

Delayed Drug Hypersensitivity Reactions

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Immune reactions to small molecular compounds, such as drugs, can cause a variety of diseases involving the skin, liver, kidney, and lungs. In many drug hypersensitivity reactions, drug-specific CD4⁺ and CD8⁺ T cells recognize drugs through their $\alpha\beta$ T-cell receptors in an MHC-dependent way. Drugs stimulate T cells if they act as haptens and bind covalently to peptides or if they have structural features that allow them to interact with certain T-cell receptors directly. Immunohistochemical and functional studies of drug-reactive T cells in patients with distinct forms of exanthema reveal that distinct T-cell functions lead to different clinical phenotypes. In maculopapular exanthema, perforin-positive and granzyme B-positive CD4⁺ T cells kill activated keratinocytes, while a large number of cytotoxic CD8⁺ T cells in the epidermis is associated with formation of vesicles and bullae. Drug-specific T cells

also orchestrate inflammatory skin reactions through the release of various cytokines (for example, interleukin-5, interferon) and chemokines (such as interleukin-8). Activation of T cells with a particular function seems to lead to a specific clinical picture (for example, bullous or pustular exanthema). Taken together, these data allow delayed hypersensitivity reactions (type IV) to be further subclassified into T-cell reactions, which through the release of certain cytokines and chemokines preferentially activate and recruit monocytes (type IVa), eosinophils (type IVb), or neutrophils (type IVd). Moreover, cytotoxic functions by either CD4⁺ or CD8⁺ T cells (type IVc) seem to participate in all type IV reactions.

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Drug-induced adverse reactions are a major health problem (1–3). Most adverse effects, so-called type A reactions, are due to the pharmacologic action of a drug. Idiosyncratic and immune-mediated side effects, which are not predictable, are called type B reactions (4). Drug hypersensitivity reactions (drug allergy) account for about one seventh of adverse reactions and manifest themselves in many diseases, some of which are severe (5, 6). The most common allergic reactions occur in the skin and are observed in about 2% to 3% of hospitalized patients (7–9).

To correlate the clinical symptoms with the underlying immune mechanism, drug hypersensitivity and other immune reactions are frequently classified into 4 categories described by Coombs and Gell (10). Type I reactions are due to IgE mediation and mainly cause urticaria, anaphylaxis, and asthma; type II reactions are based on immunoglobulin-mediated cytotoxic mechanisms, accounting mainly for blood cell dyscrasias; type III reactions are immune complex-mediated (for example, vasculitis); and type IV reactions are mediated by T cells, causing so-called delayed hypersensitivity (Table 1).

This classification system has proven to be helpful in clinical practice and can guide diagnostic decisions. However, the term *delayed hypersensitivity reactions*, originally coined to describe T-cell reactions to tuberculin, became an umbrella term for various T-cell-mediated immune mechanisms leading to clinically distinct diseases. Indeed, T cells have been found to differ in the cytokines they produce, which result in distinct disorders. T-helper 1 T cells activate macrophages by secreting large amounts of interferon drive the production of complement-fixing antibody isotypes, and costimulate proinflammatory responses (tumor necrosis factor- α , interleukin [IL]-12) and CD8⁺ T-cell responses. T-helper 2 T cells secrete the cytokines IL-4 and IL-5 (11), which promote B-cell production of IgE and IgG4, macrophage deactivation, and mast-cell and eosinophil responses. CD8⁺ T cells can produce

similarly polarized patterns of cytokines. Newer immunology textbooks have recognized this heterogeneity of T-cell function and consequently subdivide delayed hypersensitivity reactions into type IVa, type IVb, and type IVc reactions, which correspond to T-helper 1, T-helper 2, and cytotoxic reactions (Table 1) (12).

T cells recognize small peptide antigens but are also involved in immune reactions to small chemicals. Indeed, the original description of cellular immunity is based on immune responses to haptens (13). The role of T cells in contact dermatitis elicited by small chemicals has been extensively documented (14, 15), and animal models have been used to dissect the immunopathogenesis (16, 17). Understanding of allergies to orally or parentally administered drugs has, in contrast, only slowly evolved, since clinical manifestations are extremely heterogeneous and animal models do not exist for most side effects. Nevertheless, the observation of T-cell infiltrates in drug-related allergic reactions that affect the skin, liver, and kidney, as well as drug-specific reactions found in vitro or indicated in the results of skin tests (16–21), strongly suggested T-cell-mediated pathogenesis.

This review presents newer concepts of the role of T cells in drug hypersensitivity, which evolved from the study of drug-specific T cells in various drug-induced hypersensitivity diseases. On the basis of in vitro analysis of drug-specific T-cell clones, novel methods of drug presentation to T cells can be defined, extending the hapten concept (22–24). Moreover, functional analysis of T-cell clones from the peripheral blood as well as from the affected tissue, together with immunohistologic analysis, reveals that distinct types of T-cell reactions can lead to different clinical forms of drug hypersensitivity reactions (25–28).

HOW DO T CELLS RECOGNIZE DRUGS?

T cells recognize the antigen by their antigen receptors, which are heterodimers of 2 chains designated as ei-

Table 1. Relationship of Clinical Symptoms to Drug Reactivity*

Extended Coombs and Cell Classification†	Type of Immune Response‡	Pathologic Characteristics	Clinical Symptoms‡	Covalent and Noncovalent Drug Binding§	Cell Type
Type I	IgE	Mast-cell degranulation	Urticaria, anaphylaxis	Covalent drug binding	B cells/Ig
Type II	IgG and FcR	FcR-dependent cell destruction	Blood cell dyscrasia	Covalent drug binding	B cells/Ig
Type III	IgG and complement or FcR	Immunocomplex deposition	Vasculitis	Covalent drug binding	B cells/Ig
Type IVa	Th 1 (IFN- γ)	Monocyte activation	Eczema	Covalent and noncovalent drug binding	T cells
Type IVb	Th 2 (IL-5 and IL-4)	Eosinophilic inflammation	Maculopapular exanthema, bullous exanthema	Covalent and noncovalent drug binding	T cells
Type IVc	CTL (perforin and granzyme B)	CD4- or CD8-mediated killing of cells (i.e., keratinocyte)	Maculopapular exanthema, eczema, bullous exanthema, pustular exanthema	Covalent and noncovalent drug binding	T cells
Type IVd	T cells (IL-8)	Neutrophil recruitment and activation	Pustular exanthema	Covalent and noncovalent drug binding	T cells

* CTL = cytotoxic T cells; FcR = Fc receptor; IFN = interferon; Ig = immunoglobulin; IL = interleukin; Th = T helper.

† Based on reference 10.

‡ Only the dominant reaction is shown. In maculopapular exanthema, type IVb and IVc reactions can occur together; in pustular exanthema, type IVb, IVc, and IVd can occur together; and in bullous exanthema, type IVc with IVb, IVa, or both can occur together. In most instances, 1 type predominates clinically (type IVc in maculopapular and bullous exanthema, type IVd in pustular exanthema). See text.

§ Covalent binding can elicit both T-cell- and B-cell-mediated immune reactions, while noncovalent presentation may elicit exclusive T-cell reactions. See text.

|| T-cell help for Ig (e.g., IL-4, IL-5, IFN- γ).

ther $\alpha\beta$ T-cell receptors (the majority of T cells) or $\gamma\delta$ T-cell receptors (about 5% of circulating T cells). An enormous variety ($>10^7$) of T-cell receptors can be generated with distinct specificities because of different recombinations of genes related to T-cell receptors and the addition of N-region nucleotide insertions. Each T cell displays thousands of identical T-cell receptors, which bind a bimolecular complex displayed at the surface of another cell called an antigen-presenting cell. This complex consists of a fragment of a protein antigen (peptide) bound in the groove of a major MHC molecule (**Figure 1**). Two classes of MHC molecules present peptides of different origin and stimulate different T cells. Peptides that are derived from proteins synthesized and degraded in the cytosol are presented by MHC class I molecules and activate CD8⁺ T cells. The reactive CD8⁺ T cells secrete cytokines and are able to kill cells displaying foreign peptides derived from cytosolic pathogens, such as viruses. In contrast, MHC class II molecules present peptides derived from proteins degraded in endocytic vesicles. These structures interact with CD4⁺ T cells, which activate other immune effector cells as dictated by their cytokines (for example, macrophages, B cells, and CD8⁺ T cells) (11, 12). CD4⁺ T cells can also be cytotoxic (34).

The recognition of small molecules (such as drugs) by B cells and T cells is usually explained by the hapten concept. Haptens are small molecules (mostly <1000 Da) that are chemically reactive and thus able to undergo stable, covalent binding to a larger protein or peptide (13, 29, 30–33, 35, 36). This modification of a protein or peptide makes it immunogenic (**Figure 1**): Cell-bound or soluble immunoglobulins can recognize it directly, while T cells recognize a hapten–peptide fragment that is generated by intracellular processing of the hapten–protein complex and is presented to T cells by MHC molecules (**Figure 1**).

Penicillin G is a typical hapten that tends to bind covalently to lysine groups within soluble or cell-bound proteins, thereby modifying them and eliciting B-cell and T-cell reactions (36). It is also possible that the hapten may bind directly to the immunogenic peptide presented by the MHC molecule itself or alter the MHC molecule directly. In this case, no processing is required (26, 37–39) (**Figure 1**).

Alternatively, if the drug is not chemically reactive itself, it may represent a prohaptens, which becomes reactive during metabolism (26–28, 34) (**Figure 1**). Sulfamethoxazole has been proposed as a typical example of a prohaptens, since it is not chemically reactive but gains immunogenicity by intracellular metabolism. Cytochrome P450–dependent metabolism can lead to sulfamethoxazole–hydroxylamine, which becomes sulfamethoxazole–nitroso after oxidation, a chemically reactive compound that is able to bind covalently to proteins and peptides (**Figure 1**) (31, 37–40). The finding that keratinocytes might also process sulfamethoxazole to sulfamethoxazole–hydroxylamine supports this concept and may explain the manifestation of drug allergy in the skin (38).

Recently, a third possibility has been considered, namely a pharmacologic interaction of drugs with immune receptors (the “p–i concept”) (**Figure 1**) (41–46). Chemically inert drugs, unable to covalently bind to peptides or proteins, may still activate certain T cells that happen to bear T-cell receptors that can interact with the drug. This model has been expanded by in vitro studies using T-cell clones specific for such drugs as sulfamethoxazole, lidocaine, mepivacaine, celecoxib, lamotrigine, carbamazepine, and p-phenylenediamine (41–43, 47–49). It relies on the following findings: Glutaraldehyde-fixed antigen-presenting cells, unable to process, can still present the drug and stimulate specific T cells (41); inhibited generation of reactive metabolites actually enhances the reactivity of T

cells, suggesting that the inert drug but not the reactive metabolite is recognized (50); the drug is bound in a labile way since it can be washed away from the cell surface, in contrast to covalently bound drugs, which cannot (41, 42); and a drug-reactive T-cell clone reacts to the drug within seconds, before metabolism and processing can take place (42). This stimulation by inert drugs is MHC dependent, implying that for full stimulation of the T cell, the T-cell receptor needs to interact with the drug and the MHC molecule.

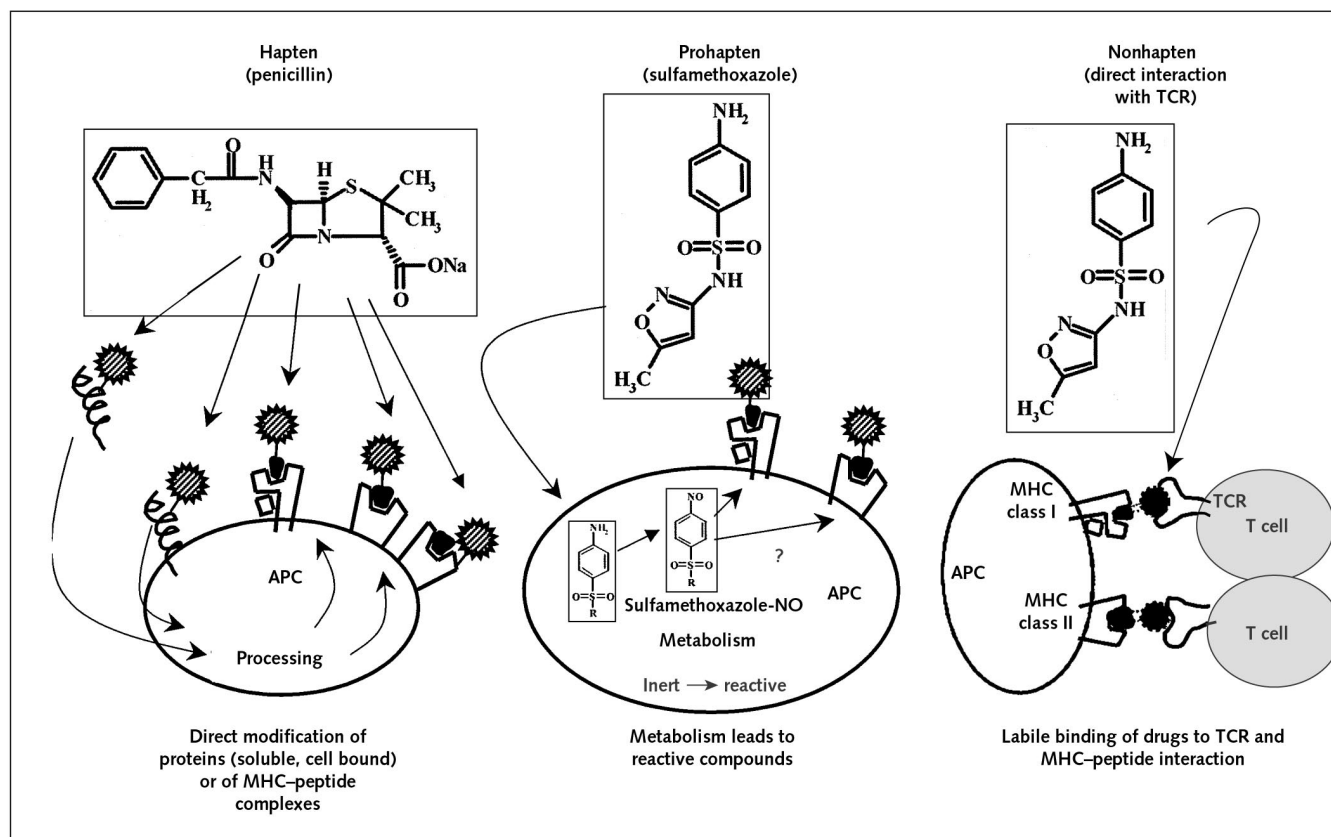
This new concept has a major impact on our understanding of drug hypersensitivity and its distinct clinical manifestations (Figure 1, Table 1). Haptens are primarily immunogenic because of their chemical reactivity. They modify peptides and make them more or newly immunogenic. In contrast, chemical inert drugs are immunogenic only because of their structural features, which enable them to interact with immune receptors (certain T-cell receptors and possibly MHC). These structural features have never

been considered in drug development but may account for a substantial portion of unforeseen side effects (51).

The clinical symptoms elicited by drugs that are immunogenic because of their chemical or structural features may well differ. A hapten-like drug (for example, amoxicillin) is able to alter many different proteins, either soluble or cell-bound, and can even modify different MHC molecules and their embedded peptides directly (Figure 1). These distinct antigenic determinants can stimulate T cells and B cells and elicit more or less all types of immune reactions. Indeed, penicillins are reported to cause different antibody-mediated diseases, such as anaphylaxis or hemolytic anemia, but also various T-cell-mediated reactions, such as maculopapular exanthema, drug-induced hypersensitivity syndrome, acute generalized exanthematous pustulosis, the Stevens–Johnson syndrome, and even toxic epidermal necrolysis.

Whether the labile binding of a drug to proteins is enough to make it immunogenic for B cells is unclear.

Figure 1. The hapten and prohaptent concept and the noncovalent drug presentation to T cells.



Hapten-like drugs (penicillins) can bind covalently to both soluble or cell-bound molecules. They can even bind directly to the immunogenic MHC-peptide complex on antigen-presenting cells (APC), either the embedded peptide or the MHC molecule itself (29). Thus, the chemical reactivity of haptens leads to the formation of many distinct antigenic epitopes, which can elicit both humoral and cellular immune responses. Other drugs are prohaptens, which require metabolism to become haptens (that is, chemically reactive). The metabolism occurs mainly inside cells (for example, from sulfamethoxazole to the chemically reactive form, sulfamethoxazole-nitroso [NO]). Metabolism may lead to modification of cell-bound or soluble proteins by the chemically reactive metabolite (30–33). A third, nonhapten pathway (pharmacologic interaction with immune receptors, also known as the “p-i concept”) does not require covalent association of the drug with the MHC molecule. The chemically inert drug seems to bind directly to the T-cell receptor (TCR). Full T-cell stimulation requires an interaction with the MHC molecule. This type of drug stimulation is restricted to certain drugs that fit into TCRs and results in an exclusive T-cell stimulation (see text) (24).

However, this possibility is rather unlikely for most small drugs. If a drug acquires immunogenicity only by its ability to fit into some of the available T-cell receptors, then the immune reaction might be restricted to an exclusive T-cell response. Consistent with this hypothesis is the observation that certain drugs appear to elicit mainly T-cell reactions, while B-cell reactions seem to be exceedingly rare or are not reported. For example, carbamazepine is known to elicit maculopapular exanthema and drug hypersensitivity syndromes (2 typical T-cell-mediated diseases) but not anaphylaxis. Some drugs, like sulfamethoxazole or p-phenylenediamine, might be immunogenic by both mechanisms (49, 52).

It is still not known why the same drug, such as amoxicillin or sulfamethoxazole, causes a maculopapular exanthema in 1 person and acute generalized exanthematous pustulosis in another. An immunogenetic disposition, such as certain MHC alleles or a polymorphism in the tumor necrosis factor- α promoter, may play a role (53–55). In addition, the genetic polymorphism of metabolizing enzymes (for example, of certain cytochrome P450 enzymes or of *N*-acetyltransferase) may contribute to the generation of chemically reactive or toxic compounds, which cause hypersensitivity (53).

CROSS-REACTIVITY OF DRUG-SPECIFIC T CELLS

The great majority of drug-specific T cells express the $\alpha\beta$ T-cell receptor (26–29, 35, 41). Both CD4⁺ and CD8⁺ T cells can be activated with highly heterogeneous functions (26, 27). In some instances, an oligoclonal T-cell reaction to a drug has been observed in vitro (56). However, most drug-specific T cells have a heterogeneous T-cell receptor repertoire (45, 56). This implies that many different T-cell receptors can interact with the same drug. The ability of these T-cell receptors to recognize structurally related compounds differs substantially (45, 56–59). In the sulfamethoxazole model, the complete sulfanilamide core structure is always required to elicit cross-reactivity to other anti-infectious drugs. Thus, the presence of a sulfonamide (SO₂-NH₂) structure, as found in furosemide or celecoxib, is never sufficient to stimulate T-cell clones originally stimulated by sulfamethoxazole; conversely, celecoxib-stimulated T cells do not recognize sulfamethoxazole (45, 54). On the other hand, if the sulfanilamide core structure is present, certain T-cell clones tolerate large alterations of the side chain of related antibiotics, while other T cells react exclusively with sulfamethoxazole itself (45). Similar bindings have been observed with lidocaine, which has no cross-reactivity with ester compounds; however, there is reactivity with bupivacaine and mepivacaine (59). Cross-reactivity of amoxicillin-specific or penicillin G-specific T-cell clones with various cephalosporins in vitro has never been observed, even if the same side chain was present (56). These in vitro data suggest that T cells recognize primarily the core structure and, to a variable degree, the side chain. The cross-reactivity of T cells and immuno-

globulins (IgE) apparently differs, since an exclusive side chain reactivity has been reported for IgE (60).

Alloantigens frequently stimulate drug-specific T-cell clones (57, 58), which suggests that drug-specific T cells also recognize peptide antigens. This drug and peptide specificity might be relevant for drug-induced autoimmunity, since it might be the mechanism by which the development of a drug allergy alters the body's tolerance to some autoantigens (57).

INNATE IMMUNITY AND DRUG HYPERSENSITIVITY

We do not understand how primary sensitization to a drug occurs. Most likely it happens in the lymph nodes and requires a sensitization phase of at least 3 to 4 days, frequently longer. Not only haptens–prohaptens but also chemically inert compounds can elicit a primary immune response, since most drug-specific T-cell clones of sulfamethoxazole-sensitized patients react with the inert parent compound (52).

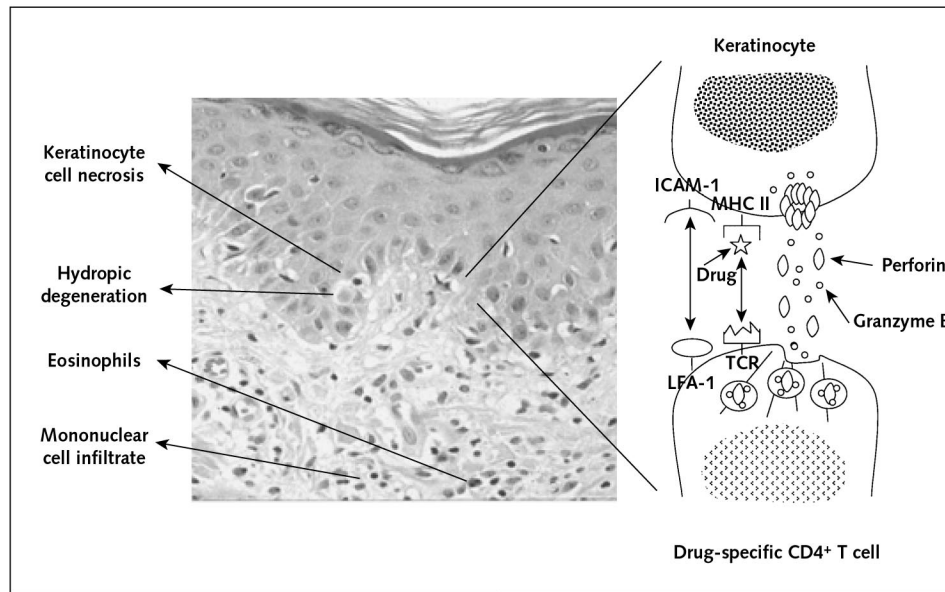
To develop an effective immune response, the innate immune system needs to be activated (61–63). The innate immune system comprises serum proteins and cells, which provide broad but relatively nonspecific host defenses. These defenses lack the properties of antigenic specificity and immunologic memory that characterize acquired immunity. Important cellular components of innate immunity are antigen-presenting cells such as monocytes–macrophages and dendritic cells, which need to be activated to appropriately present foreign antigen to the specific immune system. These cells can be activated through special receptors on antigen-presenting cells, to which structures of bacteria or other antigenic substances can bind (61). Their engagement may stimulate expression of co-stimulatory molecules and cytokine production, thereby providing important signals to activate resting T cells (62).

Similar mechanisms are probably involved in the initiation of a drug-specific immune response (64–66). Such enhancing factors might be provided by the drug or a drug metabolite itself (for example, if it activates an antigen-presenting dendritic cell by an unknown mechanism). Alternatively, the massive stimulation of innate and acquired immune systems during generalized viral infection with HIV, Epstein–Barr virus, or herpesvirus-6 or during an acute exacerbation of an autoimmune disease (such as Still disease or systemic lupus erythematosus) may provide sufficient bystander stimulation for the initiation of an immune response to drugs as well, since such patients have a substantially higher frequency of drug allergies (67–70).

DRUG-INDUCED MACULOPAPULAR, BULLOUS, AND PUSTULAR EXANTHEMA

Cutaneous reactions are the most frequent manifestation of drug allergy (6–8). They comprise a broad spectrum of clinical and distinct histopathologic features. Some

Figure 2. Typical changes of maculopapular drug eruptions and depiction of the killing of keratinocytes by drug-specific, perforin-positive and granzyme B-positive T cells.



Left. Histologic features of maculopapular exanthema: hydropic degeneration of keratinocytes, necrosis of keratinocytes, and lymphocyte and eosinophil infiltrations into dermis and epidermis. (Hematoxylin–eosin; original magnification, $\times 250$.) **Right.** Keratinocytes are stimulated to express MHC class II and intercellular adhesion molecule-1 (*ICAM-1*), a ligand for leukocyte function-related antigen-1 (*LFA-1*). Infiltrating T cells interact with the drug through their T-cell receptor (*TCR*) for antigen, together with the MHC class II molecules on keratinocytes, and kill them by a mechanism dependent on perforin and granzyme B.

of these features appear rapidly after drug intake, such as urticaria and angioedema, and others appear as delayed-type reactions, such as maculopapular, bullous, and pustular exanthemas.

Immunohistologic Characteristics of Maculopapular Exanthema

Immunohistologic work-up of maculopapular exanthema shows a superficial, mainly perivascular, mild to moderate mononuclear cell infiltrate with some eosinophilia. Typically, an interface dermatitis of varying degree is present (71), characterized by an accumulation of $CD3^+$ T cells (40% to 70%) with a predominance of $CD4^+$ T cells (72–75). $CD4^+$ T cells are mainly located in the perivascular dermis, whereas both $CD4^+$ and $CD8^+$ T cells are found at the dermoepidermal junction zone in equal numbers, together with a hydropic degeneration of keratinocytes at the basal-cell layer (74–76).

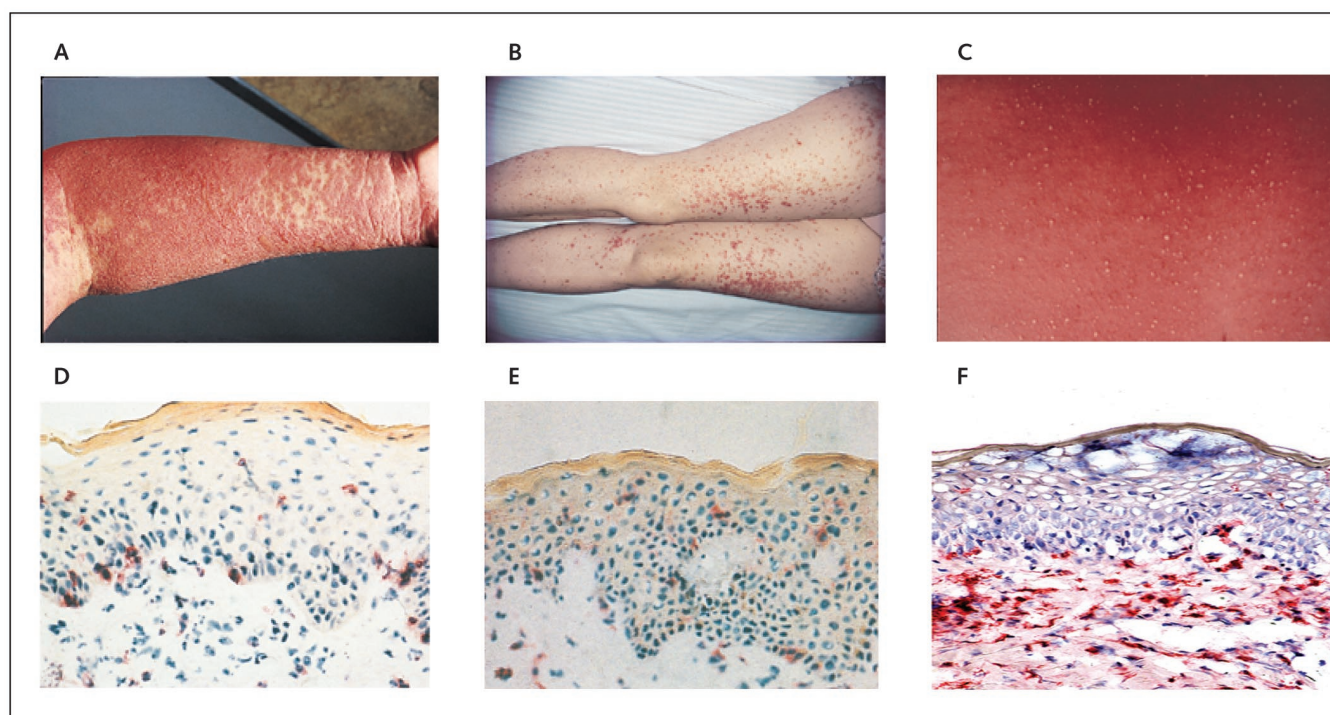
The infiltrating T cells are very active: They express the α chain of the IL-2 receptor, HLA-DR, and adhesion molecules such as the leukocyte function-related antigen-1 and L-selectin. Among the resident cells, endothelial cells are activated and express various adhesion molecules, such as E-selectin and P-selectin, platelet endothelial cell adhesion molecule-1, and intercellular adhesion molecule-1 (74, 75). Of interest, MHC class II is also found on cells of the epidermis, both on the residual $CD1a^+$ dendritic cells and on most of the keratinocytes of the basal-cell layer, which also express intercellular adhesion molecule-1 (74, 75) (Figure 2).

CD4-Mediated Cytotoxicity in Maculopapular Exanthema

Throughout the epidermis, usually close to the epidermal junction zone, dead keratinocytes showing hydropic degeneration can be found (Figure 2). Many are close to the infiltrating T cells expressing perforin and granzyme B (74). Perforin and granzyme B are important mediators of cell-mediated cytotoxic reactions (77) that are released during granule exocytosis and kill other cells in a contact-dependent way. Double-immunostaining for cytotoxic molecules and CD4 or CD8 indicates that both cell types may have cytotoxic potential (74). Perforin-containing T cells are also found in the blood of patients with drug eruptions (41, 78, 79) and in cells eluted from positive patch test reactions (80). Moreover, drug-specific $CD4^+$ T cells kill autologous keratinocytes in vitro (78). Thus, in maculopapular exanthema, cytotoxic T cells (more CD4 cells than CD8 cells) contribute to the characteristic features of interface dermatitis, such as vacuolar alteration and keratinocyte death caused by a perforin-dependent and granzyme B–dependent killing mechanism (Figure 2).

IL-5 in Maculopapular Exanthema

In addition to their cytotoxic function, drug-specific T cells orchestrate skin inflammation through the release and induction of different cytokines and chemokines (72, 81). These T cells exhibit a heterogeneous cytokine profile, including type 1 (interferon- γ) and type 2 (IL-4, IL-5) cytokines (26, 28, 82, 83). The finding that all of these cytokines can be detected by staining skin samples is consistent

Figure 3. Distinct T-cell functions in different forms of exanthemas.

Various drugs can elicit distinct forms of T-cell-mediated drug reactions. For example, amoxicillin causes bullous skin disease (A), maculopapular exanthema (B), and acute generalized exanthematous pustulosis (C). Analysis of skin infiltrates and functional analysis of drug-specific T-cell clones from these different forms of drug allergy revealed distinct contribution of CD4⁺ and CD8⁺ T cells to these disorders, as well as distinct functions of CD4 cells. In maculopapular exanthema, CD4 cells dominate. More CD8⁺ T cells are found in patients with (mild) bullous skin disease, and these cells can kill keratinocytes (D). CD4 cells secrete high levels of interleukin-5 and substantial amounts of interferon- γ and can kill activated MHC class II-expressing keratinocytes (E). CD4⁺ and CD8⁺ T cells are found in patients with acute generalized exanthematous pustulosis (F); both these cells contribute to vesicle formation through their cytotoxic activity. CD4 cells secrete granulocyte-monocyte colony-stimulating factor and interleukin-8, which leads to the recruitment of neutrophils (54, 72, 74, 75, 78, 85). (Immunostaining by the avidin-biotin complex/alkaline phosphatase method; original magnification, $\times 250$.)

with the idea that both T-helper 1 and T-helper 2 cells infiltrate the skin (72, 81). Enhanced production of IL-5 by drug-specific T cells is common in different forms of drug allergies (26, 27, 84) and is of particular importance. This cytokine is known to be a key factor in regulating the growth, differentiation, and activation of eosinophils, which frequently are increased in various forms of drug allergies and can be found in the serum during the acute stage (85, 86). The recruitment and activation of eosinophils may be further enhanced by the expression of the chemokines eotaxin and RANTES in lesions of maculopapular exanthema (72, 87).

Bullous Exanthema

The immunohistologic characteristics of mild bullous exanthema are actually quite similar to those of maculopapular exanthema. Both lesions involve T-cell infiltration, MHC upregulation on keratinocytes, and immigrating T cells and IL-5 expression in the lesions (73). The decisive difference in bullous exanthema is that there is a higher percentage of perforin-positive CD8⁺ T cells in the dermis and particularly in the epidermis (Figure 3). Previous studies have shown that these CD8⁺ T cells are killer cells,

since CD8⁺ T cells eluted from the skin of patients with amoxicillin-induced bullous skin reactions could kill other cells after mitogen stimulation (25, 88). These CD8⁺ killer T cells may cause formation of bullae because such cells not only kill MHC class II-bearing keratinocytes but also keratinocytes that express MHC class I. The latter is expressed on all, also resting, keratinocytes, making more cells vulnerable to the cytotoxic attack.

Toxic Epidermal Necrolysis

The most severe forms of drug-induced bullous skin diseases are the Stevens-Johnson syndrome and toxic epidermal necrolysis. The histologic characteristics of toxic epidermal necrolysis—many dead keratinocytes and only scarce cell infiltration—are difficult to reconcile with a killing process that depends on contact between the T cells and target cells, as is the case for perforin and granzyme B. Indeed, it has been proposed that the apoptosis of keratinocytes occurs because of Fas ligand, a soluble molecule of the tumor necrosis factor family that binds to so-called death receptors called Fas on keratinocytes (89, 90). On the other hand, a recent study of cells obtained from bullae of patients with toxic epidermal necrolysis showed that

CD8⁺ T cells with some natural killer cell–like features were present during the initial phase and that monocytes were present later in the disease (91). These cytotoxic CD8⁺ T cells express $\alpha\beta$ T-cell receptors (some express CD56⁺) and kill through perforin and granzyme B but not through the Fas-mediated pathway at this disease stage (92).

Systemic Drug Reactions: The Drug Hypersensitivity Syndrome

In a prospective study of patients with severe forms of maculopapular exanthema (73), approximately 20% had transient elevation of liver enzyme levels. This liver injury was associated with substantial activation of CD8⁺ T cells in the circulation or in the affected skin (73), which illustrates that cutaneous drug allergies are often only part of a systemic immune reaction. Therefore, patients with maculopapular or other exanthemas should have a physical examination to define the extent of cutaneous or mucosal involvement (Table 2). Those with severe forms should have laboratory tests to determine the strength of the immune reaction (reflected in elevated C-reactive protein levels, activated lymphocyte counts, and levels of eosinophils in the circulation) and the involvement of internal organs (reflected in aminotransferase levels and creatinine concentrations) (Table 2). For example, signs of liver or kidney involvement would strongly indicate that future use of the suspect drug should be avoided.

Some drugs, in particular anticonvulsants, dapsone, sulfamethoxazole, sulfasalazine, allopurinol, and minocycline, are known to cause severe systemic disease in some patients (for example, fever, lymph-node swelling, hepatitis, and various forms of exanthemas) (93–96). More than 90% of these patients have eosinophilia, and activated T cells are often found in the circulation, similar to patients with acute HIV or generalized herpesvirus infections. This syndrome has many names; the most frequently used are the drug (or anticonvulsant) hypersensitivity syndrome or drug-related eosinophilia with systemic symptoms (93, 95). The pathogenesis of the disorder has not yet been clarified. The clinical picture resembles that of a generalized viral infection, such as an acute Epstein–Barr virus infection, but it is distinguished by prominent eosinophilia. Massive immune stimulation and the presence of drug-specific T cells have been demonstrated (27, 47, 48, 97). A peculiar feature of this syndrome is its long-lasting clinical course despite withdrawal of the causative drug. There may also be persistent intolerance to other, chemically distinct drugs, leading to flare-up reactions months after the initiating drug therapy is stopped. Recently, it has been shown that human herpesvirus-6 DNA can be found in many patients with this syndrome during the third or fourth week of the disease (but not before), followed by an increase in antibodies to human herpesvirus-6 (97, 98). Other reports document reactivation of cytomegalovirus infection (99). Thus, drug-induced massive immune stim-

Table 2. Clinical Findings and Laboratory Tests and Findings in Drug-Induced Exanthema*

Clinical Findings	Laboratory Tests and Findings
Extent of exanthema, Nikolsky sign	Differential blood count†
Extent of infiltration, formation of bullae‡, pustules‡, painful skin‡	Eosinophilia (eosinophil count > 1.2×10^9 cells/L)‡; atypical (activated) lymphocytes in the circulation (>2%)‡
Possible involvement of mucous membranes‡	Elevated CRP level‡
Systemic symptoms‡ Malaise, fever‡, lymphadenopathy‡; possible liver, kidney, lung, pancreas involvement‡	Aminotransferase levels increased to 2 times the upper limit of normal‡; additional investigations (urinalysis, creatinine concentration) depend on clinical signs

* CRP = C-reactive protein.

† Should always be done in acute, extensive maculopapular drug eruption and more severe forms (bullous or pustular exanthema) or if mucous membranes are involved.

‡ Danger signs, indicating a more severe reaction.

ulation may somehow lead to a loss of control of these herpesviruses, which subsequently replicate and possibly contribute to the chronic course and persistent drug intolerance characteristic of this peculiar disease.

Drug-induced hepatitis, nephritis, interstitial lung disease, pancreatitis, or isolated fever can also be the only symptom of a drug allergy. Sometimes, eosinophilia helps to distinguish a peculiar drug reaction from other diseases and suggests a T-cell–mediated process, since these cells are the main source of the eosinophil-stimulating cytokine IL-5 (27, 67, 85, 86).

Acute Generalized Exanthematous Pustulosis

Acute generalized exanthematous pustulosis is a rare disease with an estimated incidence equal to that of the Stevens–Johnson syndrome and toxic epidermal necrolysis combined (100, 101). It is caused by drugs in more than 90% of cases (mainly aminopenicillins, sulfonamides, and diltiazem); its clinical hallmark is the presence of myriad disseminated, sterile pustules in the skin (Figure 3). Affected patients have fever and massive leukocytosis in the blood, sometimes with eosinophilia (48, 101). The involvement of T cells has been suggested by a positive patch test reaction to the causative drug (102) that resembles the morphologic characteristics of the original reaction with formation of pustules (54).

Immunohistologic work-up of the acute lesion reveals intraepidermal pustules, which are filled with neutrophilic leukocytes and are surrounded by activated, HLA-DR–expressing CD4⁺ and CD8⁺ T cells (Figure 3). In contrast to maculopapular or bullous exanthema, the keratinocytes do not express MHC class II but show an elevated expression of the neutrophil-attracting chemokine IL-8. Surprisingly, even the T cells migrating into the epidermis express IL-8 (54). Analysis of patch test reactions suggests that drug-specific T cells emigrate first, cause formation of

vesicles by killing keratinocytes, and then recruit neutrophilic leukocytes (103). In vitro analysis of drug-specific T-cell clones obtained from the blood or patch test lesions has confirmed the high IL-8 production of drug-specific CD4⁺ T cells, while samples from patients with other drug reactions have shown no or only moderate IL-8 production (54). In addition, the T cells produce high levels of granulocyte-monocyte colony-stimulating factor, which is probably important for the survival of the emigrated neutrophilic leukocytes (Figure 3).

Neutrophilic Inflammation Regulated by IL-8–Producing T Cells (Type IVd Reaction)

The existence of IL-8–producing T cells, with their in vivo correlate of pustule formation, goes beyond the T-helper 1–T-helper 2 concept, which does not consider neutrophilic inflammation orchestrated by T cells themselves. It shows that T cells may not only stimulate monocyte activation through the T-helper 1 cytokine interferon or an eosinophilic inflammation through the T-helper 2 cytokine IL-5 but might also regulate a neutrophil-rich inflammation. Since this T-cell function leads to a particular disorder with infiltrates of neutrophils, which is clearly different from T-helper 1– or T-helper 2–associated disorders, it might be considered a separate T-cell reaction (type IVd) (Table 1). Such IL-8–producing T cells might also be important in other MHC-associated chronic autoimmune diseases such as psoriasis and Behçet disease, which are hard to classify with the T-helper 1 or T-helper 2 scheme (104).

Overlapping T-Cell Functions in Exanthema

Immune reactions to small chemicals are complex and overlapping. For example, in acute generalized exanthematous pustulosis, different T-helper cell functions occur simultaneously. Some patients have both neutrophilia and eosinophilia in the blood and, in addition to the neutrophilic pustules, a massive eosinophil recruitment in the tissue (54, 101). Both IL-5– and IL-8–secreting T cells were found in such reactions. A similar overlap of various immune reactions can also occur in maculopapular exanthema, in which patients have IL-5–secreting and cytotoxic T cells together and may even have drug-specific IgE, detectable by skin tests or serologic tests (Table 1) (28, 105).

CONCLUSIONS: LESSONS FROM DRUG ALLERGY

The often surprising appearance of “bizarre” side effects and the multitude of causes have made drug allergy a difficult area of clinical research. While drug allergies are iatrogenic diseases that are unpredictable and embarrassing to the clinician, they can also be seen as unintended immunologic experiments. A closer look at these events may provide new insights into basic and clinical immunology.

First, drug hypersensitivities are more diverse and often more complicated than solely IgE-mediated reactions. Small drugs themselves (or in association with MHC mol-

ecules) can act as “antigens” for T cells and elicit an immune reaction if the drug structurally fits into a T-cell receptor. Second, the variability of the immune response to hapten-like drugs is mainly due to the formation of distinct antigenic epitopes on soluble or cell-bound proteins. Third, various drug hypersensitivity diseases can be related to the preferential activation of drug-specific T cells with distinct functions (immune reactions type IVa through type IVd) (Table 1). These T cells recruit and activate monocytes (type IVa), eosinophils (type IVb), or neutrophils (type IVd) by secreting cytokines and chemokines. Moreover, CD4⁺ and CD8⁺ T cells themselves can exert cytotoxic functions (type IVc). An overlap of these immune reactions is common in clinical manifestations of drug allergies, but frequently 1 type is dominant (Table 1, Figure 3). Fourth, drug allergies are great imitators of diseases. This has been well documented in studies of acute generalized exanthematous pustulosis, which until 1980 was considered a special form of psoriasis (von Zumbusch type) but is now recognized as a hypersensitivity reaction with a known cause, imitating psoriasis (54, 100, 101). Thus, improving our understanding of drug allergies not only allows us to better avoid them in the future but might also provide hints to understanding imitated diseases.

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