characteristics (metabolites, reactive groups) in hypersensitivity reactions. Utilize emerging technologies (eg, gene chip technology) and information (eg, Human Genome Project, SNP database, Pharmacogenetics research network) to address these issues.
- Develop better diagnostic methods to discriminate between immune and nonimmune reactions and to identify culprit drugs producing a reaction.
- Determine the basic mechanism of hapten processing, presentation, and recognition in systemic hypersensitivity responses.
- Develop mechanism-based in vitro screening methods and animal models for hypersensitivity reactions.

TABLE II. IDHR organizational needs

- Convene a consortium of stakeholders to include federal regulatory and funding agencies, the pharmaceutical industry, and university-based scientists and clinicians to discuss mechanisms to initiate and support the type of collaborative projects that will be required to impact IDHR, such as the establishment of new funding mechanisms to stimulate basic and clinical research in IDHR under the sponsorship of one or more of the stakeholders, collaborative efforts with pharmaceutical companies, and the development of IDHR centers. One of the outcomes of these collaborations would likely be the development of tissue or serum banks of samples from patients or animals with documented hypersensitivity reactions.
- Initiate new program announcements from the NIH, FDA, and perhaps the Agency for Healthcare Research and Quality to encourage and promote interdisciplinary research and training focusing on IDHR. Such initiatives should provide for collaboration between institutions, support for sharing case materials and scientific resources, as well as training and career development support for scientists and physicians committed to IDHR research.
- Organize an international dialogue under the auspices of appropriate organizations such as the WHO or ILSI, to promote consensus development and global collaboration in the areas of case definitions and classification schemes, the sharing of IDHR-relevant datasets, and the facilitation of international research networks which can share diagnostic and analytic resources and case materials.

mediated. This report focuses on immune-mediated drug hypersensitivity reactions (IDHRs) and defines IDHRs as ADRs attributable to a drug-specific immunologic response that results in pathology to the host. The immune response generated may recognize the drug or its metabolite that acts as a hapten to modify self-proteins. IDHRs are adverse events that are not qualitatively derived from the known toxicologic properties of the drug.

Impact of IDHR on public health

It has been estimated that IDHRs account for 6% to 10% of all ADRs.⁵ Although the incidence of IDHRs per drug varies among marketed drugs, the total number of IDHR cases for all marketed drugs has a significant

impact on public health. IDHRs can be serious and potentially life-threatening. They are associated with many drugs and may be manifested by a variety of clinical syndromes including urticaria or angioedema, anaphylaxis, immune complex disease, serum sickness-like reactions, Stevens-Johnson syndrome, and toxic epidermal necrolysis. IDHRs may also manifest as pulmonary, hematologic, renal, and hepatic disorders or as multisystem (organ) hypersensitivity syndromes. In general, because these reactions are unpredictable, they are difficult to prevent or manage. In addition, patients with IDHRs may need alternative therapy, but clinical treatment is complicated not only by confirmation of diagnosis but also by concerns over cross-reactivity with similar drugs.

It has been estimated that serious ADRs account for some 6.7% of hospitalized patients.⁶ In 1998, the number of hospitalized patients reached approximately 34 million in the United States. If 6% to 10% of all ADRs can be attributed to IDHRs, then IDHRs may have accounted for 137,000 to 230,000 hospital admissions in 1998. The costs for all types of ADRs that occur in hospitalized patients were likely to be in the range of \$2013 to \$2595 per patient for excess costs from an extended stay of 1.7 to 2.2 days.^{7,8} Thus, the costs due to IDHR hospital admissions would be \$275 to \$600 million. When the costs of IDHRs that occur in outpatients are added, the annual costs of IDHRs in the United States could approach 1 billion dollars.

Impact of limited progress in the prevention and management of IDHR

Clinical concerns

Although IDHRs are of great concern to public health, there has been limited progress in IDHR research because of the difficulties of studying these uncommon reactions. As a result, key information and methods for the prevention and management of most types of IDHRs are unavailable. For instance, knowledge of factors that put patients at risk for IDHRs (eg, genetic predisposition, infection) would be helpful to the practicing physician, but these factors have not been identified. Methods to diagnose IDHRs and identify the specific drug responsible for the IDHR have not been developed, and so corrective management of patients with IDHRs is often delayed or inadequate. In addition, if a physician, regulatory agency, or pharmaceutical company wanted to monitor the incidence (and type of IDHR) for a specific drug, it would be difficult because of inadequate diagnostic methods, classification schemes, and tracking or database systems.

Drug development concerns

The minimal progress in basic IDHR research has also had significant impact on the success and costs of drug development. This impact is a result of the lack of mechanism-based preclinical methods to either understand the results of animal testing or screen out drugs that may potentially produce IDHR. Thus, potentially beneficial drugs may be unnecessarily eliminated from development on the basis of preclinical findings (such

as reactive metabolite generation, covalent binding to tissues, or hypersensitivity reactions in animals). Conversely, drugs that have the potential to produce IDHRs may progress through standard preclinical toxicity testing without being detected. When this occurs, there would be increases in both the risk to subjects enrolled in clinical drug trials and the cost to industry when compounds are discontinued late in the development process as a result of IDHRs.

A recent collaborative retrospective study was conducted in which 11 pharmaceutical companies submitted coded preclinical testing data on drugs found to produce toxicity in human subjects during clinical trials.⁹ Of more than 200 drugs that produced human toxicity, 13 produced toxicities that appeared to be IDHRs. Nine of the 13 compounds were discontinued from development (3 in Phase I and 6 in Phase II). For all 13 compounds, evidence of IDHR was not detected in preclinical safety studies. (Evidence of potential IDHR in preclinical studies may or may not stop the development of a drug. Such action depends on the type and severity of the findings.) The costs of not determining the potential of a drug to produce hypersensitivity in the preclinical phase of drug development can be enormous. It has been estimated that the preclinical phase and clinical Phase I, Phase II, and Phase III costs are approximately \$6 million, \$12 million, \$12 million, and \$100 million per drug, respectively.¹⁰ Thus, it is critical that investigational drugs with the potential to produce IDHR be identified as early in the development process as possible.

IDHR research: Primary needs and barriers

Advancements in the prevention and management of IDHR are contingent on several primary needs: (1) better diagnostic methods, (2) tracking systems to determine the incidence and prevalence of IDHR, (3) identification of patient-related predisposing factors for increased risk of IDHR, (4) availability of tissue and serum samples from IDHR patients for in-depth studies, and (5) preclinical testing methods (in vitro screens and animal models) to assess the risk of potential IDHR in new drug development candidates. Each of these needs could be addressed with a better understanding of the different mechanisms of IDHR. The absence of tracking systems can be attributed in part to inadequate classification systems and diagnostic methods, but development of a better classification system depends on an understanding of the mechanisms of IDHR.

Although it is apparent that more IDHR research is needed, significant barriers have impeded progress. The major barriers include (1) the low frequency of IDHRs, which has limited the number of patients to evaluate at individual institutions, (2) limited in-depth studies of patients on clinical trials with IDHRs, (3) inadequate data collection and database systems that limit the ability to conduct retrospective analyses, (4) few collaborations between academia, industry, and regulatory agencies to investigate cases of IDHR, and (5) inadequate funding of research to study IDHRs.

BACKGROUND: MECHANISMS OF IDHR

The mechanisms of immune-mediated hypersensitivity reactions to low molecular weight drugs generally fit one of two classifications: (1) reactions that occur when a drug or metabolite acts as a hapten and the immune response is directed against the hapten or hapten-peptide conjugate, or (2) autoimmune reactions in which drug administration results in an immune response to self-antigens. After a brief review of these two mechanisms, the section Determining Risk Factors will describe critical steps in the hapten and autoimmunity hypotheses that need further examination. For more detailed background information, see reviews by Weltzein et al,¹¹ Greim et al,¹² and Park et al.¹³

Hapten hypothesis

With the exception of proteins, most drugs are too small to induce an immune response alone, but they may act as a hapten by covalently binding to a carrier protein. Drugs that act as haptens may be intrinsically reactive or they may require enzymatic or nonenzymatic conversion to a reactive intermediate. It is hypothesized that the reactive compound binds to proteins (extracellular or intracellular), forming hapten-protein conjugates that are processed to hapten-peptide fragments. These hapten-peptide conjugates bind to the major histocompatibility complex (MHC) class I or II proteins, and then they are presented to T cells with the appropriate T-cell receptor. It has been demonstrated that some haptens directly bind to MHC-associated peptides.¹⁴ With antigen stimulation, the hapten-specific T cells are activated and proliferate with costimulation (second signal) from antigen-presenting cells. The T cells may then help in antibody formation, directly kill cells with the cell-surface hapten, or secrete cytokines that possibly stimulate inflammatory reactions. Much of the data on the hapten hypothesis has been generated from studies on mechanisms of contact-hypersensitivity responses and from use of various model haptens, such as trinitrophenyl, to elucidate the molecular basis for T-cell recognition of haptens. With the ability to prepare human T-cell clones, several labs have examined drug-specific T-cell clones to address mechanistic questions.¹⁵⁻¹⁸

Autoimmunity hypothesis

Autoimmunity may be generated by drugs through at least two general mechanisms.¹⁹⁻²¹ The first mechanism is believed to involve a general stimulation of the immune response (an adjuvant effect) to produce or unmask an immune response to self-antigens. In individuals with a genetic predisposition to autoimmune diseases, general immunostimulation may trigger early onset or exacerbate the disease process. The second possible mechanism involves the binding of reactive compounds to proteins, as described by the hapten hypothesis. However, the binding may result in the formation of neoantigens on self-proteins or modification of protein processing to reveal epitopes not normally produced (cryptic epitopes). Such an outcome could lead to the generation of T cells against either neoantigens or cryp-

tic epitopes on self-proteins. Although several drugs produce autoimmune responses or autoimmune-like diseases in humans (eg, procainamide, hydralazine, penicillamine, alpha-methyl dopa), it is unclear if these reactions are mediated by an immune response against self-proteins, haptenated proteins, or both.

PREVENTING AND MANAGING IDHR

Data gaps, research needs, and recommendations for better prevention and management of IDHRs are described in this section, which is divided into three subsections: Determining Risk Factors, Clinical Issues, and Preclinical Issues.

Determining risk factors

Compound-related, treatment regimen-related, and patient-related risk factors play a major role in determining IDHR. Greater understanding of these factors and identification of new factors will be critical to preventing and managing IDHRs in the future. For instance, information gained on compound-related factors may lead to the development of preclinical screens that pharmaceutical companies can use to identify potential sensitizers. Once identified, patient-related risk factors could be used to target new diagnostic methods to high-risk groups and thus facilitate surveillance and provide early treatment. Another use would be the incorporation of risk factors in the development of preclinical animal models to provide a more mechanistic basis for preclinical studies.

Compound-related factors

Metabolism, reactivity, and covalent binding

A critical element in determining the immunogenicity of a drug is the inherent chemical reactivity of the drug or its metabolites. A reactive parent drug—or its metabolite—may covalently link to proteins or directly bind to peptides associated with MHC. The amount bound will likely be determined by the amount of reactive metabolite generated, how quickly it is inactivated, and the relative reactivity of the intermediate. A greater amount of covalent binding will probably result in the constitution of T- and B-cell epitopes and thus a potentially more robust immune response.

When a drug is found to be metabolized to a reactive intermediate during the preclinical stages of drug development, additional studies may be conducted to determine the significance of this finding. These studies would include investigating: (1) the reactive intermediates formed, (2) the enzymes involved in intermediate formation, (3) relative reactivity of the intermediate, and (4) the net amount of covalent binding observed (in vitro or in vivo). With much effort, these questions can be answered. An even more difficult problem, however, is interpreting these findings in terms of risk assessment. For example, consider the situation in which a drug development candidate is found to be metabolized to a reactive intermediate, and a significant amount of covalent binding occurs without overt toxicity. How should pharmaceutical companies and regulatory agencies use

these data to determine human risk? We would not want to impede the development of a valuable drug class such as the penicillins. Innovative approaches are needed to help determine whether relative reactivity or covalent binding data are predictive and, if they are, how this information can be used to determine risk of IDHR.

Additional investigations are needed on the characterization of the reactive intermediates of drugs that cause IDHR and the chemistry of the reactions with cellular and serum macromolecules. Such studies will help in understanding structure-activity relationship (SAR) comparisons (SAR approaches are discussed in more detail below: see *In Vitro Models under Preclinical Testing Issues*).

Because most drug hypersensitivity reactions do not occur in the liver and it is likely that reactive metabolites are formed at sites where immune reactions are manifested, extrahepatic metabolism may play a key role in the generation of IDHR. Skin, for example, has metabolizing capability²² and immunologic competence, and it is often involved in IDHRs with systemically administered drugs. In addition, cells involved with the immune and inflammatory responses (polymorphonuclear neutrophils, monocytes, macrophages, and Langerhans cells) are known to have enzymes (myeloperoxidase, prostaglandin H synthase, and cytochrome P-450 enzymes) capable of generating reactive intermediates of drugs.²³ Although the presence of extrahepatic metabolizing enzymes has been known for several years, more studies are needed to determine if these enzymes play a critical role in generating metabolites that produce the hypersensitivity reactions. Investigation is also needed regarding the roles of metabolizing-enzyme location and drug distribution in determining the organ specificity of IDHR. For example, the skin is a common target of IDHRs for a variety of compounds, but it is not known if this targeting is primarily attributed to metabolism, distribution, or factors related to the immune or inflammatory response of the skin.

Effects on antigen processing

Another compound-related factor is the ability of a hapten to alter the processing of the protein to which it is conjugated. Proteins are known to be processed into smaller peptide fragments that bind to Class I or II MHC. Because the processing of proteins into peptides is not random, only certain peptides are preferably generated. These peptides are called immunodominant peptides, whereas the nonprocessed regions contain cryptic epitopes that are not normally seen by the immune system. On the basis of findings with metal ions such as gold (III)²⁴ and mercury (II),²⁵ it was proposed that the binding of metal ions to endogenous proteins modifies antigen processing such that cryptic-epitopes are formed and presented. For gold (III), this was demonstrated with use of metal ion-treated RNase and a CD4+ T cell clone against cryptic epitopes of RNase. This hypothesis of hapten-mediated autoimmune responses needs to be investigated further with drugs known to induce autoimmune responses.

Affinity between the hapten-peptide conjugate and MHC

It is known from studies on immune responses to peptides that the binding affinity between the antigenic pep-

tide and major histocompatibility complex is a critical factor in antigen presentation. This particular affinity may also be of importance with haptens because the binding of haptens to specific regions of peptides may alter their binding affinity to the MHC and thereby increase or decrease its immunogenicity. The binding of the hapten trinitrophenyl to specific amino acids of a peptide with known anchor regions increases affinity to purified MHC class II.²⁶ Whether this occurs with drugs known to produce IDHRs needs to be investigated.

Drug-related cytotoxicity or stress

In addition to the signal produced by the binding of the hapten-peptide conjugate/MHC complex to the T-cell receptor, a second signal mediated by costimulatory molecules (eg, B7-CD28, CD154-CD40) on the surface of the antigen-presenting cells is needed to activate naive T cells.²⁷ It is known that the expression of these costimulatory molecules increases with various proinflammatory cytokines such as tumor necrosis factor- α and interleukin-1. Based on these findings, Matzinger²⁸ proposed the "Danger" hypothesis to explain the immunostimulatory effect produced in which costimulatory molecules are upregulated with production of proinflammatory cytokines. As an extension of this hypothesis, Park et al¹³ suggested that the toxicity or inflammatory response that occurs with drug exposure is needed to stimulate upregulation of costimulatory molecules and thereby enhance the immune response to either a hapten or self-antigens.

The proinflammatory cytokines produced with cytotoxicity or stress also play a key role in the migration of antigen-presenting cells from the site of hapten contact in peripheral tissues to draining lymph nodes. This action has been demonstrated for contact sensitizers that stimulate the production of proinflammatory cytokines in the skin and thereby stimulate the migration of Langerhans cells to the draining lymph node.^{29,30} As the Langerhans cells migrate, they differentiate into dendritic cells (increased MHC class II expression) and present hapten to T cells. A similar scenario may occur with some immunogenic drugs that produce toxicity that results in the production of proinflammatory cytokines. Additional studies on this potentially significant factor need to be conducted.

Drug-induced autoantibodies

For IDHRs that occur in the liver and other tissue (non-type I reactions), antibodies against certain self-antigens (eg, cytochrome P-450) are often observed.³¹ It is not known whether these autoantibodies mediate the hypersensitivity response and cause tissue damage or they form secondarily as a result of tissue destruction occurring with drug-induced hypersensitivity responses or toxicity. This question needs to be answered before undue relevance is placed on antibody formation.

Treatment regimen-related factors

Certain drugs given at higher doses or with more frequency are associated with increased rates of sensitization and expression of IDHRs.³² This association is consistent with the dose-dependent relation between hapten exposure, covalent binding, and greater T-cell epitope density. Incidence of IDHR is generally higher with intravenous admin-

istration of drugs than with oral administration. Although the difference may be caused by a higher exposure level with the intravenous route, other explanations such as antigen presentation and tolerance need to be explored. It has been hypothesized that oral administration of immunogenic drugs may result in decreased responsiveness or tolerance.³³ The hypothesis is supported by findings that oral administration of a protein or contact sensitizer to experimental animals will result in a reduced peripheral immune response to subsequent dermal or systemic exposure. This phenomenon, known as oral tolerance, is thought to be mediated by the inactivation of specific T cells or by the production of inhibitory cytokines (TGF- β or IL-10). Thus, one could speculate that the low incidence of hypersensitivity reactions to drugs may be attributed to oral tolerance, and therefore a break in that tolerance might allow an immune response to occur. Oral tolerance may also account for the difficulty in generating hypersensitivity responses in animal models. Additional studies are needed to determine if oral tolerance results from exposure to drugs known to act as haptens when orally administered. If it does, the factors that alter tolerance should be investigated.

Host-related factors

Metabolism

Several host- or patient-related factors also can influence how a drug is metabolized and can potentially predispose the host to IDHR. Allelic differences in the activity of Phase I and Phase II enzymes can affect both the level of reactive metabolites being generated and how quickly these metabolites are removed. For example, of the patients who have Stevens-Johnson syndrome and toxic epidermal necrolysis after sulfonamide treatment, 90% (compared with 45% in control patients) exhibited a slow acetylator phenotype for N-acetyltransferase-2.³⁴ Many nongenetic factors can also influence drug metabolism enzyme levels and activity (eg, coadministration of other drugs, age, diet, alcohol, and smoking). In addition, the levels of necessary cofactors and conjugating proteins (eg, glutathione) are important. Clearly, more in-depth studies of the metabolism of certain drugs by IDHR patients are needed to investigate its importance.

Individual immune response differences

Several studies have found a relation between the human leukocyte antigen genotype and incidence of certain IDHR³⁵⁻³⁸; however, no causal relationships have yet been elucidated. Patients with atopic disorders (rhinitis, asthma, eczema) are no more likely to become sensitized to beta-lactams than are nonatopic subjects,³⁹ but if sensitized, they have a much higher risk of serious anaphylactic reactions.⁴⁰ Preliminary evidence indicates that patients who react to one antibiotic are at higher risk for reactions to other non-cross-reacting antibiotics.⁴¹⁻⁴³ Only about half of the population produces an IgG antibody response to intensive beta-lactam therapy, and among the 10% of those who make an IgE antibody response, serum half-lives of specific antibodies vary over several orders of magnitude. Reasons why only half of the general population appears to be able to mount an IgG immune response to a highly sensitizing drug need to be investigated.

Concurrent disease

Underlying disease states may also influence susceptibility to IDHRs. By mechanisms not clearly determined, patients with AIDS (acquired immunodeficiency syndrome) have high rates of reactions to some drugs (eg, sulfonamide antimicrobials).^{44,45} Similarly, aminopenicillins induce very high rates of rash in patients with Epstein-Barr virus infections.⁴⁶ Hepatic damage with various infections may affect IDHRs by compromising hepatic detoxification of drugs.^{13,47} Certain diseases and concurrent infections may also result in the upregulation of costimulatory molecules and act as adjuvants in IDHRs.

Other

Individual patients with prior drug reactions (or a history of IDHRs to other non-cross-reacting drugs) are at greater risk for IDHRs.^{38,42} Increasing age is a risk factor, though pediatric populations are not spared, and it is uncertain that the effect of age is independent of cumulative drug exposure. According to a study by Tran et al,⁴⁸ there may also be gender differences in IDHR prevalence, but additional studies are needed.

Summary of data gaps and research needs

Compound-related risk factors

- Interpretation of reactivity and covalent binding data in terms of immunogenicity and risk to humans
- Structure of reactive intermediates and reactions with cellular macromolecules
- Role of cryptic epitope formation as a mechanism of hypersensitivity reactions that occur with exposure to drugs known to produce autoimmune reactions in human beings
- Role of hapten binding on hapten-peptide affinity with MHC
- Role of drug-mediated upregulation of costimulatory molecules and dendritic cell migration
- Pathogenic role of drug-induced autoantibodies

Treatment regimen-related risk factors

- Significance of the route of administration in determining immunogenicity

Patient-related risk factors

- Role of differences in drug metabolism, induced and genetic
- Role of genetic difference in the immune response of individuals
- Role of disease or concurrent infections
- Role of gender and age

Recommendations

Clearly, many basic mechanism questions remain to be answered. Studies should focus on determining which of the factors or processes are critical to the induction of IDHR. Emphasis should be placed on understanding the genetic basis for the individual differences. This knowledge is necessary to understand the factors determining immunogenicity, develop new diagnostic methods, identify predisposing host characteristics, and inform clinical management practices and predictive preclinical testing methods.

Clinical issues

Better diagnostic tests, new mechanism-based classification systems, and a tracking system or database for IDHRs are needed to help support the prevention and management of IDHR.

Diagnostic tests

Good diagnostic tests for drug hypersensitivity reactions, in addition to a patient's detailed history and physical examination, are critical to establishing the type of reaction (classification), determining patient treatment, identifying which drug is responsible for the reaction, and tracking the incidence of hypersensitivity reactions to that specific drug. These same methods may be useful to pharmaceutical companies in conducting clinical trials of drugs and in postmarketing monitoring. Following are some of the currently used diagnostic tests and their limitations.

Type I hypersensitivity testing

Skin testing

Skin prick or intradermal skin testing for evaluating the presence of drug-specific IgE antibodies is useful for only a few LMW drugs (penicillin, muscle relaxants, barbiturates) because the relevant immunogenic drug determinants have not been elucidated for most other drugs. For drugs for which commercially available reagents are not available, allergists may prepare their own reagents from the formulated drug. Because the antigenic determinants of antibiotics may differ, one type of antibiotic reagent cannot necessarily be used to test for specific IgE antibodies against another type of antibiotic. Thus, there is a critical need for a wide variety of commercially available skin test reagents that contain the immunogenic determinants clinically relevant to the drug in question. More research is also needed to identify potential immunogenic determinants for drugs known or suspected to produce IgE-mediated hypersensitivity reactions.

Radioallergosorbent test

The radioallergosorbent test (RAST) is a solid-phase radioimmunoassay that measures circulating allergen-specific IgE antibodies. It has limited usefulness as a diagnostic test in drug allergy since, as with skin testing, the immunochemistry of most allergenic drugs is unknown. RAST assays have been developed for the major penicillin determinant (penicilloyl moiety), insulin, chymopapain, some muscle relaxants, thiopental, protamine, and latex. These assays are limited, however, by a lower sensitivity than skin tests, potential interference by drug-specific IgG antibodies, and in some cases high nonspecific binding. In comparison to skin testing, the predictivity of the RAST for IgE-specific antibodies varies between drugs. The UniCAP assay, a variation of the RAST, has the same limitations as the RAST, but radiolabeled antibody is not needed.

Type II and III hypersensitivity testing

Hemagglutination assays (direct or indirect Coomb's assay) have been used for many years to determine the presence of drug-specific IgG and IgM antibodies to aid in the diagnosis of immune-mediated hemolytic anemia. However, because of limitations of this test (especially the need to maintain fresh stocks of drug-conjugated ery-

throcytes), enzyme-linked immunosorbent assays are now used routinely. Of greater importance, the clinical relevance of drug-specific IgG and IgM needs to be determined, since these antibodies may be present without immunopathologic significance.

Type IV hypersensitivity testing

Patch testing may be useful in determining the etiology of T-cell-mediated reactions, specifically eczematous, drug-induced eruptions. In addition, it may have broader applicability to other drug-induced cutaneous diseases and systemic reactions. Patch or topical testing has been used with varying success in the investigation of systemic drug hypersensitivity reactions. The utility of this method may depend on the vehicle for the drug and location of application. The rationale is based on the compound's being metabolized and presented via the skin to the immune system. Patch testing has been most useful for anticonvulsants, such as carbamazepine and penicillins.⁴⁹⁻⁵¹ In light of recent data that support an immunopathogenic role for specific T cells in morbilliform eruptions, fixed drug eruptions, and bullous eruptions, patch testing may be useful in the evaluation of other drug-induced cutaneous reactions. Romano et al⁵² found that most patients at risk for maculopapular eruptions caused by ampicillin, amoxicillin, or penicillin demonstrated positive delayed intradermal skin test responses as well as positive patch test responses. Additional studies need to be conducted to determine the selectivity and sensitivity of this and other delayed-hypersensitivity methods for other drug classes. Like the skin test assay for IgE, this method may also be limited by the lack of validated test reagents with relevant immunogenic drug determinants.

Miscellaneous

In some instances, a tissue biopsy may be helpful to determine the diagnosis of the reaction and the nature of the inflammatory response, but only general information will be obtained (type of cellular infiltrate, presence of edema fluid). Immunohistochemical studies may yield additional useful information.

Tryptase is a mast cell-specific protease that may be elevated in serum after drug-induced anaphylactic reactions. Elevated levels have been found after systemic reactions to anesthetic agents, latex, and various antibiotics. Other tests that may be helpful in the evaluation of drug-induced hypersensitivity include basophil histamine release, lymphocyte proliferation, complement activation, and the lymphocyte cytotoxicity assay. Most of these should be considered research tools at this time, and their role in the evaluation of IDHR has not been fully elucidated.

New technologies

A key factor in determining whether a reaction is immune-mediated is the presence of antigen-specific T cells against the hapten. The recent development of MHC class I and II tetramers for quantifying the number of antigen-specific T cells by flow cytometry⁵³ may also be applicable for evaluating IDHRs. The application of tetramer technology to haptens and self-proteins needs to be investigated. Significant advances in genomic and

proteomics technology may also provide leads to the identification of protein expression patterns indicative of IDHR. As a greater understanding of the immune response to haptens is gained, perhaps new diagnostic biomarkers can be identified.

Summary of data gaps and research needs

- Identity of immunogenic determinants of drug haptens
- Commercially available diagnostic reagents with known immunogenic determinants
- More sensitive methods to measure drug-specific IgE, IgG, and IgM antibodies
- Methods to determine if a clinical reaction is non-immune-mediated or immune-mediated
- Evaluation of new technology for applicability to new diagnostic tests
- Clinical studies to evaluate the specificity and sensitivity of new diagnostic test methods

Recommendations

To address the data gaps and research needs itemized above, better diagnostic tests are required. The data gaps and research needs must be tackled by clinicians, immunochemists, chemists, and companies that make the diagnostic reagents or testing kits. It is recommended that a workshop be convened to develop a process to better address these goals. In addition, research funding is needed to better understand immune responses to haptens, novel targets, and analytical methods.

Classification systems

Most disease classification systems are based on differences in either mechanism or pathogenesis. However, because of the uncertainty of the mechanisms involved in drug hypersensitivity reactions and the inability to determine the mechanism by currently available diagnostic tests, these reactions are classified by descriptive-based systems (ie, organ system classification) or are grouped in simplified mechanism-based schemes (ie, Gell and Coombs) without a full understanding of the mechanism. Thus, the various terminologies that have evolved over the years make it difficult to describe and track these reactions.

Current classification systems

All ADRs belong to one of two major types: those that are common and predictable (type A reactions) or those that are uncommon and unpredictable (type B reactions). Type A reactions, which account for approximately 80% of ADRs, are usually dose-dependent, are related to the pharmacologic actions of the drug, and occur in otherwise normal individuals. Type B reactions typically are unrelated to a drug's pharmacologic properties, and they occur in susceptible individuals.⁵⁴ Drug hypersensitivity reactions, which are type B, can be further divided into immune-mediated and non-immune-mediated (pseudoallergic) reactions. For instance, penicillin elicits drug-specific IgE antibodies that cause mast cell mediator release after receptor crosslinking, whereas opiates cause nonspecific mast cell mediator release.⁵⁵

Mechanism-based system

Immune-mediated hypersensitivity reactions are classified in four categories according to the mechanism

involved or thought to be involved (Gell and Coombs):

Type I — immediate type hypersensitivity reactions mediated by IgE

Type II — cytotoxicity reactions mediated by antigen-specific IgG or IgM

Type III — immune complex reactions mediated by antigen-specific IgG antibodies

Type IV — T cell-mediated reactions (delayed-type hypersensitivity response)

Classification according to organ system

Presumed hypersensitivity reactions are also classified according to the organ systems involved in the reaction. Systemic reactions include anaphylaxis, vasculitis, serum sickness-like reactions, drug fever, drug-induced autoimmune disease, and the hypersensitivity syndrome. Organ-specific reactions are numerous and include cutaneous reactions, hematologic reactions, pulmonary reactions, hepatic reactions, and renal reactions.

Problems with the current systems

One of the primary problems in using a mechanism-based classification system is that the mechanisms of most IDHRs are not clearly understood. Thus, the problem may not be the classification system itself but the difficulty in obtaining the necessary information to classify the reaction appropriately. Still, improvements in the Gell and Coombs scheme are needed. For instance, reactions mediated by multiple immunologic mechanisms or a combination of immune-mediated and non-immune-mediated reactions do not fit into the Gell and Coombs classification. In the case of drug-induced urticaria, the mechanism responsible appears to depend on the particular drug involved.

Although it is easier to classify a reaction in an organ-based system, this system also has its faults. For example, use of different terminology to describe similar reactions that occur in different tissues makes it difficult to classify the reaction and track it in a database.

Recommendations

The development of a classification scheme depends on the development of better diagnostic methods and a greater understanding of mechanisms. Until we reach that point, however, a tentative classification system should be put in place for better tracking of incidence rates. This system could be developed by an international panel of experts. Another approach would be to fund a grant or contract to develop a classification system, similar to the Request for Proposals from the National Institute of Arthritis and Musculoskeletal and Skin Diseases for the "Development of a Dermatology Lexicon" (NIH-NIAMS-01-03).

Drug hypersensitivity databases

Because of the low incidence rate of IDHR per drug, it is difficult to determine the true incidence and significance of reactions produced by a specific drug during postmarketing surveillance without a comprehensive and accurate database system. A thorough review of approaches for the surveillance of all ADRs and future needs in this area has been recently published.⁵⁶ Much of that information on tracking ADRs is applicable to IDHR cases. In brief, data

on ADRs may be obtained from four sources: (1) data from premarketing clinical trials, (2) spontaneous reporting of ADRs of marketed drugs to the US FDA's Medwatch system or to the US FDA's Adverse Event Reporting System (AERS) and in published case reports, (3) database systems in hospitals and insurance institutions, and (4) postmarketing cohort studies.

The major drawback to the premarketing clinical testing databases and those developed by hospitals and insurance agencies is the limited subject or patient population. Thus, ADRs that occur with low frequency are unlikely to be detected. The major problems with the Medwatch system are that it is a volunteer system and the reporting rate may not be representative of the problem. For the AERS, any adverse effects during a drug's first three years must be reported to the FDA within 15 days of the sponsor's becoming aware of the event. After 3 years, the sponsor may report ADRs on an annual basis. Although the Medwatch system and AERS are designed to pick up serious adverse reactions early, reporting biases limit their utility in estimating IDHRs. These biases include underreporting after the first few years of marketing experience (as adverse effects become common knowledge and lead to labeling changes), lack of perceived relationship between the reaction and drug use, and lack of incentives for reporting. Information can be reported to Medwatch before the sponsor is aware of the problem. Also, once a drug is widely used and the medical community becomes aware of possible reactions, reporting to either the sponsor or AERS decreases significantly. Because some of the reported IDHR data in ADR databases do not differentiate between IDHRs and nonimmune (pseudoallergic) or unrelated reactions with drug use, it is clear that better databases for IDHRs also depend on better diagnostic and classification methods.

Recommendations

Data sets from premarketing and postmarketing sources need to be organized onto a database system in which IDHR cases can be readily identified and tabulated. Although the Medwatch and AERS systems have provided important information, they are spontaneous or passive reporting systems and therefore of limited value. An international workshop should be convened to initiate the development of a new database that does not rely on spontaneous reporting and incorporates a large number of patients. The database system should be developed in conjunction with efforts to set up a working classification system.

Preclinical testing issues

Animal models to identify potential systemic sensitizers

Currently, no animal models have been developed and adequately validated to identify potential systemic sensitizers. Two models, however, have been recommended and used. As described below, these two approaches are poor predictors and are not based on mechanisms relevant to IDHRs mediated by systemically administered drugs.

The current use of these models, in spite of their known limitations, accentuates the need for predictive animal models. A review of animal models was recently reported by Choquet-Kastyleusky and Descotes.⁵⁷

Models currently used

Antigenicity assays

Although there is no animal model for systemic hypersensitivity with LMW compounds, the Japanese Ministry of Health and Welfare and recently the Korea Food and Drug Administration⁵⁸ support the use of what are generally referred to as antigenicity assays to determine the ability of new pharmaceutical agents to induce specific antibody responses *in vivo*. To clarify terms, antigenicity refers to the ability of a compound to bind to antibody or T-cell receptors. Immunogenicity strictly refers to the ability of a molecule to induce an immune response (antibody or T-cell-mediated response). In the antigenicity assays, compounds are tested for their ability to induce an antibody response and elicit reactions mediated by antigen-specific antibodies. Thus, antigenicity assays would be more properly termed immunogenicity assays.

According to the Japanese Ministry of Health and Welfare guidelines, antigenicity studies are relevant for the following test situations: (1) drugs that form covalent bonds with proteins (haptens), (2) peptides or proteins, (3) polymers, (4) beta-lactam antibiotics, (5) compounds belonging to a class shown to cause hypersensitivity in human beings, (6) compounds with suspected allergic potential in repeated-dose nonclinical studies, and (7) compounds for injection or infusion.

In general, antigenicity assays involve administering the test compound either subcutaneously with complete Freund's adjuvant (CFA) or by the clinical route of exposure. Guinea pigs and mice are the most commonly used species for these assays. It is thought that during this initial drug treatment phase (also referred to as the sensitization phase), an immunogenic drug will act as a hapten and induce the generation of a specific antibody. The second phase of the assay involves measuring the amount of the antigen-specific antibody generated by using the active systemic anaphylaxis and passive cutaneous anaphylaxis reactions.⁵⁹⁻⁶¹

The usefulness of antigenicity assays to assess the potential of LMW compounds to produce antibody-mediated hypersensitivity has been questioned. A primary criticism is the use of CFA given in conjunction with a drug-protein conjugate. Pharmaceuticals are not given with adjuvants, nor are most drugs administered as protein conjugates. Consequently, false-positives potentially may result from this approach. Another criticism is that most compounds administered orally to guinea pigs or mice are not immunogenic, including compounds known to produce IDHRs in human beings. Given these two major problems, antigenicity assays should not be recommended until adequately validated with the use of a wide variety of known human sensitizers and negative compounds when administered by the clinical route of exposure.

Contact hypersensitivity methods

Other methods such as the Buehler guinea pig assay, the guinea pig maximization test, and the mouse local lymph node assay (LLNA) are designed to detect chemicals that can produce immunologically mediated skin sensitization.⁶²⁻⁶⁴ These assays are designed to detect haptens when applied to the skin (Buehler assay and LLNA) or injected subcutaneously with CFA (guinea pig maximization test). Although it seems reasonable to conclude that compounds found to be positive in these assays could also act as systemic sensitizers, an analysis of numerous compounds demonstrated a poor correlation between the potentials for contact hypersensitivity and systemic hypersensitivity.⁶⁵ The reason for this poor correlation is unknown but may be due in part to the complex nature of IDHRs in comparison to contact hypersensitivity reactions. As discussed earlier in Mechanisms of IDHR, it has been proposed that tolerance induced with oral exposure to contact allergens may account for this phenomenon. In addition, organ-specific metabolism resulting in immunogenic products probably could not be adequately modeled with dermal exposure.¹³ Neither is adjuvant-like nonspecific immunostimulation, which is considered a potential factor in chemically induced autoimmunity, likely to be modeled. Finally, the models for contact allergy detect cell-mediated (type IV) immunopathy and do not adequately model other types of immune reactions (such as IgE- or IgG-mediated processes) that are often involved in systemic hypersensitivity.

Future animal models

Development and validation of a stand-alone whole-animal model for hazard identification of potential systemic sensitizers is obviously a daunting task. However, the information gained from studies on mechanisms of these reactions in human beings and the key risk factors involved in these mechanisms (discussed above) will help direct the development of new methods. Because IDHRs encompass a wide variety of mechanisms, it is likely that different types of animal models will be needed to assess the potential of compounds to produce hypersensitivity reactions. Still, given the immediate needs for an animal model, efforts to develop and validate potential models must move forward even if the mechanisms that allow these models to detect sensitizers are unclear. Future animal models may use the following approaches: (1) preclinical rechallenge, (2) popliteal lymph node assay, (3) transgenic mice, (4) drug-induced autoimmune responses, (5) hypersensitivity responses, and (6) DNA array technology.

Rechallenge phase in preclinical safety assessment

Rechallenging a sensitized individual to a drug is one of the most convincing ways to verify a person's allergic reaction. This approach has also been used in preclinical safety testing of biotechnology products (eg, recombinant proteins, monoclonal antibodies) to determine if animals may have generated reagenic antibodies that could induce anaphylaxis. This procedure would also be simple to perform at the end of multiple repeat-dose non-clinical safety studies for LMW drugs if there was a con-

cern about sensitization. The obvious advantage of this approach is that the animals will be exposed through the anticipated clinical route of exposure and the sensitization data can be analyzed in relation to the other standard toxicity end points (physical signs, body weight, food consumption, clinical pathology, organ weights, and histopathology). The best approaches to measure specific responses in these studies need to be determined. In addition, false-positive reactions that may occur with the direct release of histamine from mast cells or basophils by some compounds need to be considered. If this approach appears to be helpful, extensive validation studies would be needed.

Popliteal lymph node assay

The popliteal lymph node assay (PLNA) arose from studies that found that subcutaneous administration of phenytoin resulted in an enlargement of the popliteal lymph node and that the effect could be transferred with lymph node cells in naïve animals.^{66,67} Subsequently, various compounds known to produce autoimmune-like diseases in human beings were tested and modifications of the assay were developed. In general, this method has shown some promise but needs to be modified and then fully validated. In parallel, the mechanism responsible for the chemically induced increase in lymph node cellularity needs to be examined. The response has been proposed to be the result of a hapten-mediated reaction like the LLNA, immunodysregulation that results in an autoimmune response or general immunostimulation of lymph node cells.

The PLNA is subject to both false-positive and false-negative results. Subsequent modifications in the assay have been developed, but additional validation studies are needed to determine specificity and sensitivity. Among the modifications developed are (1) coinjection of a "reporter" antigen with the test compound to detect T-cell activation or adjuvant-like activity,^{68,69} (2) administration of a secondary challenge with test compounds into the footpad to determine if the PLNA response is caused by an antigen-specific immune response,⁶⁶ and (3) because many drugs require metabolic activation to produce hypersensitivity reactions, use of *ex vivo* metabolic pretreatment (liver S-9) of the test compound before administration into the footpad.⁷⁰ Although the modifications of the PLNA have improved the prospects of this assay, two primary concerns need to be addressed. The first concern is that the PLNA is known to readily detect compounds that are also contact sensitizers. For orally administered drugs, subcutaneous injections into the footpad may not be relevant. As discussed earlier, it is well known that contact sensitizers when administered orally do not produce systemic hypersensitivity reactions. The second concern is the need to investigate the mechanisms for lymph node response with a wide variety of compounds that may produce IDHRs via different mechanisms (eg, autoimmunity, hapten).

Transgenic mouse models

Rodents (primarily mice) that have been genetically engineered to be deficient in specific traits ("knock-out

mice") have been used to define specific immune mechanisms underlying various immunopathies. Although these models have been used primarily in mechanistic studies, they may be further developed as models to identify potential systemic sensitizers. The selection of the type of transgenic mouse will stem from basic research on mechanisms of IDHR in human beings and animals. For example, the use of IL-10 knock-out mice may allow a greater immune response against drugs that act as haptens because IL-10 normally downmodulates immune responses. This has been shown to be true for contact hypersensitivity responses to a known contact sensitizer in IL-10 knockout mice in comparison to wild-type control animals.⁷¹

Animal models of autoimmune diseases

Most of the work with chemical-induced autoimmune responses in animals has been focused on metals (mercury, gold, cadmium, lithium). There are a few drugs (eg, hydralazine, L-canavanine, penicillamine) that are known to produce autoimmune responses or diseases in animal models.²¹ Many of these response do not mimic the response seen in human beings. On the other hand, there is a much longer list of drugs that have been associated with autoimmune disease in human beings. The reasons why autoimmune responses/diseases are difficult to reproduce in animal models are not known. Thus, much more work with animal models for drug-induced autoimmune responses or diseases must be conducted before a suitable animal model is available to predict the potential of compounds to produce autoimmune responses.

Drug-induced autoimmune diseases may be related to genetic predisposition, possibly caused by an acceleration of the autoimmune process. Thus, animal models of spontaneous and chemically induced autoimmune disease may be used. Studies with the brown Norway rat and NZB mouse strain have shown an accelerated autoimmune response (autoantibodies) with exposure to certain chemicals such as mercury. Similarly, this idea can be applied to other animal models of spontaneous autoimmune diseases to identify drugs that may stimulate the autoimmune response. Recent studies with NZB mice have shown some promise.⁷² To explore this approach, additional studies are needed in these models with various compounds known to stimulate the autoimmune response.

Building on known animal models of hypersensitivity reactions

Animal models have reproduced in part some hypersensitivity responses observed in humans. One example is the use of guinea pigs or rats in modeling certain biochemical and immunologic parameters known to be associated with halothane hepatitis, a type of systemic hypersensitivity.^{73,74} A better understanding of these models may lead to their further development for hypersensitivity testing. In the future, if a hypersensitivity response in an animal model is found to mimic the clinical reactions, further investigation of the mechanism of that response should be encouraged.

Application of DNA array technology in animal models

The development of DNA array technology is being applied to many areas of toxicology. The attraction of

this approach is its ability to quickly generate data on the expression of many genes after chemical exposure and thereby assist in elucidating mechanisms of action. Specific patterns of gene expression might be used to identify potential systemic sensitizers. Although DNA array technology is still in fairly early stages of development, it has enormous potential to explore and understand the role of specific genes in hypersensitivity reactions.

In vitro models

Reactivity and covalent binding

The ability of a LMW drug to induce an immune response often depends on its ability to bind to proteins via covalent or coordinate binding. Therefore, the potential of a drug to act as a hapten may be determined by examining the structure of the molecule and its metabolites for reactive groups. If a chemical does act as a hapten, its ability to bind proteins can be further assessed in vitro by testing whether potentially immunogenic chemical-protein conjugates can be formed. For drugs that do not require metabolism, this question can easily be answered by assessing the ability of the drug to conjugate a protein such as ovalbumin.⁷⁵⁻⁷⁷ However, because many drugs are not intrinsically reactive, it may be necessary to add metabolic activating systems, such as S9 or liver microsomes, to detect haptens formed by biotransformation. This approach requires the use of either radioactive drugs or antibodies specific for the drug to be able to detect the protein adducts. Thus, a valuable tool would be a method to easily assess the ability of a drug to bind proteins after metabolic activation in vitro without the use of radioactivity or the need for specific antibodies.

The primary limitation of using covalent binding data in determining risk is knowing how much covalent binding should be of concern. If one compound exhibits high levels of covalent binding, does this indicate a greater level of concern than low levels of binding? Because immunogenicity probably depends on several factors in addition to covalent binding (discussed above in Determining Risk Factors), the role of covalent binding in determining overall immunogenicity needs to be clarified.

Structure-activity relationship approaches

Several SAR models of allergic contact dermatitis have been reported.⁷⁸⁻⁸⁰ The skin-sensitizing potential of unknown chemicals can be predicted on the basis of the presence of substructures associated with this reactivity as well as their physiochemical property of lipophilicity. No SAR model for systemic hypersensitivity has yet appeared, but once an understanding of its mechanism or an animal model for predicting it has been obtained, this valuable tool should be developed as well.

There are few SAR studies of drug-induced autoimmunity, and the challenges are formidable. For example, more than 60 compounds, including drugs with a variety of pharmacologic activities, have been implicated in systemic lupus erythematosus-like syndromes in humans, but there is no obvious unifying structural relation.²¹ The biochemical and immunologic mechanisms involved in autoimmune responses (generation of autoantibodies and T cells) are complex, and a clearer understanding of drug metabo-

lism, target autoantigens, and specific-organ immunotoxicity are needed to enable a rigorous SAR analysis.

Validation process of new methods

The concept of “validation” is complex, and the standards for establishing that a proposed predictive model is valid for a specific purpose are controversial. Within the US government, a center has been established to set validation standards and to coordinate validation of toxicology methods. The National Toxicology Program Center for the Evaluation of Alternative Toxicological Methods and its associated advisory committee, the Interagency Coordinating Committee on the Validation of Alternative Methods, have been developing a standard approach to assessing the validation status of proposed toxicological methods.

One important element of validation is the testing, based on clinical data, of several positive and negative controls of different chemical classes to determine sensitivity and specificity. However, assembling a sufficient list of positive control compounds may be problematic because of the limitations of the available databases. For example, with certain drugs it is unclear whether allergic-like reactions are true immune-mediated responses or pseudo-allergic reactions. Also, a list of positive compounds probably would need to be separated into subclasses according to the mechanism or target organ of the hypersensitivity response. Another problem with establishing such a list is that often the most potent or troublesome hazards have been removed from the market or were identified in the development process and therefore never marketed. It would be helpful if such drugs could be made available for evaluation of proposed methods. Clearly, extensive collaboration among scientists from industry, academia, and government is needed to assemble a list of positive controls and to provide test compounds for future studies.

Summary of data gaps and research needs

- Additional modification and validation of guinea pig and mouse antigenicity assays
- Studies examining the possibility of rechallenging animals in standard preclinical safety studies to assess potential to produce IDHR
- Modification and validation of the mouse popliteal lymph node assay, along with studies to examine the mechanism of the lymph node response
- Testing of certain transgenic and autoimmune models as approaches to determining the potential of compounds to produce hypersensitivity reactions
- Studies to investigate the significance of covalent binding data in determining overall immunogenicity of a test compound
- Studies to examine the structure-activity relationships of compounds known to produce hypersensitivity reactions

Recommendations

Because of the complexity of IDHRs, it is unlikely that a single stand-alone animal model would be completely adequate for predicting all types of systemic hypersensitivity reactions. A tiered or “tool box” approach might be more appropriate for hazard identi-

cation and subsequent risk assessments. A tiered, “tool box” approach could include several methods to identify drugs that are potential systemic sensitizers; the selection of specific tools would be decided on a case-by-case basis. Such an approach has been applied to identification of immunosuppressive compounds and respiratory sensitizers. With respect to systemic hypersensitivity, the approach might include SAR, biotransformation studies, antigenicity determinations, and whole animal models.

FUTURE RESEARCH FUNDING, TRAINING, AND ORGANIZATIONAL NEEDS

The long list of data gaps and issues in the clinical and preclinical areas suggests that a significant amount of research is needed before progress can be made in the prevention and management of IDHRs. To move forward, increased funding for basic and applied research is needed along with increased training and the development of novel cooperative efforts between government, academia, and industry.

Research funding

History

To date, investigators seeking support for basic or clinical research in IDHR have been frustrated by the inconsistent and minimal amount of funding available. On one hand, industry has looked to academia to solve basic research questions supported by funds from government sources; on the other hand, private and federal funding organizations have often viewed such studies as applied problems that should be resolved through industry funding. Because these research projects require a long-term commitment and significant levels of funding, individual companies have rarely supported them. Groups of companies have not collaborated to identify key needs and pursue long-term projects, a lack of cooperation that can be attributed in part to the low incidence of these types of reactions per compound and the large effort required for practical solutions. As a consequence, IDHR research remains limited in scope and depth, whereas other comparably important areas of research have advanced.

In the United States, funding for studies of IDHRs has come largely from the National Institutes of Health and ad hoc funding from individual pharmaceutical companies. Investigator-initiated research grants on clinical and immunologic aspects of allergic drug reactions have been periodically funded by the National Institute of Allergy and Infectious Diseases' Division of Allergy, Immunology, and Transplantation (DAIT). The portfolio in DAIT has always been small, often including only one or two active grants. Other awards from a variety of Institutes have funded targeted drug allergy problems (eg, drug sensitivity in AIDs). The National Institute of General Medical Sciences has a program in clinical pharmacology that funds basic research on drug metabolism, reactive metabolites, and pharmacogenetics; these disciplines directly or indirectly advance the current understanding of drug hypersensitivity states. The National Institute of General Medical Sciences, US FDA,

Canadian Food and Drug Directorate, and other organizations contributed funding for a long-term systematic survey of hospital-based ADRs in Boston during the 1970s and 1980s.⁸¹ Important epidemiologic information about IDHRs came from this pioneering project.

At one time, the US FDA had an extramural research program that supported several grants on allergic drug reactions, but the extramural research program was discontinued. Currently there is little if any intramural research on drug hypersensitivity within the FDA. Some intramural FDA attention has recently been placed on efforts to identify marketed drugs with the highest incidence of IDHR-like reactions.⁸²

Individual pharmaceutical and biotechnology companies have funded academic research targeted at investigations of immunologic reactions to their licensed products or therapeutics under development. The trade organizations for these industries, however, have not been a major source of funds for basic or clinical research in IDHRs. Some large pharmaceutical companies internally have relevant expertise in many of the disciplines related to drug hypersensitivity, and they occasionally conduct product-specific research on mechanisms for hypersensitivity reactions (eg, immune versus nonimmune mechanisms). Unfortunately, most of the findings from these studies are unpublished.

Within European Union countries, there appears to be more focus on supporting drug hypersensitivity research, which is largely conducted in state-supported research institutes. In particular, the European Union has a Working Group on Drug Hypersensitivity that has been instrumental in providing resources for multicenter and multinational clinical studies of allergic drug reactions. Similar national networks of cooperating investigators have been organized under the National and Medical Research Institute in France and other European countries. As a result of these overlapping international funding mechanisms, studies of immunologic drug reactions in Europe are much more common than in other parts of the world, and many if not most of the landmark observations for allergic drug reactions in the past decade have come from European research centers. Yet, given the opportunities, a much larger world-wide investment in IDHR research is needed.

Current difficulties

In the absence of flexible and varied sources of funding for IDHR research in the United States, research efforts have been fragmented and episodic. Although case studies and small-scale studies of IDHRs continue to appear from American academic centers, very few involve interdisciplinary efforts that bring together the complementary disciplines of clinical allergy, immunology, toxicology, drug metabolism, and genetic epidemiology—collaboration needed to advance understanding of pathogenesis and risk factors for IDHRs. Most studies on predictive models for drug hypersensitivity have been undertaken within the pharmaceutical industry in an effort to refine drug development. Fundamental studies on the immunopathogenesis and genetics of multiple drug allergy syndromes are virtually nonexistent in the United States.

The paucity of drug allergy research in the United States over the past two decades has multiple origins. The federal agency with the greatest interest and involvement in IDHRs, the US FDA, has discontinued its support of extramural research of any kind, and has no internal research efforts in IDHR in general. At the NIH, clinical pharmacology and immunologic diseases fall under the purview of numerous Institutes, but there have been no program announcements for interdisciplinary projects in IDHR research. Pharmaceutical and biotechnology industries express interest from time to time, especially as part of their commitment to rational drug development, but there has been only limited funding for clinical or basic research (including animal models) in this area. Patient advocacy groups for allergic disorders often devote their limited resources to more prevalent allergy problems, such as bronchial asthma. Private foundations have not targeted IDHRs as an important health research priority. Thus, investigators who might be willing and able to invest scientific effort on problems related to IDHRs have not been given the incentive to do so by broad multidisciplinary program announcements. The time now has largely passed when an investigator can study the pathogenesis or epidemiology of an immunologic drug reaction within a single institution. Most drug allergy research has focused on penicillins and sulfonamides because of the relatively high prevalence of reactivity to these broadly administered drugs. Studies of less prevalent drug reactions invariably require multisite participation through structured networks of investigators. The framework for such studies is lacking in the United States. High quality clinical research also requires the involvement of a multidisciplinary team including clinical immunologists, clinical pharmacologists, toxicologists, pharmaceutical chemists in some cases, and genetic epidemiologists. Requisite expertise rarely lies within a single institution, and thus funding mechanisms are needed to include multiple disciplines and institutions to ensure sufficient expertise and patient materials.

Finally, funding mechanisms are needed for applied research projects such as the development of new diagnostic methods or preclinical animal models. Traditional investigator-initiated grants from the NIH are not amenable to these types of projects. Moreover, the specific needs of and benefits to clinicians and pharmaceutical companies are not always clearly communicated to academicians who may have the expertise for these applied projects.

Recommendations

We recommend that the following approaches for supporting collaborative research funding for IDHR research be seriously considered.

US FDA extramural funding

Because of its regulatory and programmatic responsibilities, the US FDA should consider reactivation of its extramural research program to allow investigator-initiated applications for research grants dealing with various aspects of immunologic drug reactions, both fundamental and clinical. These grants would fund applied research (eg, diagnostics, preclinical model development) and organizational needs (eg, meetings, database

initiative), whereas NIH grants would continue to focus on basic research.

NIH initiatives

As requests for proposals, requests for applications, or program announcements are developed by the NIH that relate to investigations of adverse events, it is suggested that the areas of interest include IDHR studies. Investigators should be encouraged to use currently existing NIH-funded programs and resources to conduct IDHR studies (eg, general clinical research centers, various disease specific and pharmacogenomics and toxicogenomics research networks, and clinical trials programs). Supplemental awards to investigators should be encouraged to broaden the use of currently existing NIH-supported, patient-oriented research resources on IDHR mechanisms. Highly innovative, hypothesis-based research applications should be encouraged to stimulate the development and application of tools and technologies. Center or consortium grants, including a multidisciplinary approach provided by several institutions and perhaps including a clinical network, will be needed to overcome the low incidence IDHRs in a single medical center. The sharing of case material and diagnostic and analytic capabilities will be an essential component of any successful effort. New funding opportunities for IDHR could also readily fit in with concurrent efforts for pharmacogenetics research from the National Institute of General Medicine Sciences and single nucleotide polymorphisms (SNP) research funding from the Human Genome Research Institute.

Funding of applied research

Funding of applied research for the development of diagnostic or preclinical testing methods may usefully come from a consortium of pharmaceutical companies or from the Small Business Innovation Research program of the NIH and FDA. Funds from the SBIR program go to innovative projects that have commercial potential. These grants are divided into three phases. Phase I explores the technical merit and feasibility of the idea (up to \$100,000 for 6 months). If Phase I is successful, the developer applies for Phase II support to evaluate the commercialization potential of the method (up to \$750,000 for 2 years). Phase III is the actual migration of the method from the laboratory to the marketplace. To make the transition from Phase I to II, the developers need to work closely with industry to determine if the method developed will be used for drug development or in the clinics. Channels are needed by which developers can discuss their ideas with industry representatives for the Phase I to II transition.

An all-stakeholder funding consortium

Large projects that require sustained funding, such as a network of IDHR centers, might best be funded jointly by all the relevant stakeholders. These would include both pharmaceutical and biotechnology industries and appropriate federal health agencies (NIH, FDA, and perhaps the Agency for Healthcare Research and Quality). A previous model for this type of consortium funding was the very successful Boston Collaborative Drug Surveillance Program.⁸³

Without new funding mechanisms to stimulate and support basic and clinical research, the portfolio of research on IDHRs in the United States will continue to decline. This would be most unfortunate since progress in immunology, genetics, and pharmacogenetics has created new research opportunities that could advance the field rapidly, given adequate resources.

Training opportunities: Current difficulties and recommendations

To increase the level of active IDHR investigation, it will be necessary to establish opportunities for interdisciplinary training for research in allergic drug reactions. Concomitantly, support is needed for research training in the disciplines essential to this work: pharmacology, medicinal chemistry, drug metabolism, the immunology of haptenic sensitization, and clinical allergy.

Various funding mechanisms will be necessary to achieve the goal of increasing the level of research activity and training related to understanding immunologic drug reactions. Fellowships providing stipends and tuition to support study in any one of the related disciplines would be an attractive funding opportunity for the pharmaceutical industry. Even more effective might be the establishment of training programs as part of large center grants focused on IDHR research. Such programs would link training in a flexible way to a critical mass of relevant scientists and might fruitfully be incorporated in a multi-institutional consortium or network. An organizational framework of this sort would recognize that training, like research in this field, can benefit from interinstitutional and even international collaboration.

Consideration should also be given to providing career development support for entry- and mid-level scientists and physicians who are committed to a career involving IDHR research. Once again, the multidisciplinary focus of this field makes such an initiative unlikely to be undertaken by any one or two of the existing NIH institutes. Several mechanisms are possible. One would be targeted individual K awards. Senior Fellowships for established investigators committed to this line of endeavor could be supported by pharmaceutical consortia or multiple-stakeholder agreements that should include the FDA, which has an important interest in advancing this research area. For clinical training in IDHR, investigators should be encouraged to consider application to K08, K23, and K24 training and mentoring programs. Without long-term support of training for IDHR research in academia, it will be difficult if not impossible to nurture the growth of research in this area.

Organizational strategies: Current difficulties and recommendations

Two key concerns discussed under Clinical Issues—improvements on classification and database systems—require the cooperation of many individuals from different organizations and countries. Although this monumental task will take many years to complete, some type of organizational structure and funding needs to be put into place

to initiate discussions. Organizations such as the World Health Organization or the International Life Sciences Institute (ILSI) could assist in coordinating these meetings.

Organizations that implement cooperation between industry, academia, and government are also critically needed to conduct basic preclinical and clinical research. During preclinical and clinical stages of drug development, pharmaceutical companies identify compounds that appear to produce IDHRs. For those compounds for which development has been stopped, perhaps the compound, patient samples, and background data could be shared with colleagues in academia who might investigate mechanisms of action. Given the limited number of compounds that are able to produce IDHRs in experimental animals when given by the clinical route of exposure (without adjuvant), these models are very valuable. Some issues, such as confidentiality and the rights to information or technology developed, will be difficult to resolve, but these types of collaborations are critical to progress in IDHR research.

We also strongly recommend the development and fostering of networks of qualified investigators who can work together to conduct valuable studies of IDHRs of low frequency, individuals and families with tendencies for multiple drug hypersensitivity, and studies of drug reactions in high-risk subpopulations (eg, those with AIDs, connective tissue disease, or viral infections).

PRIORITIES AND CONCLUSIONS

Although there are multiple barriers that must be overcome to advance understanding of IDHR, the public health benefits of understanding this work and successful strategies for prevention and management of IDHR are clear. The following general points summarize the top priorities.

Priorities for research

- Identify key host risk factors (metabolism, infection, genetic background) and drug characteristics (metabolites, reactive groups) in hypersensitivity reactions. Emphasize research efforts to better understand the genetic basis for individual differences. Utilize emerging technologies (gene chip technology) and information (Human Genome Project, SNP databases, Pharmacogenetics Knowledge Base) to address these issues.
- Develop better diagnostic methods to discriminate between immune and nonimmune reactions and to identify culprit drugs producing a reaction
- Determine the mechanism of hapten processing, presentation, and recognition in systemic hypersensitivity responses
- Develop mechanism-based in vitro screening methods and animal models for hypersensitivity reactions

Priorities for organizational and support mechanisms

- Work with international organizations and profession-

al societies to develop a more uniform terminology and classification system.

- Develop a worldwide or regional tracking and database system for IDHR
- Develop a tissue or serum bank of samples from patients and animals with documented hypersensitivity reactions
- Develop networks of investigators to conduct studies on IDHR patients
- Set up a consortium for industry, academia, and government to share information and compounds to better understand drug hypersensitivity reactions discovered in preclinical testing
- Define criteria for the development and validation of in vitro models and animal models for preclinical hypersensitivity testing
- Define and develop new funding strategies for IDHR research

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