

Current reviews of allergy and clinical immunology

(Supported by a grant from Glaxo Wellcome, Inc, Research Triangle Park, NC)

Series editor: Harold S. Nelson, MD

Drug metabolism, danger signals, and drug-induced hypersensitivity

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One of the most difficult challenges for the practicing allergist/immunologist today is that of evaluating and managing patients who present with histories of drug-induced reactions. Adverse drug reactions are heterogeneous, and a single drug can often cause a multitude of reactions. Because the mechanisms responsible for many of these reactions are not known, they can be, and often are, difficult to classify. Moreover, for those that have features consistent with immune-mediated mechanisms, our diagnostic tools remain limited, because little is known about the relevant immunogenic determinants of most drugs. Despite these challenges, management approaches must be devised for patients who present with histories of drug-induced disease. Simply telling such a patient to avoid all drugs that have been associated with previous adverse events leaves both the patient and the referring physician frustrated. The initial part of this review focuses on exciting current research that is furthering our understanding of the mechanisms responsible for drug-induced reactions. Because it will take time to translate this new information into clinical practice, the latter part of the review focuses on ways to evaluate and manage patients who present with drug-induced reactions using the tools and the knowledge that are currently available. (*J Allergy Clin Immunol* 2001;108:475-88.)

Key words: Adverse drug reactions, allergic drug reactions, drug determinants, drug metabolism, danger hypothesis

Adverse drug reactions continue to be a major clinical problem. They account for approximately 5% of all hospital admissions, and they occur in approximately 10% to 20% of hospitalized patients.¹⁻⁵ Over 80% of these reactions are common, predictable, and related to the pharmacologic actions of the drugs. Reactions of this type have been termed *type A reactions*; they are typically dose-dependent and are readily reversed when the drugs are discontinued.

In contrast to type A reactions, type B reactions are neither predictable nor common. Thus, because type B reactions are idiosyncratic in nature, they usually do not

Abbreviations used

APC: Antigen-presenting cell
SMX: Sulfamethoxazole
S-NOH: SMX-hydroxylamine

manifest themselves until after a drug is marketed. Although reactions of this type account for a minority of adverse drug reactions, they are extremely important, because they are often serious and can result in death.

Most pharmacologists classify drug-induced hypersensitivity reactions as type B/idiosyncratic reactions that are immunologically mediated. However, even though the time-course and clinical manifestations of many idiosyncratic reactions support the involvement of an immune mechanism, proving its existence can be quite a challenge. Despite the fact that we have a much better understanding of human immune responses in general, we still know little about human immune responses to drugs. There are 2 major stumbling blocks that are hindering progress in this area. First, we have insufficient knowledge of the relevant antigenic/allergenic drug determinants that elicit human immune responses. Second, we have no animal models that would allow us to dissect the nature of these responses.

In this review, I do not belabor the fact that many data gaps exist in this important area of human disease. In contrast, the first part of the review focuses on some exciting new scientific developments that will, it is hoped, set the stage for more rapid progress in this critically needed area. The second part provides management strategies involving use of the diagnostic tools that currently are available.

IgE-binding determinants on β -lactam drugs

Despite the fact that the immunochemistry of penicillin has been known for many years,⁶⁻⁸ until recently, little information existed about either the antigenic structures recognized or the fine specificity of the IgE antibodies produced in β -lactam-allergic people. Over the past several years, Baldo et al⁹⁻¹² have performed much work in this area. Not only have they identified the IgE binding sites on β -lactam drugs; they have also identified IgE binding sites on a variety of other agents as well, includ-

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Received for publication May 8, 2001; revised July 5, 2001; accepted for publication July 6, 2001.

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0091-6749/2001 \$35.00 + 0 1/10/118509

doi:10.1067/mai.2001.118509

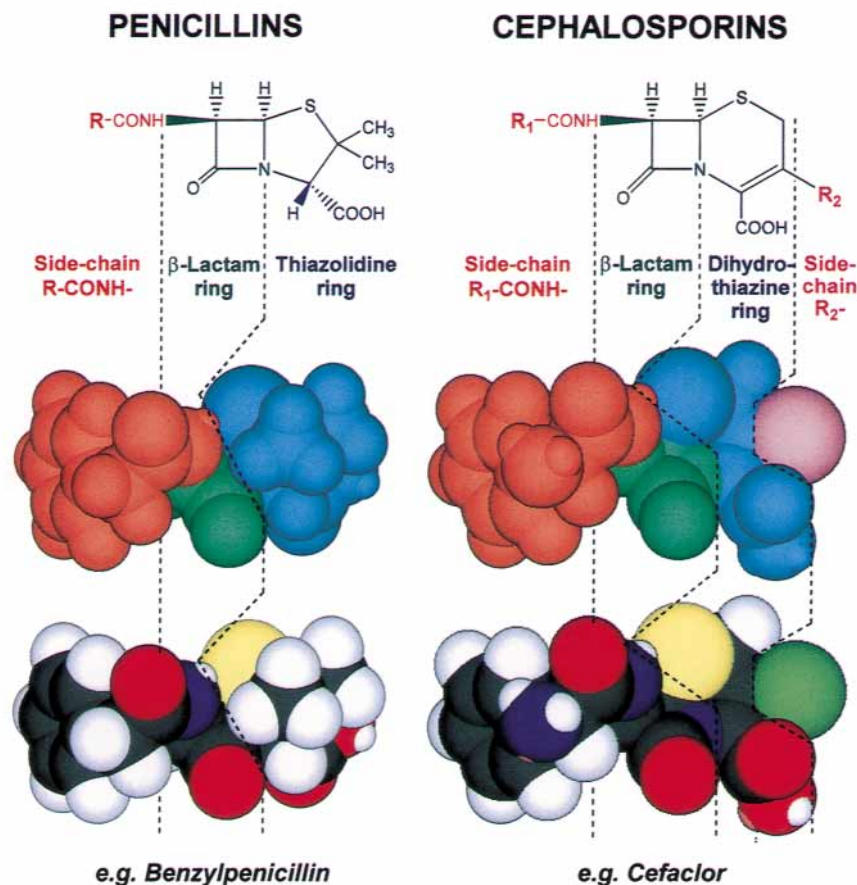


FIG 1. General structures of penicillins and cephalosporins. Structure of backbone and positions of side-chain groups are shown. For benzylpenicillin: R , benzyl. For cefaclor: R_1 , aminobenzyl; R_2 , chlorine. Reproduced with permission from ACI international, vol. 12, 2000, pp. 206-212 © 2000 by Hogrefe & Huber Publishers/Seattle, Toronto, Bern, Göttingen.

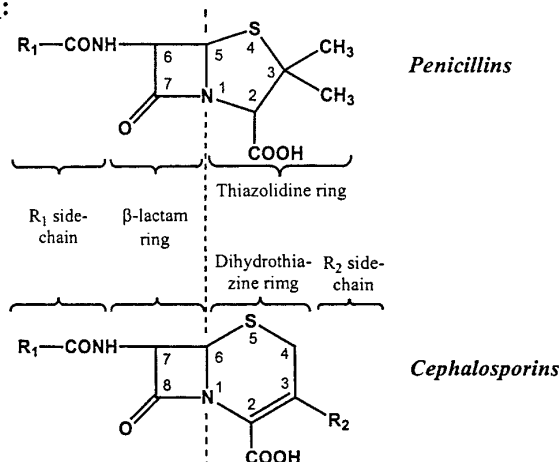
ing alcuronium, tubocurarine, thiopentone, suxamethonium, and sulfamethoxazole (SMX).¹³⁻¹⁹ Both penicillins and cephalosporins possess a β -lactam ring as well as a second sulfur-containing ring structure. For penicillins, this second ring structure is a thiazolidine ring; for cephalosporins, it is a dihydrothiazine ring (Fig 1). Both of these drugs are distinguished from others in their class by the R_1 side chain that is attached to the β -lactam ring by an amide linkage. The cephalosporins have an additional R_2 group at the 3-position of the dihydrothiazine ring that further distinguishes members of this group.

Over the last 30 years, a number of clinically relevant penicillin determinants have been identified.^{6,8,20} Importantly, both penicillins and cephalosporins are reactive substances that by virtue of certain ring modifications and the point of attachment to protein carriers are capable of giving rise to multiple different antigenic determinants. In the case of penicillin, because the β -lactam ring is unstable, it readily opens and acylates lysine residues in proteins forming the penicilloyl determinant.²¹ In light of the fact that approximately 95% of penicillin molecules covalently bind to proteins in this manner, the penicilloyl determinant is termed the "major" penicillin determinant. However, numerous other conjugates are

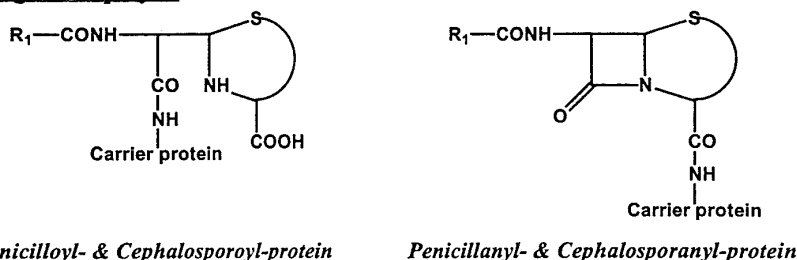
also formed, and though these are classified as "minor" determinants, they too are capable of eliciting the production of IgE antibodies.

Few studies have focused on the identification of the IgE binding sites on β -lactam drugs. Most of what we know about the chemical nature of β -lactam "allergens" has been derived from studies in which hemagglutinating and precipitating antibodies, rather than IgE antibodies, have been measured.^{7,8} Recently, using several structural conjugates, including the penicilloyl and cephalosporoyl "major" determinants as well as the penicillanoyl and cephalosporanoyl "minor" determinants (determinants produced via coupling to carrier proteins through the thiazolidine/dihydrothiazine ring carboxyl; Fig 2), Baldo et al²² found that penicillin-specific IgE antibodies found in some allergic individuals were highly selective in their recognition patterns. Those that recognized the *-oyl* determinant did not recognize the *-anyl* determinant, and vice versa.^{23,24} However, the picture is much more complex than this. Baldo et al also showed that antibodies that react to β -lactam drugs demonstrate a spectrum of specificities. Whereas some are directed to a structure as small as a methylene group in a benzylpenicillin and cephalothin side chain,²⁵ others are directed to a complete side chain

General Structures:



Antigen Employed:



Heterogeneity of Allergenic Determinants:

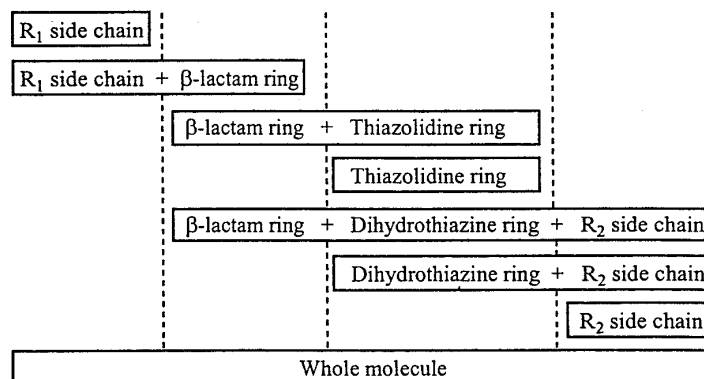


FIG 2. General structures of penicillins, cephalosporins, and the *-oyl* and *-anyl* antigens important in IgE antibody recognition of β-lactam drugs. The broad spectrum of β-lactam allergenic determinants recognized by IgE antibodies is also presented. Reproduced with permission from *Current Opinions in Allergy and Clinical Immunology*.²²

or an entire penicillin or cephalosporin molecule.^{9,10} Thus, as shown in Fig 2, drug-specific IgE antibodies, at least in the case of the β-lactam drugs, are heterogeneous, recognizing a multitude of antigenic determinants.

The fact that IgE antibodies to β-lactam drugs are heterogeneous and side chain-specific antibodies have been found suggests that antibodies other than those directed to the β-lactam ring might also be important in β-lactam-induced allergic disease. The importance of these antibodies was recently demonstrated by Baldo et al²⁶ in a

study in which the specificity of the IgE antibodies produced by patients who had demonstrated reactions to flucloxacillin was evaluated. Quantitative hapten inhibition studies revealed that only dicloxacillin, cloxacillin, and oxacillin—penicillins that have an R group similar to that in flucloxacillin—demonstrated potent inhibition of IgE binding. Penicillins that did not possess a methyl-phenylisoxazolyl side chain determinant were poor inhibitors (Fig 3). These results, along with those of others,²⁷⁻²⁹ demonstrate that at least for some β-lactam allergic individuals,

R group recognized R group not recognized

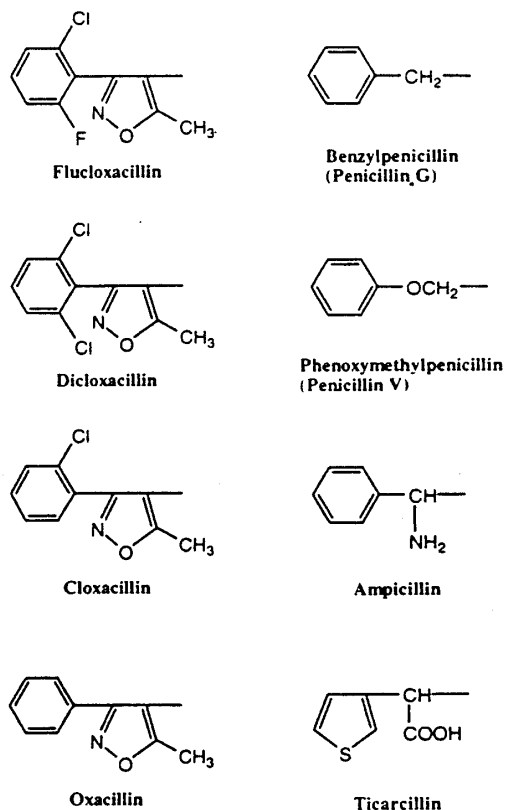


FIG 3. Recognition of the methyl-phenyl-isoxazolyl side-chain determinant on various penicillins by IgE antibodies in the sera of subjects who reacted to flucloxacillin. Reprinted from: Baldo BA. Penicillins and cephalosporins as allergens—structural aspects of recognition and cross-reactions. *Clin Exp Allergy* 1999;29:744-9. Copyright 1999, with permission from Blackwell Science, Inc.

IgE antibodies might be directed to the R group of the β -lactam drug and not to the determinants formed by the β -lactam ring.

Because β -lactam-induced IgE responses are heterogeneous, no single in vivo or in vitro test will be useful in the clinical evaluation of β -lactam-allergic patients. It is clear that a panel of diagnostic reagents will be needed. However, before this panel can be developed, the relevant allergenic determinants of each of the β -lactam drugs will have to be determined, and this will be no easy feat.

Drug metabolism, cellular cytotoxicity, and immunogen formation

Most drugs are not immunogenic in their native state. Many are of low molecular weight (<1000 Da) and must, to become effective immunogens, be covalently bound to high-molecular-weight carrier proteins. The fact that certain low-molecular-weight drugs do elicit allergic reactions indicates that drug-protein complexes are formed in vivo and that successful antigen processing and presentation occurs.³⁰

Because most drugs that cause idiosyncratic reactions are chemically inert, the prevailing thought is that these agents must undergo metabolism or bioactivation to chemically reactive forms before an immune response can be initiated. However, in addition to binding to carrier proteins and eliciting an immune response (the hapten hypothesis³¹⁻³³), these reactive forms might also be directly toxic (eg, acetaminophen toxicity). The most important enzymes involved in bioactivation are those that constitute the cytochrome P450 enzyme system.^{34,35} However, other enzymes, such as those that generate reactive oxygen species, might also be very important with respect to immunotoxicologic reactions.^{36,37} To protect the organism, bioactivation or phase I reactions are typically followed promptly by phase II detoxification processes, such as sulfation, glucuronidation, and acetylation. However, if bioactivation exceeds bioinactivation, reactive metabolites might "escape" and cause direct cellular necrosis or hypersensitivity responses through the formation of immunogenic drug-protein complexes.³⁸

Why only certain individuals develop drug-induced hypersensitivity reactions is not clear. However, it appears that both drug-related and host-related factors are important. One important drug-related factor (others are reviewed by deShazo and Kemp³⁹ and Adkinson⁴⁰) is the type of functional group that a drug possesses. Penicillins and cephalosporins each have a β -lactam ring structure that because of its strained state readily reacts with nucleophilic groups on carrier proteins.²¹ In contrast, as described previously, most other drugs must undergo metabolism before haptentation can occur. Accordingly, it would seem that pharmaceutical companies should avoid developing therapeutic agents containing functional groups that promote the development of metabolic intermediates. Unfortunately, avoidance of some of these structures is not always possible. There are numerous drugs that contain aromatic amines, and oxidative metabolism of these agents leads to the production of hydroxylamines and nitrosoamines that are unstable, toxic, and, through covalent binding with carrier proteins, potentially immunogenic. Drugs possessing this functional group include the sulfonamides, procainamide, and the aromatic anticonvulsants; each of these has been associated with idiosyncratic reactions thought to be elicited through oxidative metabolite formation.^{33,38,41,42}

There are at least 2 important host-related factors in the development of hypersensitivity reactions: (1) the drug-metabolizing capability of the individual and his or her ability to form reactive intermediates and (2) the elicitation of an immunologic response and the subsequent development of clinical pathology once drug-carrier complexes have been formed. Genetic polymorphisms exist in drug-metabolizing enzymes, and some of these can be associated with the development of idiosyncratic reactions.⁴³ Although some individuals have been shown to have an increased ability to form metabolically active intermediates (increased phase I metabolism), others have a decreased ability to detoxify reactive species once they have formed (decreased phase II metabolism).^{44,45}

In addition to differences in drug metabolism, it is likely that individuals differ in their ability to form immunogenic complexes and their ability to mount an immune response to these complexes once they have formed. Gruchalla et al⁴⁶ demonstrated that patients with AIDS who were taking trimethoprim/SMX for *Pneumocystis carinii* pneumonia prophylaxis differed in their ability to form sulfonamide-serum protein conjugates. Moreover, the presence of these conjugates was in some instances associated with drug-induced hypersensitivity reactions; in other instances, it was not. Thus it appears that the ability to form drug-carrier complexes might be necessary but not sufficient for the development of drug-induced hypersensitivity responses.⁴⁷

Certain tissues are known targets of drug-induced hypersensitivity responses, and it is still not entirely clear why this is the case. Park et al^{35,37,48} have stressed that drug antigen "disposition" is a critical factor in determining the type of immune response that is generated and the particular tissue that is affected in drug-induced reactions. Thus, given that the liver is the major drug-metabolizing organ, it would seem to be the most likely target for these reactions. However, because of the liver's great detoxifying ability, drug-induced hepatic reactions, though they do occur, are relatively rare. In contrast, many drug-induced reactions involve the skin, and because the skin is so frequently involved, an important question is whether there is sufficient drug metabolism occurring in the skin to elicit these reactions. Initial studies demonstrated that though the skin does possess drug-metabolizing enzymes, the concentration of these enzymes is quite low^{49,50}—probably too low for the generation of adequate numbers of clinically significant reactive drug intermediates. In contrast to the findings in these early studies, Reilly et al⁵¹ recently demonstrated that normal human epidermal keratinocytes are capable of bioactivating sulfonamides to reactive hydroxylamines. The authors conceded that their findings do not prove that sufficient hydroxylamine levels are generated in the skin to provoke drug-induced reactions; nevertheless, given the large numbers of keratinocytes in the skin, the unknown dose-response relationship that leads to the development of hypersensitivity reactions, and the possibility that bioactivation might be inducible under a range of conditions (including infection), keratinocyte-mediated hydroxylamine formation within the skin might have important implications for drug-induced toxicologic and/or immunologic reactions.⁵¹

After demonstrating that drug metabolites can be generated by keratinocytes, Reilly et al⁵¹ evaluated the ability of these metabolites to induce keratinocyte cell death. Interestingly, they found that though human keratinocytes demonstrated concentration-dependent cytotoxicity when incubated with dapsone hydroxylamines, the cells were resistant to the cytotoxic effects of SMX hydroxylamines (S-NOHs). However, when glutathione was depleted, cell death by S-NOHs was potentiated. Moreover, prior depletion of glutathione caused normal human keratinocytes to become susceptible to S-NOH-induced toxicity at concentrations that were previ-

ously not cytotoxic. These findings together suggest that in the skin, glutathione plays an important role in preventing keratinocyte toxicity by SMX metabolites.

The same investigators also found that in addition to inducing keratinocyte death, hydroxylamine metabolites form covalent adducts with keratinocyte proteins, and this covalent association occurs at relatively low drug concentrations.⁵¹ Moreover, because covalent binding occurred in the absence of keratinocyte toxicity, it appears that the cytotoxic effects of S-NOHs are unrelated to their ability to covalently associate with cellular proteins.

In summary, many haptenic drugs that are incapable of directly binding to proteins undergo metabolism to reactive intermediates. Once formed, these reactive metabolites might cause cellular cytotoxicity and/or covalently link to cellular proteins. With the generation of drug-protein adducts, potential immunogens are formed that might lead to the elicitation of an immune response.

Processing and presentation of drug antigens

In theory, drug-protein conjugates can both induce an immune response and elicit effector responses after there has been prior immune recognition or sensitization. Dendritic cells play an important role in the antigen recognition process as specialized antigen-presenting cells (APCs). They initiate T-cell responses, including cytotoxic responses, to both virally infected and malignant cells,^{52,53} and they might also play a role in the induction and elicitation of T-cell responses to drug-hapten-modified cells as well.³⁷ In experimental models of contact hypersensitivity, tissue damage in the skin results from haptenation of dermal proteins, transfer of antigen to regional lymph nodes for presentation to CD4⁺ and CD8⁺ T cells, and the subsequent return of primed hapten-specific CD8⁺ T cells to the dermal/epidermal junction, where cytotoxicity can occur by either the Fas/FasL pathway or the perforin pathway.⁵⁴

During the last decade, a large amount of research has focused on dissecting the immunologic mechanisms that are responsible not only for drug-induced contact hypersensitivity reactions but also for hypersensitivity reactions elicited by drugs administered by the oral and parenteral routes. It appears that like haptenic contact allergens, other drug haptens are capable of being processed by APCs and presented to antigen-specific T lymphocytes. If presentation occurs in the context of the appropriate activation signals, an immune-initiated inflammatory cascade ensues that leads to the development of clinically relevant drug-induced disease.

Whereas most of our knowledge of the mechanisms of drug-induced hypersensitivity responses has accumulated over the last several years, the initial discovery that drugs might induce drug-specific T-cell activation was made by deWeck et al⁵⁵ in the 1970s. This group demonstrated that patients who had penicillin-induced hypersensitivity reactions, but not normal controls, possessed peripheral blood lymphocytes that proliferated in an antigen-specific manner when cultured with penicillin. More

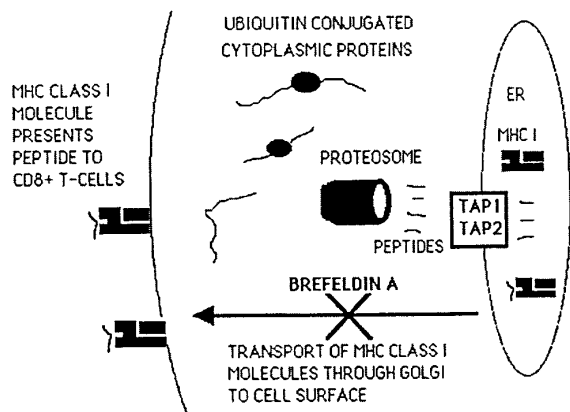


FIG 4. Processing and presentation of endogenous antigens.

recent studies have extended these findings and have shown that numerous therapeutic agents have the capability of eliciting drug-specific T-cell responses.⁵⁶⁻⁵⁹

The type of T-cell response that is generated to haptenic agents is dependent on the pathway by which the hapten is processed and presented to T cells, and the particular pathway involved is dictated by the chemical properties of the hapten. Small lipid-soluble molecules (eg, urushiol) can enter the cytoplasm and be presented on MHC class I molecules for recognition by CD8⁺ cells via the "endogenous" pathway (Fig 4).^{30,60} In contrast, polar haptens such as nickel and cobalt are more likely to be presented on MHC class II molecules for recognition by CD4⁺ cells^{61,62} (exogenous pathway; Fig 5). Some haptens, such as those that are both chemically reactive and lipid soluble (eg, dinitrofluorobenzene), might be processed by both endogenous and exogenous pathways for presentation to both CD8⁺ and CD4⁺ T cells.³⁰ The situation is probably much more complex than this, however. As more data emerge, it appears that though one pathway might be the preferred antigen-presenting pathway, drug haptens might undergo antigen processing by both pathways.^{63,64} Moreover, alternative pathways in which there is an absence of drug metabolism or processing might be occurring as well.⁶⁵⁻⁶⁷

In 1993, Hertl et al⁶⁸ found that T-cell clones derived from the peripheral blood of β -lactam-allergic patients with maculopapular exanthems were CD3⁺, CD4⁻, CD8⁺, and HLA-DR⁺ and produced IL-2 and IFN- γ on stimulation. In addition, immunohistologic studies of β -lactam-induced vesiculobullous exanthems revealed that CD8⁺ T lymphocytes were the predominant T-cell subset in these lesions. Epidermal T-cell clones derived from these lesions proliferated in response to penicillin-pulsed autologous APCs but not allogeneic APCs, indicating that these clones were both antigen-specific and MHC-restricted. Moreover, they were cytotoxic for human epidermal cells.⁶⁹ These studies were some of the first to demonstrate that in human beings, therapeutic drug haptens can be processed and presented to CD8⁺ T lymphocytes and that antigen-specific T cell-cytotoxic responses might be responsible for the clinical pathology demonstrated in drug-induced vesiculobullous exanthema.

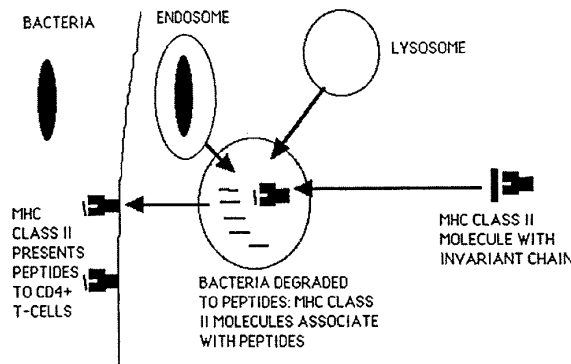


FIG 5. Processing and presentation of exogenous antigens.

Interestingly, it was found in subsequent studies that like penicillin-specific B-cell responses,^{11,22,23} penicillin-specific T-cell responses are quite heterogeneous as well. Padovan et al⁵⁸ and Mauri-Hellweg et al⁷⁰ found that T cells from penicillin-allergic patients are of 2 types. One group of patients that they studied had T cells that proliferated only in the presence of the drug that elicited the reaction, whereas T cells from the other group of patients proliferated both to penicillin and to related penicillins. Thus, because T cells from the first group of patients could not proliferate to other β -lactam antibiotics even if the side chain was identical to that of the stimulating drug, the recognition structure for these T cells appeared to be composed of both the penicilloyl determinant and a segment of the side chain. In contrast, for the second group of patients, there was a broader recognition profile; because T cells from these individuals were stimulated both by penicillin G and by related penicillins, the penicilloyl moiety, which is common to all of these agents, appeared to be the predominant T-cell recognition structure. Unfortunately, in light of the finding that both penicillin-specific T cells and B cells from allergic individuals have a heterogeneous antigen recognition profile, determining what other β -lactam drugs will be tolerated by penicillin-allergic patients will continue to be a substantial challenge.

Unlike penicillin, sulfonamides must undergo metabolic transformation into chemically reactive metabolites before a drug-induced response is elicited. Although oxidation by the cytochrome P450 system or by macrophage myeloperoxidase⁷¹ can lead to the production of oxidative products, in usual circumstances these products are promptly detoxified by glutathione transferase or acetylation.⁷²⁻⁷⁴ The fact that certain drugs, such as the sulfonamides, must undergo metabolic activation suggests that they might act as intracellular haptens and thus might be processed by the endogenous pathway for presentation to CD8⁺ T cells.³⁰

Sulfonamides can cause a variety of adverse drug reactions, including fever, hepatitis, nephritis, urticaria, anaphylaxis, maculopapular exanthems, and severe skin reactions. In at least some of these reactions, an immune mechanism is presumed to be involved, and more and

more data are supporting this involvement. One of the initial studies that demonstrated the presence of sulfonamide-specific T cells in patients with drug-induced reactions was performed by Kalish et al.⁷⁵ Using limiting dilution analysis, they showed that SMX-specific lymphocytes are present in the peripheral blood of allergic individuals at a frequency similar to that observed for urushiol-specific lymphocytes in patients with poison ivy-induced eruptions.⁶⁰ Since this original observation, several groups have identified sulfonamide-specific CD4⁺ and CD8⁺ T cells in drug-allergic patients.^{57,76} Immunohistologic studies have shown that CD8⁺ T cells are the predominant cell type in SMX-induced bullous exanthems⁷⁷; in addition, it has been shown that T cells can mediate cytotoxicity against keratinocytes in SMX-induced skin lesions.⁷⁸ More recent immunohistochemical studies have shown that a large percentage of T cells found in various drug-induced cutaneous eruptions express perforin and granzyme B. These findings further support the role of cytotoxic T cells, not only in sulfonamide-induced eruptions but also in other drug-induced cutaneous reactions.⁷⁹

Despite the fact that haptenation of cell surface and serum proteins occurs at therapeutic drug concentrations,^{46,48,80} extensive haptenation is most likely prevented by the presence of plasma thiols and “intraorgan” glutathione.^{37,48} If, however, “bioactivation” exceeds “bioinactivation,” extensive cell surface haptenation might occur and might lead to the development of an immune response. Perhaps a more likely situation is that immunogens are being formed that are recognized by specific T lymphocytes, but because there are no additional signals, T-cell stimulation does not occur and the inflammatory cascade does not ensue. This latter scenario might be the “rule” rather than the “exception,” inasmuch as even though drug-protein conjugates appear to be generated under normal circumstances, drug-induced hypersensitivity reactions are in most instances not elicited.

The danger hypothesis and its relation to drug-induced hypersensitivity

It is thought that one of the major functions of the adaptive immune system is to distinguish “self” from “non-self.” If the immune system encounters self proteins, tolerance results, whereas encounter with nonself, or foreign, proteins leads to an immune response. However, it is now clear that the adaptive immune response does not work in isolation. The innate immune system appears to play a critical role in determining whether an immune response is generated through its regulation of costimulatory molecules that are necessary for T-cell and B-cell responses.⁸¹⁻⁸⁴ Thus, the classic self/nonself model of immune reactivity has been expanded to include the important role of the innate immune system and costimulatory molecules.^{81,82}

More recently, Matzinger^{83,84} has proposed an alternative explanation, which she terms the *Danger model*, of how immune responses are generated. She writes:

Self-nonspecific models assume that the “foreignness” of a particular entity is what triggers both the innate and adaptive response, whereas the Danger model assumes that what really matters, from an evolutionary point of view, is whether the entity causes damage or not. If it does not, and the cells in its environment are healthy or if they die normal quiet deaths and are scavenged, no immune response ensues. Only if a cell dies messily, or becomes stressed or damaged, is an immune response initiated.⁸⁴

With the advent of the Danger model, the hypotheses that have been generated to explain the events responsible for various immunopathologic reactions, such as transplant rejection and autoimmune responses, are being reconsidered.⁸⁴ Even drug-induced hypersensitivity responses are being reevaluated in the context of this new model.^{37,51,85,86} A basic tenet of the Danger model is that the immune response is controlled by endogenous signals rather than exogenous signals and that alarm signals are generated by “stressed” cells or those undergoing injury (eg, “bad death”⁸⁴). These signals are perceived as danger signals by resident APCs (Kupffer cells in the liver, Langerhans cells in the skin) and result in antigen uptake and upregulation of costimulatory molecules on APCs. If T-cell recognition of antigen (signal 1) occurs in conjunction with costimulation (signal 2), T-cell activation results.³⁷ If, however, cells undergo normal cellular death, danger is not perceived, signal 2 is not provided, and tolerance results, according to this hypothesis.

Thus, according to Matzinger, a foreign substance, including a drug, will not evoke an immune response unless it is associated with cell stress or “danger.” When pharmaceutical agents are administered, there are several circumstances whereby danger signals might be elicited. It has already been shown that many drugs, through metabolism, are converted into chemically reactive intermediates. These metabolites could serve 2 functions: (1) to act as haptens and provide signal 1 to antigen-specific T cells, and (2) to provide a costimulatory or danger signal (signal 2) through the activation of signaling pathways that are linked to oxidative stress or protein damage.^{37,86} It is also possible that the costimulatory factor is completely independent of the drug and is instead a host-related factor, such as a viral or bacterial infection. Inflammatory cytokines that are produced by the innate immune system in response to a pathogen might provide a danger signal that directly⁸⁷ or indirectly activates antigen-specific T cells,⁸⁸ and if the antigen is a drug, this mechanism might account for how drug-induced hypersensitivity responses are generated.

Whether the Danger model is correct is not known. Even if it is proved to be true, additional data will be required to determine whether it applies to drug-induced reactions. Despite the paucity of data at the current time, several groups working in this area are beginning to attempt to explain their findings in the context of the Danger model.^{37,51,85,86} Most recently, Reilly et al⁵¹ devel-

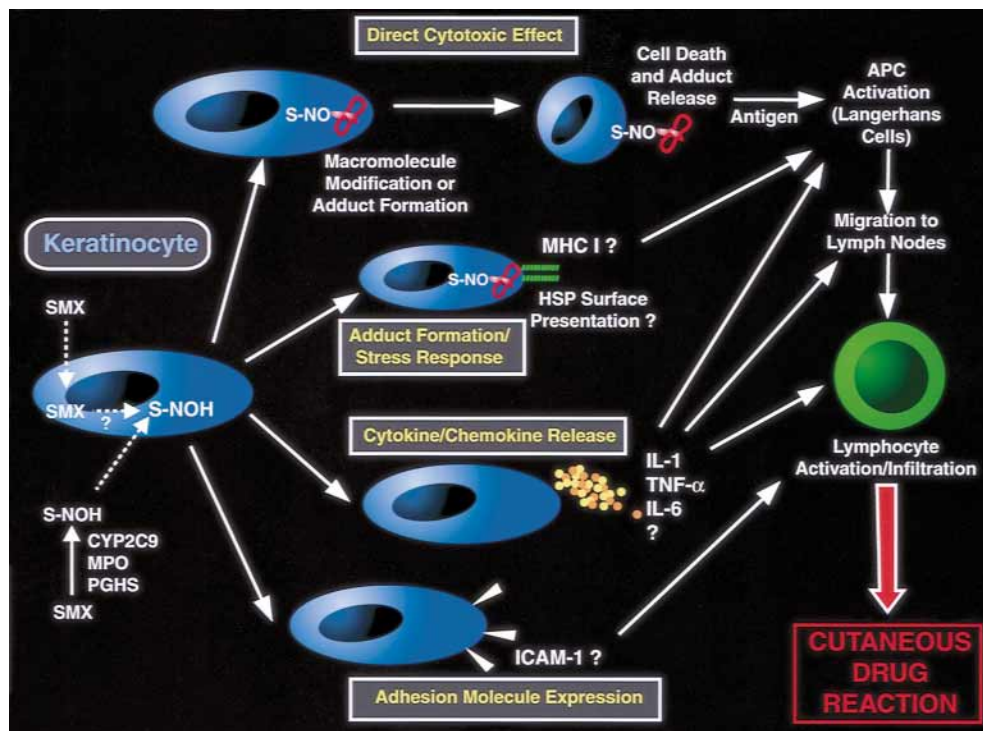


FIG 6. Proposed mechanism of sulfonamide-induced cutaneous drug reactions. APC, Antigen-presenting cell(s); CYP2C9, cytochrome P450 2C9; HSP, heat shock protein(s); ICAM-1, intercellular adhesion molecule 1; MPO, myeloperoxidase; PGHS, prostaglandin H synthase. Reprinted from: Reilly T, Lash L, Doll M, Hein D, Woster P, Svensson C. A role for bioactivation and covalent binding within epidermal keratinocytes in sulfonamide-induced cutaneous drug reactions. *J Invest Dermatol* 2000;114:1164-73. Copyright 2000, with permission from Blackwell Science, Inc.

oped a working hypothesis for the mechanisms of cutaneous drug reactions to sulfonamides that incorporates features of the Danger model (Fig 6). Their hypothesis suggests that reactive drug metabolites that are generated both locally (in the skin) and systemically (possibly in the liver) might do one or more of several things: (1) they might be directly cytotoxic; (2) they might stimulate a "stress" signal that manifests as the expression of cell-surface activation molecules and/or the release of cytokines; (3) they might stimulate the expression of adhesion molecules that promote lymphocyte infiltration and activation.⁵¹ Thus, in the presence of cell "stress," "danger signals" are generated, signal 2 is provided, and lymphocyte activation and target cell damage occur, whereas in the absence of this stress, no signal 2 is provided and tolerance of the drug results (Fig 7). Although this is an intriguing hypothesis, only through further research will it be determined whether it is correct.

Cytokine responses and clinical manifestations of drug-induced disease

Some drugs elicit a characteristic immunologic response, whereas others produce more heterogeneous reactions. Thus, for some drugs, a humoral response is favored; for others, a cellular response is more likely. As noted previously, the way in which antigen is presented

clearly influences the immune response. In addition, because cytokines are known to be important orchestrators of the immune response through their actions on several cell types, including APCs and antigen-specific T cells, it is not surprising that recent data suggest that they play a critical role in determining the type of immune response to drug antigens that is generated. In the case of sensitizing chemicals, it is known that IL-1 β , TNF- α , and GM-CSF all regulate the activation, maturation, and migration of epidermal Langerhans cells.⁸⁹ Moreover, cytokines from both APCs and helper T cells are critical to the activation, proliferation, and differentiation of drug-hapten-specific T lymphocytes.³⁷

The type of drug administered influences the cytokine profile elicited. In the mouse, dinitrofluorobenzene and oxazolone elicit a strong IFN- γ response, whereas topical administration of trimellitic anhydride and diisocyanates elicits a T_H2 response characterized by IL-4 and IL-10 production.^{90,91} These particular chemicals are known to react directly with proteins, unlike most drugs that are administered to human beings. However, at this time, neither the nature of their target proteins nor the signaling pathways that dictate the influence of the chemical on cytokine production are known.³⁷ Similar to what has been demonstrated in mice, drug-specific T-cell clones from patients who have had presumed immune-mediated drug-induced adverse reactions have been shown to have

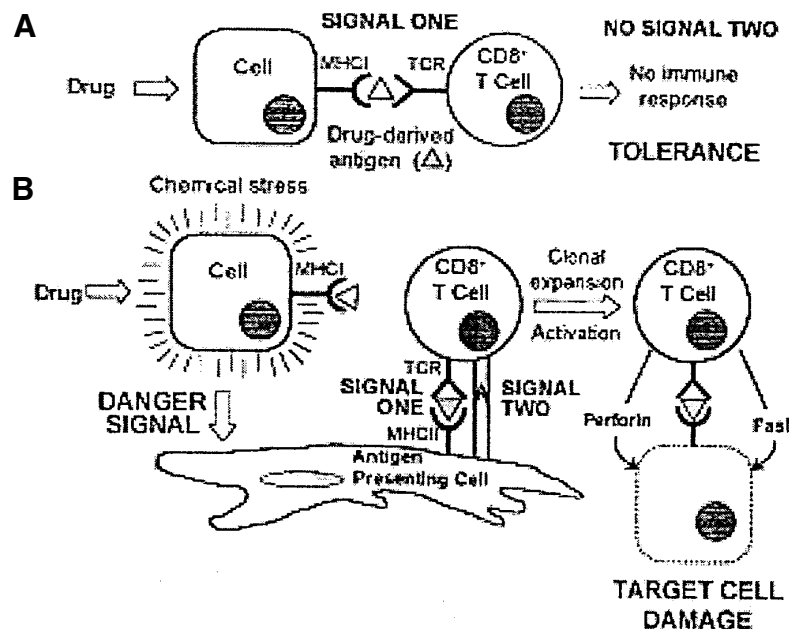


FIG 7. Application of Matzinger's Danger model to drug hypersensitivity. **A**, Development of tolerance when antigen is presented to T cells in the absence of signal 2. **B**, "Danger signal" from a "stressed" cell is detected by the APC; as a result, signals 1 and 2 are passed to the T cell, which becomes activated. Reprinted from *Toxicology*, vol 153, Park B, Kitteringham N, Powell H, Pirmohamed M. Advances in molecular toxicology—towards understanding idiosyncratic drug toxicity, Pages 39-60, Copyright 2000, with permission from Elsevier Science.

heterogeneous cytokine profiles.⁵⁶⁻⁵⁸ This finding is not surprising, because different drugs, as a result of differences in antigen processing and presentation, most likely lead to the activation of heterogeneous T-cell subsets.

It is also likely that the types of cytokines produced are related to whether a drug-induced reaction is immediate or delayed. To examine this question, Posadas et al⁹² evaluated the pattern of cytokine expression and production in PBMCs both from patients who had immediate drug-induced reactions and from patients who had nonimmediate or delayed reactions. Using RT-PCR and ELISA methods, they found that PBMCs from patients who had immediate reactions, such as urticaria and anaphylaxis, expressed and produced increased amounts of IL-4 soon after the reaction. In contrast, PBMCs from those patients who had delayed drug-induced skin eruptions showed an overexpression of IL-2, IFN- γ , and TNF- α , with no expression of IL-4. Although the investigators were not able to demonstrate elevated IL-5 production in PBMCs from either of the 2 groups, others have demonstrated *in situ* expression of IL-5 in drug-induced nonimmediate maculopapular and eczematous skin lesions.^{93,94}

Hertl et al^{69,95} also demonstrated a similar relationship between cytokine profile pattern and clinical manifestations. They found that T-cell clones from patients with penicillin-induced bullous exanthams produced T_H1-like cytokines whereas those from patients who experienced penicillin-induced urticarial exanthams produced a T_H2-like pattern. In contrast, other researchers have been unable to demonstrate such clear T_H responses in drug-induced reactions. Gaspard et al⁹⁶ found that some

patients with well-documented histories of β -lactam-induced immediate reactions had PBMCs that produced high levels of IFN- γ but little IL-4. Moreover, though Brugnolo et al⁹⁷ found that β -lactam-specific T-cell lines from individuals who had drug-induced immediate reactions had high intracellular levels of IL-4, IL-5, and IL-13, they found this T_H2-skewed cytokine profile in patients with late-onset reactions as well.

Although it is difficult at present to reconcile some of these results, new data are being generated that should help sort out some of the contradictory findings. Recently, Naisbitt et al⁹⁸ demonstrated through use of a rat model that drug metabolism and cell surface binding are necessary for the development of a primary immune response after administration of SMX and its metabolites. This is an exciting development, inasmuch as it might now be possible to further elucidate the cellular and molecular mechanisms responsible for drug-induced immune responses in an *in vivo* animal system. Although admittedly we do not yet have an animal model of drug-induced hypersensitivity, this group has made an extremely important step in the right direction.

Management approaches for patients with drug-induced disease

Because of important research efforts, we are starting to learn much more about the immunopathologic character of drug-induced disease. However, it will still be some time before this information is translated into clinical practice. Until such time, clinicians must be provided with manage-

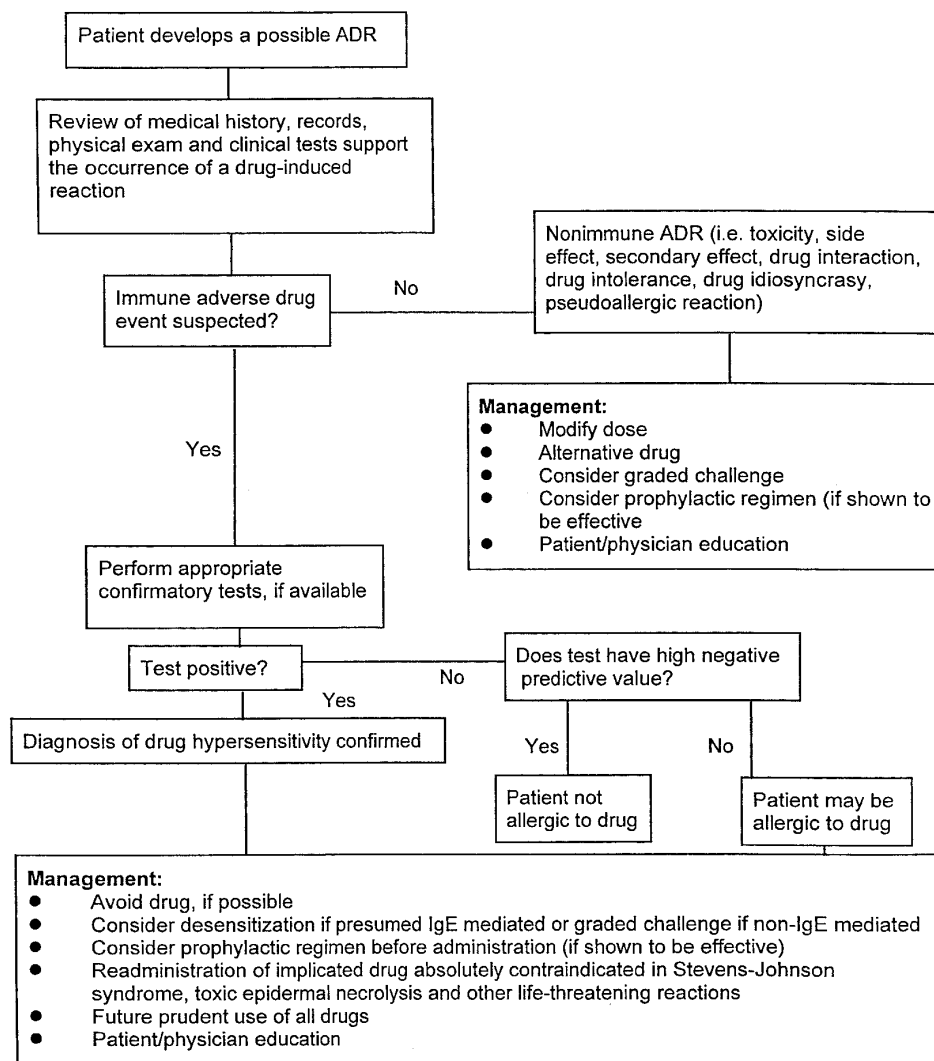


FIG 8. Algorithm for management of adverse drug reactions. Reprinted with permission of *Ann Allergy Asthma Immunol* 1999;83:665-700. Copyright 1999.

ment strategies that can be used today. In addition to several recent reviews on the topic,^{39,40,99} an article entitled "Disease management of drug hypersensitivity: A practice parameter" has been published recently; in it, an algorithm for management of adverse drug reactions is outlined (Fig 8).¹⁰⁰ Clearly, an important first step in the evaluation of patients who present with drug-induced disease is to determine the reaction type. Type A reactions are not immunologically mediated, and for some of these reactions, drug readministration might be considered, especially when the reactions are caused by a drug interaction or by toxicity that is reversible and presumed not to occur at lower doses. Idiosyncratic type B reactions must be approached with more caution, however. For severe reactions, such as coumadin-induced skin necrosis in the presence of protein C deficiency, the drug should not be readministered. For mild to moderate idiosyncratic reactions not thought to be immune-mediated, a provocation challenge, by a specialist trained in this procedure, might be considered.

IgE-mediated reactions

For those reactions that are thought to be immunologically mediated, confirmatory tests should be performed if they are available. Patch testing is used to determine the culprit drug responsible for drug-induced contact allergic reactions, and prick testing might be done to determine the etiology of drug-induced urticaria, angioedema, and anaphylaxis. Although prick testing is useful for the detection of IgE antibodies to large-molecular-weight proteins such as insulin, antilymphocyte globulin, and streptokinase,¹⁰⁰ reliable skin test reagents are not available for determining the presence of IgE antibodies to low-molecular-weight agents other than penicillin. However, despite the fact that valid reagents do not exist, some information might be gleaned by skin testing with the native drug form. Recently, our group determined the nonirritating intradermal skin test concentrations for 16 different antimicrobial agents.¹⁰¹

Drug:	Dapsone
Previous reaction:	Severe hemolysis, glucose-6-phosphate dehydrogenase deficiency determined
Future recommendation:	Avoid drug, severe nonimmunologic idiosyncratic reaction
Drug:	Amoxicillin
Previous reaction:	Morbilliform rash that occurred during a viral exantham
Future recommendation:	Drug may be readministered. Reaction is not uncommon and is not immunologically mediated. The patient is not considered to be at risk for developing a life-threatening reaction to amoxicillin or other penicillins.
Drug:	Intravenous gamma globulin
Previous reaction:	Headache and chills
Future recommendation:	Drug may be readministered at a lower infusion rate after premedication with antihistamines and aspirin. Reaction is not immunologically mediated.
Drug:	Cephalexin
Previous reaction:	Maculopapular rash only. No urticarial lesions or angioedema
Future recommendation:	If drug is required for treatment, it may be readministered via slow graded challenge <u>by a specialist trained in this procedure</u> . Previous reaction not consistent with an IgE-mediated mechanism.
Drug:	Trimethoprim-sulfamethoxazole
Previous reaction:	Urticaria
Future recommendation:	If drug or another sulfonamide is required for treatment, a desensitization procedure may be performed <u>by a specialist trained in this procedure</u> . Previous reaction consistent with an IgE-mediated mechanism.

FIG 9. Drug allergy action plan.

To enable this information to be useful to the practicing allergist, the nonirritating concentration was expressed as a dilution of the full-strength drug. Although a positive wheal and flare response suggests that drug-specific IgE antibodies might be present, a negative response cannot be interpreted to mean that they are absent. However, because both the concentration of the drug and the quantity of material injected are known, the drug amount that generated a negative skin test response can be calculated and used as a starting point for desensitization.

Probably the most common drug-allergy-related clinical dilemma plaguing clinicians today is that of the degree of cross-reactivity between penicillins and cephalosporins. Do all patients who present with histories of penicillin allergy need to undergo penicillin skin testing

before cephalosporin administration? Anne and Reisman,¹⁰² after analyzing available published reports and postmarketing data, found that the incidence of cephalosporin reactions is increased minimally, if at all, in patients with histories of penicillin allergy. In the light of this low incidence, the authors stated that it is safe to administer cephalosporin antibiotics to penicillin-allergic patients. More recently, however, Pumphrey and Davis¹⁰³ examined the incidence of drug-induced fatal anaphylaxis in the United Kingdom between 1992 and 1997; they found that 12 of 33 drug-induced fatal anaphylactic reactions that had been reported to the Medicines Control Agency were attributed to antibiotics. Six of these 12 reactions followed the first dose of a cephalosporin; the patient was known to be allergic to amoxycillin in each of

3 of these cases and to penicillin in 1 case. In the light of these data as well as of other reports suggesting that cross-reactivity between penicillins and cephalosporins is highly variable,^{104,105} it is recommended that patients with penicillin allergy histories undergo penicillin skin testing before a cephalosporin is administered. If the skin test result is positive, desensitization to the cephalosporin should be performed.¹⁰⁰

Non-IgE-mediated reactions

The diagnostic evaluation of non-IgE-mediated reactions is also challenging. Not only are the relevant drug antigens not known; in most cases, the mechanisms responsible for many of the reactions have not been elucidated. Although drug-specific IgG and/or IgM antibodies might play a role in disease pathogenesis (eg, hemolytic anemia), there is often no correlation between the existence of these antibodies and the presence of drug-induced disease. Similarly, T lymphocytes from patients taking a certain drug might proliferate when cultured with that drug. However, though drug-specific T-cell proliferation is a sign of sensitization, it is not necessarily clinically relevant.

As noted above, patch testing is an important diagnostic tool that is used to evaluate drug-induced contact dermatitis. Given that specific T lymphocytes appear to participate in various drug-induced cutaneous reactions, patch testing might become important in the evaluation of these reactions as well. Romano et al¹⁰⁶ found that most patients who presented with maculopapular eruptions to ampicillin or amoxicillin demonstrated both positive delayed intradermal skin test and patch test responses when tested with the drug responsible for the reaction. A more recent study by this group corroborated the previous findings. In addition, in the subsequent study, all patients who had reported a maculopapular eruption to one of the aminopenicillins but who had negative delayed intradermal skin test or patch test responses tolerated drug challenge with the suspect aminopenicillin.¹⁰⁷ These data suggest that specific T cells might play a role in several different delayed-type drug-induced cutaneous reactions and that patch testing might become useful in the diagnostic evaluation of these reactions.

Until more is learned about the pathogenesis of non-IgE-mediated reactions, our diagnostic tools as well as our management approaches will remain limited. As indicated in the drug allergy practice parameter,¹⁰⁰ if the previous reaction was determined not to be IgE-mediated and it was not life-threatening, readministration may occur via cautious graded challenge. However, as Solensky et al¹⁰⁸ recently pointed out, it is important to realize that if a patient gives a vague drug-reaction history, it cannot be assumed that the previous reaction was not IgE-mediated. Our group reviewed the penicillin allergy literature from 1966 to 1998 and found that of 1063 history-positive/skin test-positive individuals described, 347 (33%) had vague histories of penicillin allergy. Thus these data suggest that if the drug allergy history is not known or is vague, at least in the case of penicillin, skin testing should be performed.

Concluding remarks

Despite the fact that our diagnostic tools for drug allergy remain limited, we still have patients who must be managed. Some of these individuals, especially those who have had more than 1 antibiotic-induced reaction, have been quite traumatized because they have been told (typically by physicians) that they might die if they develop a serious infection. Such a patient often has "Drug allergy" written in more than one place on his or her chart. For this reason, the physician takes the "better safe than sorry" approach¹⁰⁹ and tells the patient that in the light of the multiple drug sensitivities, all of the implicated drugs and all others in their respective classes should be avoided. It is no wonder that these individuals come into the allergist's office feeling that they are doomed should a serious infection arise.

Perhaps a type of drug-allergy "action plan" should be developed for our patients who present with drug reaction histories. If a game plan were provided to the patient and the referring physician, the patient's fears, as well as the fears and frustrations of the physician, might be lessened. Through use of information provided in the drug allergy practice parameter,¹⁰⁰ a sample potential action plan is presented in Fig 9.

Because drug reactions are heterogeneous, it is not always easy to determine the mechanism responsible for their elicitation, and this lack of knowledge makes it difficult to determine whether a particular drug can be administered again. However, as was recently outlined,¹⁰⁰ implicated drugs may be readministered under certain circumstances. If the previous reaction was not life-threatening and it was not consistent with an IgE-mediated process, a cautious graded challenge with the drug or another in its class may be attempted. If the previous reaction was not life-threatening and the reaction was consistent with an IgE-mediated process, or if the history is not known, desensitization may be performed.

Although it takes a great deal of time to gather the relevant historical information that is required to develop treatment recommendations, we must not lose sight of the fact that we are doing our patients a great service. They are not doomed. Treatment options exist.

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