

Immune mechanism of drug hypersensitivity

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Drug-induced adverse reactions are common and normally classified as type A reactions, owing to the pharmacologic action of the drug, or type B reactions, which comprise idiosyncratic and immune-mediated side effects that are not predictable [1]. Drug hypersensitivity reactions (drug allergy) account for about 1/7 of adverse drug reactions. They can manifest themselves in a great variety of diseases, some of which are severe [2–5]. The most common allergic reactions occur in the skin and are observed in approximately 2% to 3% of hospitalized patients [6–8].

Drug hypersensitivity and other immune reactions are frequently classified according to Gell and Coombs into four categories that reflect a distinct immune mechanism, explaining the heterogeneous clinical presentation [9]. Type I reactions are caused by the formation of drug/antigen-specific IgE. These IgEs are cytophilic and bind to high-affinity Fc-IgE receptors on mast cells and basophils. Cross-linking of these receptors leads to the liberation of various mediators, eliciting symptoms such as urticaria, anaphylaxis, rhinitis, and bronchoconstriction. Type II reactions are based on IgG-mediated cytotoxic mechanisms, accounting mainly for blood cell dyscrasias such as hemolytic anemia and thrombocytopenia. Type III reactions are immune complex-mediated and may, via involvement of complement activation and stimulation of Fc-IgG receptor-expressing inflammatory cells, lead to vasculitis. Type IV reactions are mediated by T cells, causing so-called “delayed hypersensitivity reactions,” the most typical example being contact dermatitis or delayed skin tests to tuberculin.

This classification has been helpful in clinical practice but has its limitations, because it was established before a detailed analysis of T-cell subsets and functions was available. Immunologic research has revealed that all four types of reactions require an involvement of T cells, which provide help by generating cytokines acting as switch factors for immunoglobulin isotype switch [10]. Moreover, T cells have been found to differ in the cytokines produced, which

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results in distinct pathologies [11]. Th1-type T cells activate macrophages by secreting large amounts of interferon- γ , drive the production of complement-fixing antibody isotypes, and are costimulatory for proinflammatory responses (tumor necrosis factor, IL-12) and CD8+ T-cell responses. Th2 T cells secrete the cytokines IL-4, IL-13, and IL-5 [11,12], 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 textbooks of immunology have considered this development and subdivided delayed hypersensitivity reactions into type IVa, IVb, and IVc reactions, which correspond to Th1, Th2, and cytotoxic reactions [12].

Drug hypersensitivity reactions were always considered typical examples of these hypersensitivity types, being able to cause type I, II, III, and IV reactions [13]. On the other hand, many drug-induced hypersensitivity reactions did not seem to fit into the older Gell and Coombs classification. For example, the Gell and Coombs classification did not account for bullous or pustular skin eruption or hepatitis, because cytotoxic T-cell mechanisms were not yet known, and the regulation of neutrophils by T cells was not yet investigated. The ignorance in the field, the unpredictable and sometimes bizarre nature of the adverse reaction, and the fact that animal models did not exist (or were not published by the pharmaceutical companies) made drug hypersensitivity a neglected area of research, particularly in North America [14].

During the last few years, the author's group has tried to clarify some issues in this rather enigmatic field. We investigated particularly the role of T cells in drug hypersensitivity and asked two main questions: (1) how do T cells recognize drugs, and (2) is there a relationship between T-cell function and the clinical presentation? Our results were surprising and, initially, seemed to contradict prevailing dogmas [15]. On the other hand, the clinical data illustrate well that drug allergies are the great imitators of disease. This review focuses mainly on T-cell reactions to drugs, an area of research where the most progress has been made.

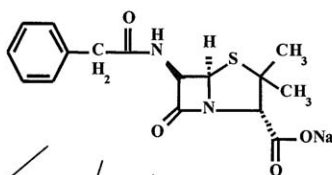
T-cell recognition of drugs

The hapten and prohapten concept

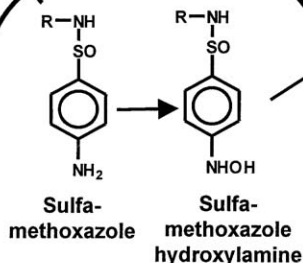
The recognition of small molecules, such as drugs, by B and T cells is usually explained by the hapten concept. Haptens are chemically reactive small molecules (mostly <1000 D) that are able to undergo a stable covalent binding to a larger protein or peptide [16–21]. This modification of a protein or peptide is thought to make the small molecule immunogenic (Fig. 1). By itself, it is too small to elicit an immune response. Cell-bound or soluble immunoglobulins can recognize the hapten–carrier complex directly, and an immunoglobulin-based response can develop. T cells recognize a peptide fragment of the hapten–carrier complex, which is generated by intracellular processing of the complex and presented to T cells by MHC molecules (Fig. 1). A typical hapten is penicillin G,

Penicillin G

Hapten



Prohaptan



metabolism

Fig. 1. Hapten and prohapten concept and noncovalent drug presentation to T cells. Drugs are haptens if they can bind covalently to molecules, whether they are soluble or cell bound (eg, penicillin G). They can even bind directly to the immunogenic MHC/peptide complex on antigen-presenting cells, either to the embedded peptide or to the MHC molecule itself. The chemical reactivity of haptens leads to the formation of many distinct antigenic epitopes, which can elicit humoral and cellular immune responses. Examples of a B-cell-mediated immune response are anaphylaxis (IgE) and hemolytic anemia and thrombocytopenia (IgM/IgG). T-cell-mediated immune responses include exanthema (maculopapular, bullous, and pustular) and hepatitis, nephritis, pancreatitis, and interstitial pneumonia. Other drugs are prohaptenes, meaning that they require metabolism to become haptens (chemically reactive). The metabolism leads to the formation of a chemically reactive compound (eg, from sulfamethoxazole [SMX] to the chemically reactive form SMX-NO). It may lead to modification of cell bound or soluble proteins by the chemically reactive metabolite, similar to a real hapten.

which tends to bind covalently to lysine groups within soluble or cell-bound proteins, thereby modifying them and eliciting B- and T-cell reactions [20,21]. It is also possible that the hapten binds directly to the immunogenic peptide presented by the MHC molecule. In this situation, no processing is required [20,22] (Fig. 1). Alteration of the MHC molecule directly is also possible, but data in the mouse model using trinitrophenol (TNP) suggest that this is less frequent, because most T-cell clones generated to TNP recognize the peptide together with the hapten but not the hapten-modified MHC molecule [23]. This feature of a hapten, namely, the possibility of binding to many proteins or peptides, may explain the great heterogeneity of immune reactions to it, resulting in a great variety of clinical symptoms (Fig. 1) [24].

Many drugs are not chemically reactive but can still elicit allergic side effects. The prohaptens hypothesis tries to reconcile this phenomenon with the hapten hypothesis. A drug that is not chemically reactive, per se, may become reactive upon metabolism [18,19,21,25–29] (Fig. 1). Sulfamethoxazole is a prototype of such a prohaptens [26]. By itself, it is not chemically reactive; it gains immunogenicity by intracellular metabolism. A cytochrome p450-dependent metabolism leads to the production of sulfamethoxazole-hydroxylamine, which can easily be transformed to the highly reactive sulfamethoxazole-nitroso by (extracellular) oxidation. This chemically reactive compound can bind covalently to proteins/peptides (Fig. 1) [26–28]. The resulting clinical presentation may be as variable as the effects of haptens, if the hapten is generated extracellularly in different parts of the body. Indeed, sulfamethoxazole is known to cause many different diseases affecting many organs. These side effects are mediated by antibodies or T cells. On the other hand, the transformation of the prohaptens to the reactive hapten may occur only in the liver, causing hepatitis, which is most likely true for tienilic acid [29].

The p-i concept

Recently, the author and his colleagues elaborated a third possibility, namely, a pharmacologic interaction of drugs with immune receptors (p-i concept, Fig. 2) [30–36]. We found that chemically inert drugs, unable of covalently binding to peptides or proteins, could directly activate certain T cells if they happened to bear T-cell receptors that could interact with the drug. This model does not require biotransformation to a chemically reactive compound. It has been elaborated by in vitro studies using T-cell clones specific for drugs such as sulfamethoxazole, lidocaine, mepivacaine, celecoxib, lamotrigine, carbamazepine, and p-phenylenediamine [30–40]. The p-i concept relies on various findings. First, glutaraldehyde-fixed antigen-presenting cells, unable to process, can still present the drug and stimulate specific T cells [30]. Second, the formation of the reactive metabolite of sulfamethoxazole (sulfamethoxazole-hydroxylamine) can be inhibited by adding glutathione (see Fig. 1). If the reactive metabolite were the “real” antigenic substance, this treatment should have reduced the reactivity. In contrast, it actually enhanced the reactivity of T cells, suggesting that the inert

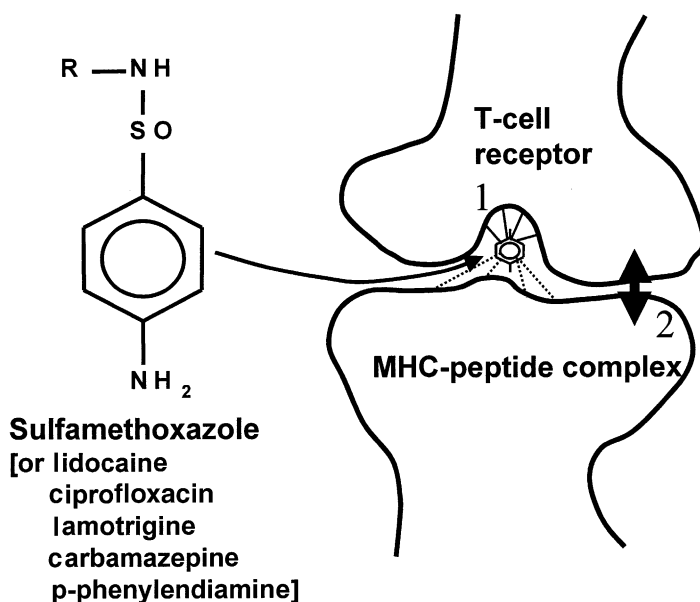


Fig. 2. Pharmacologic interaction with immune receptors, the p-i concept. Drugs are often designed to fit into certain proteins/enzymes to block their function. Some drugs may also happen to bind into some of the available T-cell receptors. Under certain conditions (see text), this drug–T-cell receptor interaction may lead to an immune response of the T cell with a “fitting” T-cell receptor (1). For a full T-cell stimulation by such an inert drug, an interaction of the T-cell receptor with the MHC molecule is required (2). This type of drug stimulation (1 + 2) results in an exclusive T-cell stimulation.

drug but not the reactive metabolite was recognized [35]. Third, covalently bound drugs are stably bound to the carrier molecule and are not washed away. In contrast, chemically inert, labile-bound drugs are removed by a simple washing step [30–32,34,37–39]. Fourth, a drug-reactive T-cell clone reacts to the drug within seconds, before metabolism and processing can take place [31].

The full stimulation of T cells by inert drugs is MHC dependent, implying that, for a complete stimulation of the T cell, the T-cell receptor needs to interact with the drug and the MHC molecule. Nevertheless, the extent of MHC dependence for T-cell stimulation depends on the affinity of the T-cell receptor for the drug. If the T-cell receptor is widely cross-reactive, such that it reacts with a wide panel of related sulfonamide structures, the affinity for the original drug might be relatively low, and optimal stimulation would rely on the interaction with an exactly fitting MHC allele. In contrast, if the T-cell receptor reacts in a highly specific manner with a single molecule only, the affinity for it may be rather high, and different MHC alleles would be sufficient to serve as a scaffold to supplement the stimulation [32,41,42]. In agreement with this peculiar nature of drug-specific immune responses is the observation that, in the same individual, T-cell responses to a certain drug can be observed that are restricted by either HLA DR or DQ molecules [43].

Variability of the clinical manifestation of a drug hypersensitivity reaction

The clinical manifestations of drug hypersensitivity depend on various factors:

- The chemical or structural features of a drug, as well as its metabolism, are crucial factors in the type of reactions it will elicit, whether it is immunogenic by the hapten/prohapten or p-i mechanism (Figs. 1 and 2).
- The genetic background of the affected individual, in addition to pharmacogenetic, immunogenetic, and hormonal factors, can affect the immune response. Tissue-specific genes might also be involved.
- The specificity and function of the drug-induced immune response, which is not only directed to the drug but also to some protein antigens, will affect the clinical manifestations.

Effects linked to chemical reactivity versus structural features

Haptens are primarily immunogenic owing to their chemical reactivity. They modify peptides and make them more or newly immunogenic. In contrast, chemically inert drugs are immunogenic based only on their structural features that enable them to interact with immune receptors (certain T-cell receptors and possibly MHC). These structural features have never been considered in the development of a drug [44] but may account for a substantial portion of unforeseen side effects, because many drugs seem to be able to interact with the T-cell receptor directly [30,32,37–40,45].

The clinical symptoms elicited by drugs that are immunogenic by their chemical or structural features may differ. A haptenlike drug (eg, amoxicillin) can alter many different proteins, either soluble or cell bound, and can even modify different MHC molecules and their embedded peptides directly (see Fig. 1). These distinct antigenic determinants can stimulate T and B cells and elicit more or less all types of immune reactions. Indeed, the classic drugs that act as haptens, namely, the 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 (AGEP), Stevens-Johnson syndrome, and even toxic epidermal necrolysis.

Whether the labile noncovalent binding of a drug to proteins is sufficient to make it immunogenic for B cells is unclear, but this mechanism seems unlikely for most small drugs. Heparin may be an exception, causing antibody-mediated thrombocytopenia without covalent binding to the gpIIb/IIIa on thrombocytes [46]. If the drug acquires immunogenicity only by its ability to fit into some of the available T-cell receptors, the immune reaction is naturally restricted to an exclusive T-cell response. Consistent with this hypothesis is the observation that certain drugs (similar to nickel) appear to elicit mainly T-cell reactions, whereas B-cell reactions appear to be exceedingly rare or are not reported. Carbamazepine is known to elicit maculopapular exanthema and drug hypersen-

sitivity syndromes (two typical T-cell-mediated diseases) but not anaphylaxis. As outlined previously, some drugs, such as sulfamethoxazole or p-phenylendiamine, might be immunogenic by both mechanisms, and a hapten-based as well as a p-i pathway-based immune response may occur together in the same individual [34,39].

Effects linked to genetic factors

It is well known that certain drugs elicit preferentially a certain pattern of disease. For example, antiepileptics (carbamazepine, phenytoin, lamotrigine) can elicit a rather severe reaction called drug hypersensitivity syndrome (also called anticonvulsant hypersensitivity syndrome or DRESS, drug-induced eosinophilia with systemic symptoms) [45,47,48]. A similar symptomatology can also be induced by other drugs, in particular, allopurinol, sulfasalazine, certain quinolone derivatives, or abacavir. It is tempting to speculate that this severe symptomatology is caused by some peculiar features of these drugs, either a particular metabolism or a highly immunogenic presentation in a certain tissue. In this context, it is interesting that abacavir reactions are highly associated with the presence of the HLA-B*57 allele, suggesting that, in this disease, this HLA locus (or a very close gene) is involved in the reaction [49]. Recently, it was reported that in Han-Chinese the appearance of Stevens-Johnson syndrome and toxic epidermal necrolysis after carbamazepine therapy is strongly linked to HLA-B*1502 [50].

On the other hand, for most haptens, such as amoxicillin or sulfamethoxazole, no association with immunogenetic or pharmacogenetic phenotypes has been found. These drugs do not need a special metabolism to become immunogenic and bind as haptens most likely to many different peptides and proteins, which contain the relevant binding amino acid (ie, lysine or cysteine) in an accessible position.

Other genes have also been suggested to be important for the localization, type, and strength of the hypersensitivity reaction [21,51]. The polymorphism in the tumor necrosis factor promoter region may have a role in the severity of the reaction [51]. In addition, the genetic polymorphism of metabolizing enzymes (eg, of certain cytochrome p450 enzymes or of N-acetyltransferase) may contribute to the generation of chemically reactive or toxic compounds, which cause or contribute to the development of hypersensitivity [21,51]. Still unknown is the role of tissue-specific genes, which lead to the manifestation of an immune response in a particular organ.

Effects linked to specificity

In the p-i concept, T cells are primed and selected by immune recognition of protein/peptide antigens in the thymus and peripheral lymphoid organs. It is logical to assume that such a T cell has additional specificities and possibly already a committed function, which might be important for the clinical manifestation and type of drug hypersensitivity.

Cross-reactivity of drug-specific T cells

General features

Most drug-specific T cells express the $\alpha\beta$ T-cell receptor [38,39,42,45,52,53]. CD4⁺ and CD8⁺ T cells can be activated, with highly heterogeneous functions [45,53]. In some instances, an oligoclonal T-cell reaction to the drug has been observed in vitro [22]. Nevertheless, the vast majority of drug-specific T cells show a heterogeneous T-cell receptor repertoire if TCR β are analyzed and when the CD3-binding region is sequenced [33,42,43].

It is surprising that exotic “antigens” such as drugs can react with so many different T cells and function as antigen. Perhaps, a small drug might fit into many different T-cell receptors of an individual, and the T-cell receptor repertoire would not be a limiting factor for the development of an immune response to a drug. This concept that it is not the T-cell receptor repertoire but the control of T-cell responses that is crucial in preventing drug hypersensitivity is supported by the following clinical observations:

Altering the immune status, for example by a viral infection (Epstein-Barr virus or HIV), leads to a high incidence of hypersensitivity reactions, most likely by overcoming the normal tolerance mechanism to the drug. Because such patients have positive skin tests and lymphocyte transformation tests to the drug, the reaction appears to be drug specific and is not an “unspecific” epiphenomenon of the viral infection [54].

Certain drug allergies occur mainly in woman, similar to autoimmune diseases, suggesting that similar control mechanisms are not functioning optimally in drug allergy and autoimmunity [55].

The author and others have observed that a previous drug hypersensitivity reaction enhances the risk of a further drug allergy to a chemically distinct compound, and that 10% of patients with drug hypersensitivity reactions react with more than one structurally distinct compound [56–58]. Some individuals seem to be particularly susceptible to drug hypersensitivity, and previous drug hypersensitivity is a major risk factor for further allergies.

Cross-reactivity with structurally related compounds

The author and his colleagues have analyzed in detail the ability of drug-specific T-cell clones raised against sulfamethoxazole, lidocaine, penicillin G, and quinolones to recognize structurally related compounds (22, 33, 58, D. Schmid, unpublished data, 2004). In the sulfamethoxazole model, the complete sulfanilamide core structure is always required to elicit cross-reactivity to other anti-infectious sulfonamides. The presence of a sulfonamide ($\text{SO}_2\text{-NH}_2$) structure, as found in furosemide or celecoxib, is not sufficient to stimulate T-cell clones originally stimulated by sulfamethoxazole; conversely, celecoxib-stimu-

lated T cells do not recognize sulfamethoxazole [33,40,43]. 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, whereas other T cells react exclusively with sulfamethoxazole itself [33]. Interestingly, some T-cell clones recognize sulfamethoxazole, which binds in a labile way to MHC, and sulfamethoxazole-NO, stably interacting with the MHC–peptide complex [34]. This observation implies that the same T-cell receptor can be stimulated by a hapten (sulfamethoxazole-NO) or directly by the inert drug (sulfamethoxazole).

Similar data have been obtained for lidocaine, a local anesthetic of the amide type. There was no cross-reactivity with ester compounds, but there was reactivity with bupivacaine and mepivacaine [59]. Cross-reactivity of amoxicillin- or penicillin G–specific T-cell clones to various cephalosporins in vitro was never observed, even if the same side chain was present [22], whereas high cross-reactivity with different penicillins was observed. These in vitro data suggest that T cells recognize primarily the core structure and, to a variable degree, the side chain. In contrast, an exclusive side-chain reactivity has been reported for IgE [60]. This observation suggests that the cross-reactivity of T cells and B cells differs. The advice to be given to a patient with clearly T-cell–mediated reactions does not have to be as strict as for a patient with IgE-mediated anaphylaxis, in which symptoms elicited owing to the same side chain but a different core (penicillin or cephalosporin) have been reported [60].

Drug–peptide cross-reactivity of drug-specific T-cells

The real specificity of drug-specific T-cell clones is still enigmatic. Peripheral blood T cells are selected in the thymus based on their ability to recognize peptides, and the author and his colleagues have observed that drug-specific T cells frequently are stimulated by alloantigens as well [41,42,61]. This drug and peptide specificity might be relevant for drug-induced autoimmune disease and might be the mechanism by which the development of a drug allergy alters the body's tolerance to some autoantigens [61]. In the mouse model with TNP, it was shown that under certain circumstances an immune response to a hapten-modified peptide may also be directed to the unmodified peptide [23]. We observed that human T-cell clones raised from a patient with drug allergy in an autologous mixed leukocyte reaction reacted with one autologous allele but also were able to recognize the second drug-modified allele [61]. This observation suggests that an immune response to a drug may break the tolerance to an unrelated protein and direct a response to an autoantigen.

Innate immunity and cofactors for drug hypersensitivity

The primary sensitization to a drug most likely happens in the lymph nodes. It requires a sensitization phase of at least 3 to 5 days, most likely dependent on the available amount of T cells with “fitting” T-cell receptors. Clinical symptoms

after the first encounter often develop at day 8 to 10 of treatment, but, in previously sensitized individuals, a re-exposure can cause symptoms on the first day.

To develop an effective immune response, the innate immune system needs to be involved and activated, providing important signals to activate resting T cells [62,63]. The activation of these cells can occur via special TOLL-like receptors on antigen-presenting cells, such as monocytes/macrophages and dendritic cells, to which products of bacteria can bind [62]. Their engagement may stimulate the expression of costimulatory molecules and cytokine production, facilitating an antigen-specific T-cell response.

Similar mechanisms are probably involved in the initiation of a drug-specific immune response [64,65] but are not yet deciphered. The factors enhancing unspecific immunity might be provided by the drug itself or by a drug metabolite. If the drug metabolism of the affected individual leads to a slightly toxic/irritative metabolite, this toxic effect may activate an antigen (drug)–presenting dendritic cell by an unknown mechanism. The drug or drug metabolite might be recognized by a T cell, and a hypersensitivity reaction might evolve. This mechanism would combine pharmacogenetic dispositions with immunogenetic aspects. The pharmacogenetic disposition may be an enhanced production of a certain metabolite to activate the innate immune system, whereas the immunogenetic background of the individual may explain the involvement of the specific immune system (T-cell repertoire, eventually MHC allele). This dichotomy would also explain why the analysis of a single genetic factor is often disappointing in drug hypersensitivity research.

Drug-induced maculopapular, bullous, and pustular exanthema

The most frequent manifestations of drug allergy are cutaneous reactions [5–8]. These reactions comprise a broad spectrum of clinical and distinct histopathologic features, some appearing within hours after drug intake, such as the immediate-type reactions (eg, urticaria and angioedema), and others appearing in a delayed fashion after 6 hours to 10 days (eg, maculopapular, bullous, and pustular exanthemas).

Immunohistology of maculopapular exanthema

The immunohistology of maculopapular exanthema shows a superficial, mainly perivascular, mild-to-moderate mononuclear cell infiltrate with some eosinophilia. The mononuclear cell infiltrate consists mainly of CD3+ T cells with a predominance of CD4+ T cells. CD4+ T cells are mainly located in the perivascular dermis, whereas CD4+ and CD8+ T cells are found at the dermoepidermal junction zone in equal numbers close to keratinocytes at the basal cell layer, which undergo hydropic degeneration [64–69].

The infiltrating T cells are activated. They express the α -chain of the IL-2 receptor, HLA-DR, and adhesion molecules such as the leukocyte function–

related antigen 1 (LFA-1) and L-selectin. Among the resident cells, endothelial cells are activated and express various adhesion molecules, such as E- and P-selectin, platelet endothelial cell adhesion molecule 1, and intercellular adhesion molecule 1 (ICAM-1) [67,68]. Interestingly, MHC class II molecules are also found on cells of the epidermis on the residual CD1a+ dendritic cells and on the majority of the keratinocytes of the basal cell layer, which also express ICAM-1 [24,67,68].

CD4-mediated cytotoxicity in maculopapular exanthema

Dead keratinocytes can be found throughout the epidermis, predominantly close to the epidermal junction zone [24,68]. Many of these cells show hydropic degeneration and are close to the infiltrating T cells expressing perforin and granzyme B [68]. Perforin and granzyme B control cell-mediated cytotoxic reactions [70] as they are released during granule exocytosis and kill other cells in a contact-dependent way. Double immunostaining for cytotoxic molecules and CD4 or CD8 indicate that both cell types may have cytotoxic potential [68]. Perforin-containing T cells are also found in the blood of patients with drug eruptions [30,71,72] and in cells eluted from positive patch test reactions [73]. Moreover, drug-specific CD4+ T cells kill autologous keratinocytes presenting the drug in vitro [71]. In maculopapular exanthema, cytotoxic T cells (CD4 more than CD8) contribute to the characteristic features of interface dermatitis, such as vacuolar alteration and keratinocyte death, by a perforin/granzymeB-dependent killing mechanism (Fig. 3).

IL-5 in maculopapular exanthema

Drug-specific T cells also orchestrate skin inflammation through the release and induction of different cytokines and chemokines [65,74]. Drug allergic reactions are mainly acute reactions and are not associated with an atopic predisposition. The T cells exhibit a heterogeneous cytokine profile, including type 1 (IFN- γ) and type 2 (IL-4, IL-5) cytokines [35,49,75,76], suggesting that Th1 and Th2 cells infiltrate the skin [72,77].

An enhanced production of IL-5 by drug-specific T cells is a frequent finding in different forms of drug allergies [45,78] and is of particular importance. This cytokine is known to regulate 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 [79,80]. The recruitment and activation of eosinophils may be enhanced further by the expression of the chemokines eotaxin and RANTES in lesions of maculopapular exanthema [65,81].

Bullous exanthema

The formation of bullae is an ominous sign and requires careful clinical supervision, because it may be a precursor of a severe hypersensitivity reaction

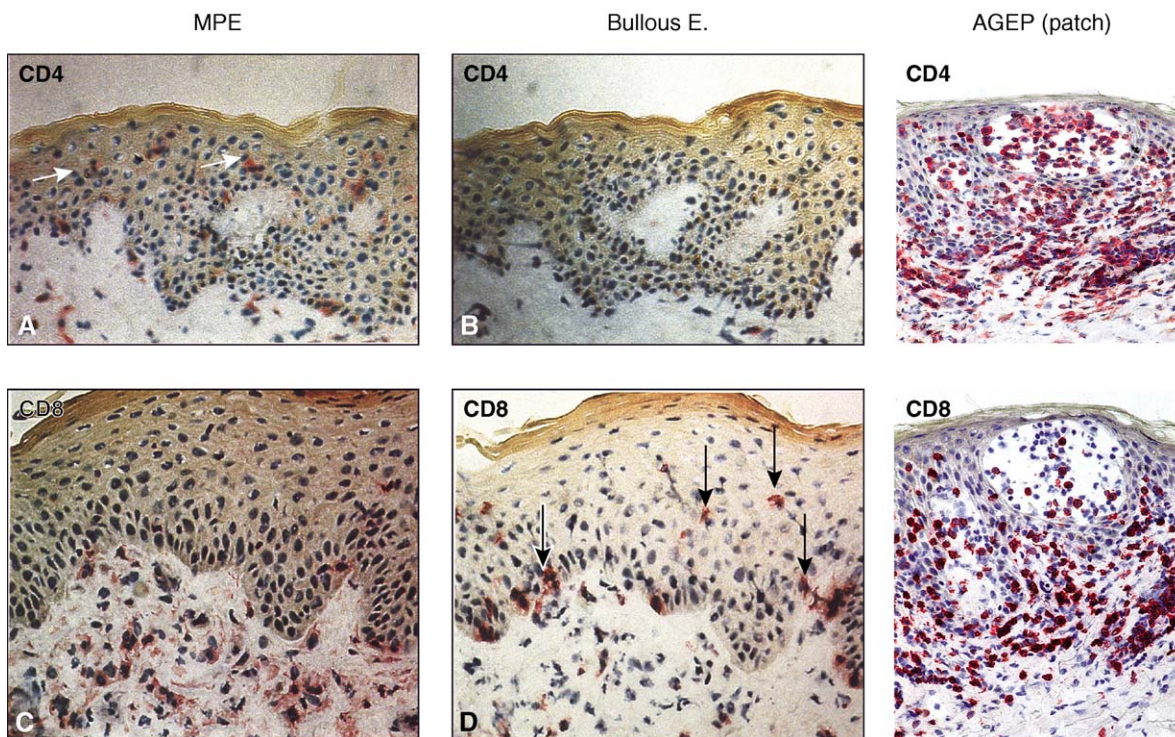


Fig. 3. Immunohistologies of three representative drug exanthemas (maculopapular, bullous exanthema, and patch test reaction of a patient with AGEP). The immunohistologies reveal infiltration of CD4 (in all three) and CD8 (mainly in bullous and pustular reactions) cells of the dermis and epidermis. These cells express perforin and granzyme G and, to a variable degree, FasL. Strong IL-8 staining is found in pustular eruption, and high IL-5 and eotaxin in maculopapular and bullous exanthema. For details, see the text and [24]. CD4 T-cells in epidermis (white arrows); CD8 T-cells in epidermis (black arrows).

(Stevens-Johnson syndrome or toxic epidermal necrolysis). Some bullous exanthemas remain relatively mild. Their immunohistology is actually similar to maculopapular exanthema, because both lesions involve T-cell infiltration, MHC upregulation on keratinocytes and immigrating T cells, and IL-5 expression in the lesions [66]. The decisive difference in bullous exanthema is a higher percentage of perforin-positive CD8+ T cells in the dermis and particularly in the epidermis. Previous work has shown that these CD8+ T cells are killer cells. When they were eluted from the skin of patients with amoxicillin-induced bullous skin reactions, they could kill other cells after mitogen stimulation [52,77]. These CD8+ killer T cells may cause formation of bullae, because such cells kill not only MHC class II-bearing keratinocytes but also resting keratinocytes that express MHC class I, which means that more cells are potential targets of the cytotoxic attack.

Toxic epidermal necrolysis

The most severe forms of drug-induced bullous skin diseases are Stevens-Johnson syndrome and toxic epidermal necrolysis. The histology of toxic epidermal necrolysis is distinct from that of maculopapular exanthema. Many dead keratinocytes are found, but only scarce cell infiltration is seen. This histology is hard to reconcile with a killing process that depends on T-cell–target cell contact, as is true for perforin/granzymeB–mediated cytotoxicity. Indeed, it has been proposed that the apoptosis of keratinocytes occurs owing to FasL, a soluble molecule of the TNF family that binds to keratinocytes via Fas, which functions as a so-called “death receptor” [75,76]. On the other hand, a recent study of patients with toxic epidermal necrolysis revealed a high amount of lymphocytes in the early bullae, which were CD8+ and had some natural killer cell–like features, whereas later in the disease, monocytes were present [82]. These cytotoxic CD8+ T cells express $\alpha\beta$ T-cell receptors, are partly CD56+, and kill via perforin/granzymeB but not via the Fas-mediated pathway at this stage of the disease [82,83]. The recently proposed treatment of patients with toxic epidermal necrolysis with anti-Fas antibody containing immunoglobulin preparations [82] is controversial and has not been confirmed, which also refutes a dominant role of FasL-mediated killing in this disease [84].

Systemic drug reactions in drug hypersensitivity syndromes

The maculopapular rashes are normally seen as benign, self-limited diseases. This may indeed be true for the more erythematous, not papular reactions, which are short lasting (<48 hours) and often negative in skin or lymphocyte transformation tests. On the other hand, in a prospective study of patients with more severe forms of maculopapular exanthema, the author and his colleagues found that approximately 25% of these patients had a transient elevation of liver enzymes (ALAT/ASAT) [66]. This liver injury was associated with a substantial activation of CD8+ T cells in the circulation, or an enhanced presence of CD8+ T cells in the affected skin. This observation illustrates that drug hypersensitivity

reactions are often systemic diseases, and cutaneous manifestations may only be the tip of the iceberg. Patients with exanthemas should undergo a physical examination defining the extent of cutaneous or mucosal involvement (Table 1), and patients with severe forms should undergo laboratory tests to define the severity of the reaction and the involvement of internal organs. One should screen for the strength of the immune reaction (reflected in the presence of activated lymphocytes and indirectly in the amount of eosinophilia in the circulation) and the involvement of internal organs (level of transaminases or creatinine, Table 1) [24]. Signs of liver or kidney involvement would be a strong argument to avoid use of the suspect drug in the future, even when only a moderate rash is seen on the skin. C-reactive protein is elevated in certain forms of hypersensitivity reactions (eg, interstitial lung or kidney diseases), but I have also observed normal levels of C-reactive protein in severe generalized drug hypersensitivity reactions affecting the skin, mucosa, and liver, indicating that it is not a marker of severity in all types of reactions.

Some drugs, in particular, anticonvulsants, dapsone, sulfamethoxazole, sulfasalazine, allopurinol, and minocycline, are known to cause a severe systemic disease in some patients, with fever, lymph node swelling, hepatitis, and various forms of exanthemas [37,38,45,47,48,85–89]. More than 90% of these cases have eosinophilia, and activated T cells are often found in the circulation, similar to acute HIV or generalized herpes virus infections. This syndrome has many names. The most frequently used are drug (or anticonvulsant) hypersensitivity syndrome (DHS) or drug-related eosinophilia with systemic symptoms (DRESS) [42,90]. Patients with this syndrome have a massive immune stimulation with many activated T cells in the circulation. The T cells can react with the parent compound and sometimes with some metabolites. They can secrete high amounts of IL-5, but in carbamazepine and lamotrigine hypersensitivity syndrome, the reactive T-cell clones secrete high levels of IFN- γ [37,38,45]. The clinical presentation resembles a generalized viral infection, such as an acute Epstein-Barr virus infection, but is distinguished by prominent eosinophilia. A peculiar

Table 1
Clinical and laboratory investigations in drug-induced exanthema

Clinical findings	Laboratory tests
Extent and type of exanthema (infiltration, bullae, pustules) pain of skin, Nikolsky sign	Eosinophilia ($>1000-1200/\mu\text{L}$) ^a , atypical (activated) lymphocytes in the circulation ($>2\%$) ^a
Involvement of mucous membranes	CRP elevation
Systemic symptoms (malaise, fever), lymphadenopathy, hepatosplenomegaly	ALAT/ASAT (increase $>2-3\times$) ^a Additional investigations depend on clinical signs of liver, kidney, lung, pancreas involvement (eg, urine analysis, creatinine)

Abbreviations: ALAT/ASAT, alanine transaminase/aspartate transaminase; CRP, creative protein.

^a The cut-off values of the laboratory parameters are estimates and are not based on prospective studies. Severe reactions can develop in the absence of these signs.

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 stopping the initial drug therapy. Recently, it has been shown that, in many patients with this syndrome, human herpes virus 6 DNA can be found during the third or fourth week of the disease (but not before), followed by a rise of antibodies to human herpes virus 6 [86,88]. Other reports document a reactivation of a cytomegalovirus infection [89]. Similar to HIV infection, in which T-cell activation can also enhance virus production, the drug-induced massive immune stimulation may somehow reactivate these lymphotropic herpes viruses, which subsequently replicate and possibly contribute to the chronic course and persistent drug intolerance in 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, because these cells are the main source of the eosinophil-stimulating cytokine IL-5 [35,45,78].

Acute generalized exanthematous pustulosis

Acute generalized exanthematous pustulosis is a rare disease with an estimated incidence equal to that of Stevens-Johnson syndrome and toxic epidermal necrolysis combined [76,91]. It is caused by drugs in more than 90% of cases (mainly, aminopenicillins, sulfonamides, and diltiazem). Its clinical hallmark is the presence of myriads of disseminated sterile pustules in the skin (Fig. 3). Patients have fever and massive leukocytosis in the blood, sometimes with eosinophilia [40,92]. The involvement of T cells has been suggested by a positive patch test reaction to the causative drug [90], which resembles the morphology of the original reaction with formation of pustules [40,93].

Immunohistology of the acute lesion reveals intraepidermal pustules, which are filled with neutrophilic leukocytes and surrounded by activated, HLA-DR-expressing CD4+ and CD8+ T cells. In contrast to maculopapular or bullous exanthema, the keratinocytes do not express MHC class II molecules but show an elevated expression of the neutrophil-attracting chemokine IL-8 (CXCL-8). Surprisingly, even the T cells migrating into the epidermis express IL-8 [40]. Analysis of sequential patch test reactions at 48 to 96 hours suggests that drug-specific T cells emigrate first, cause formation of vesicles by killing keratinocytes, and then recruit neutrophilic leukocytes into the vesicles, which are transformed to pustules [93]. In vitro analysis of drug-specific T-cell clones obtained from the blood or patch test lesions confirmed the high IL-8 production of drug-specific CD4+ T cells, whereas those from patients with other drug reactions had no or only moderate IL-8 production [40]. In addition, the T cells produce high levels of granulocyte-monocyte colony-stimulating factor (GM-CSF), which is probably important for survival of the neutrophilic leukocytes in the pustules. Because this T-cell function leads to a particular pathology

Immune reactant	Type I	IgE	Soluble antigen	Type IV a	Type IV b	Type IV c	Type IV d	Example of hypersensitivity reaction
Antigen	Type II	IgG	Cell- or matrix-associated antigen	IFN γ , TNF α (T $_H$ 1 cells)	IL-5, IL-4/IL-13 (T $_H$ 2 cells)	Perforin/GranzymeB (CTL)	CXCL-8 GM-CSF (T-cells)	Allergic rhinitis, asthma, systemic anaphylaxis
Effector cells	Type II	IgG	FcR $^+$ cells (phagocytes, NK cells)	Macrophage activation	Eosinophils	T cells	Neutrophils	AgEP Behçet disease
	Type III	IgG	Soluble antigen	Soluble antigen presented by cells or direct T cell stimulation	Soluble antigen presented by cells or direct T cell stimulation	Cell-associated antigen or direct T cell stimulation	Soluble antigen presented by cells or direct T cell stimulation	Chronic asthma, chronic allergic rhinitis, maculopapular exanthema with eosinophilia
	Type III	IgG	FcR $^+$ cells Complement immune complex	Chemokines, cytokines, cytotoxins	Cytokines, inflammatory mediators	Cytokines, inflammatory mediators	Cytokines, inflammatory mediators	Tuberculin reaction, contact dermatitis (with IVc)
	Type II	IgG	Platelets	Blood vessel	Chronic asthma, chronic allergic rhinitis, maculopapular exanthema with eosinophilia	Contact dermatitis, maculopapular and bullous exanthema, hepatitis	Behçet disease	Serum sickness, Arthus reaction
	Type II	IgG	Platelets	Hemolytic anemia, thrombocytopenia	Chronic asthma, chronic allergic rhinitis, maculopapular exanthema with eosinophilia	Contact dermatitis, maculopapular and bullous exanthema, hepatitis	Behçet disease	Hemolytic anemia, thrombocytopenia
	Type I	IgE	Mast-cell activation	Allergic rhinitis, asthma, systemic anaphylaxis	Chronic asthma, chronic allergic rhinitis, maculopapular exanthema with eosinophilia	Contact dermatitis, maculopapular and bullous exanthema, hepatitis	Behçet disease	Allergic rhinitis, asthma, systemic anaphylaxis

with infiltration of neutrophils, which is clearly different from Th1- or Th2-associated pathologies, it might be considered a separate T-cell reaction.

Subclassification of type IV reactions

Drug hypersensitivity reactions are a highly interesting topic of immunologic research. The cause of the disease is often known. The eliciting drug is often harmless and does not normally cause any damage or inflammation, implying that the inflammation seen is caused only by the immune response to the drug. The immune-mediated pathomechanism is closely related to the clinical presentation. Drug hypersensitivity reactions can serve as a model for various types of allergic/immune reactions and have been classified as type I to IV reactions (Fig. 4) [9]. Identical mechanisms are involved in infectious or autoimmune diseases, which emphasizes the importance of drug hypersensitivity as a model for other diseases, sometimes of unknown origin.

Research by the author and others has revealed that different T-cell functions in drug hypersensitivity lead to distinct clinical presentations, suggesting that the type IV reaction, which comprises cellular but not antibody-mediated reactions, can be further subclassified. The subclassification proposed in Fig. 4 considers the distinct cytokine production by T cells and incorporates the well-accepted Th1/Th2 subclassification of T cells. It includes the cytotoxic activity of both CD4 and CD8 T cells and emphasizes the participation of different effector cells, such as monocytes and CD8 T cells, eosinophils (IVb), T cells themselves (IVc), or neutrophils (IVd), which at the end are the cells causing the inflammation and tissue damage.

Type IVa reactions correspond to Th1-type immune reactions and are characterized by the activation of monocytes. The T cells promote it by IFN- γ secretion and possibly other cytokines (eg, TNF, IL-18). An *in vivo* correlate would be the granuloma formation owing to tuberculin in tuberculosis or skin tests to tuberculin, but also other reactions involving monocyte activation. These Th1 cells are also known to activate CD8 cells, which might explain the common combination of IVa and IVc reactions.

Fig. 4. Revised Gell and Coombs classification of drug reactions. Drugs can elicit all types of immune reactions. Actually, all reactions are T-cell regulated, but the effector function relies mainly on antibody-mediated effector functions (type I–III) or more T-cell/cytokine-dependent functions (type IVa to IVd). (Scheme adapted from Janeway CA, Travers P, Walport M, Shlochik M. Immunobiology. New York: Garland Publishing; 2001; with permission.) Type IVa reactions correspond to Th1 reactions with high IFN- γ /TNF- α secretion and involve monocyte/macrophage activation. Often, one can see a CD8 cell recruitment (type IVc reaction). Type IVb reactions correspond to eosinophilic inflammation and a Th2 response with high IL-4/IL-5/IL-13 secretion. They are often associated with an IgE-mediated type I reaction. Type IVc cytotoxic reactions (by CD4 and CD8 cells) rely on cytotoxic T cells themselves as effector cells. They seem to occur in all drug-related delayed hypersensitivity reactions. Type IVd corresponds to a T-cell-dependent, sterile neutrophilic inflammatory reaction. It is clearly distinct from the rapid influx of neutrophils in bacterial infections and seems to be related to high CXCL-8/GM-CSF production by T cells (and tissue cells).

Type IVb reactions correspond to the Th2 cellular immune response and use the eosinophil as an effector cell of inflammation. High production of the Th2 cytokine IL-5 leads to an eosinophilic inflammation. In addition, IgE production by IL-4/IL-13 is boosted. An *in vivo* correlate might be an eosinophil-rich maculopapular exanthema, but also infestations with nematodes or an allergic inflammation of the bronchi or nasal mucosa (asthma and rhinitis).

In type IVc reactions, T cells themselves act as effector cells. They emigrate to the tissue and can kill tissue cells such as hepatocytes or keratinocytes in a perforin/granzymeB-dependent manner. Such reactions occur in most drug-induced delayed hypersensitivity reactions, such as maculopapular or bullous skin diseases, as well as neutrophilic inflammations (AGEP) and contact dermatitis.

In type IVd reactions, the concept that T cells cause neutrophilic inflammation as well is rather new. A typical example would be AGEP. Such IL-8 (and GM-CSF)–producing T cells recruit neutrophils and prevent apoptosis of these inflammatory cells. Some of these T cells do not produce IFN- γ or IL-4/IL-5. They are clearly distinct from Th1 or Th2 T cells and may represent a T-cell subset with a unique function (P. Schörli et al, unpublished data, 2004). These mechanisms might also be important in other MHC-associated, chronic autoimmune diseases such as psoriasis and Behçet's disease, which are not classifiable by the Th1 or Th2 scheme [94].

Overlapping T-cell functions in drug hypersensitivity

The immune system has evolved different strategies to combat real or assumed aggressors. The different types of immune responses are determined by various factors, such as the type of antigen (virus, bacteria, fungus), its uptake into the antigen-presenting cell, the stimulation of dendritic and other antigen-presenting cells via different TOLL-like receptors or a certain cytokine milieu, the processing of the antigen, and its presentation on MHC class I or II. Consequently, the evolving immune reactions follow certain rules and can be dissected based on different pathways into different T cell regulated responses.

The natural immune response often uses different effector pathways simultaneously and sequentially to achieve an optimal and effective defense. Even the immune response to simple protein antigens, such as pollen allergens, frequently relies on antibody (type I; IgE) and cellular responses (type IVb, eosinophils), both regulated by Th2 T cells. Nevertheless, these protein-specific immune reactions follow certain predictable rules.

The bizarre nature of some drug hypersensitivity reactions is most likely explained by the fact that drug reactions do not follow the “normal” rules of immune responsiveness to protein antigens and appear contradictory to prevailing concepts elaborated with protein antigens. In drug allergy, antigen processing can be bypassed, many different antigenic determinants can be formed in one individual, different MHC molecules can serve simultaneously as presenting structures for the same antigen (drug), and the inert drug itself as well as a metabolite with haptentlike characteristics could be the target structures of the immune

As shown in **Figs. 4 and 5**, in AGEp, different T-helper cell functions (IVb, IVc, and IVd) occur simultaneously, because some patients have a neutrophilia and eosinophilia in the blood. Beside neutrophilic pustules, a massive eosinophil recruitment into the tissue can sometimes be observed, and vesicles are formed owing to cytotoxic activity of T cells [40,93]. IL-5 and IL-8-secreting T cells as well as T-cell-mediated cytotoxicity are found in such reactions. A similar overlap of various immune reactions can 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 serology [95].

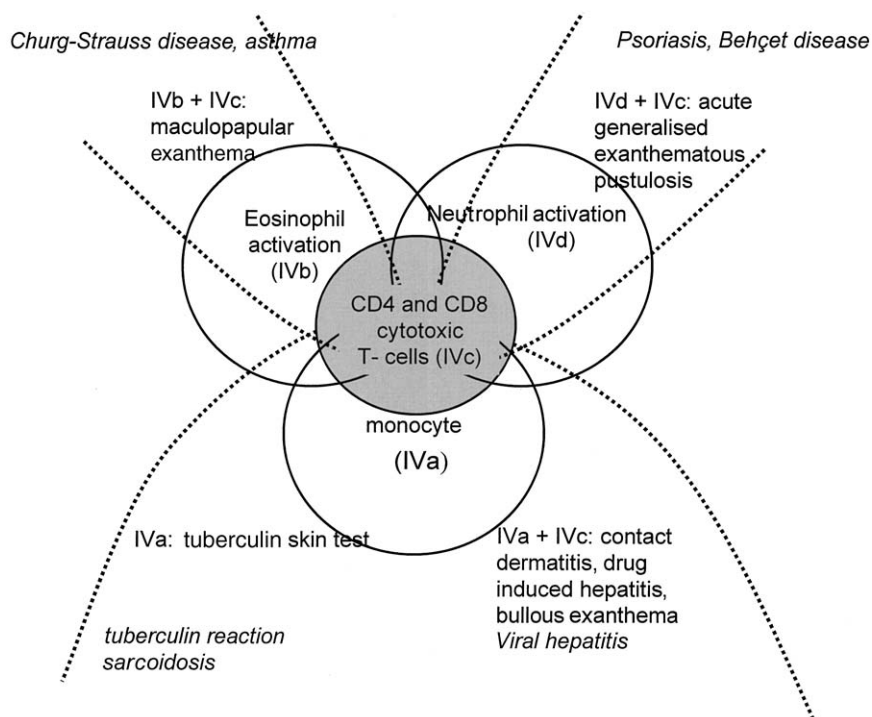


Fig. 5. Schematic representation of overlapping immune functions. The clinical symptomatology of diseases is often complex and can be traced back to various immune functions. These immune mechanisms may occur together or sequentially and lead to different forms of drug hypersensitivity reactions (or phenotypically related diseases, shown in *italics*). Type IVc reactions can occur in association with all other reactions, leading to maculopapular (IVc and IVb), bullous (IVa and IVc), and pustular exanthema (IVc and IVd). Type IVa reactions involve monocyte activation and are often associated with a CD8 cell activation. Maculopapular reactions are a combination of cytotoxic CD4 cell and IVb reactions (eosinophils), and bullous skin reactions can be seen as combinations of IVa, sometimes IVb, and IVc reactions.

These considerations have an impact on clinical practice. They are important for drug hypersensitivity testing. One should consider a drug hypersensitivity reaction if the patient has a bizarre clinical presentation that is not compatible with an infectious cause.

Outlook: a chance to learn from the immune-mediated side effects of drugs

Drug hypersensitivity reactions can be seen as unintended experiments of the treating physician [24]. What can we learn from such experiments? Do we actually take the chance to learn from them? Two obvious aims of a better understanding of hypersensitivity reactions would be (1) to improve the treatment of patients with these drug-induced side effects, remembering that these are actually iatrogenic diseases; and (2) to prevent these side effects by predicting the immunogenicity of a drug during its development.

The finding that drugs can directly interact with T-cell receptors (p-i concept) may open new possibilities for specific interventions in immune-mediated diseases. The p-i concept implies that each of the millions of different antigen-specific T-cell receptors could be a potential target of a drug therapy by stimulating or blocking the T cell. The sometimes massive and even lethal immune stimulation seen in drug hypersensitivity teaches us that such interactions between the drug and the T-cell receptor can be extremely powerful. In addition, this interaction can mediate its effect by a variety of mechanisms.

In contrast to usual treatments of immune-mediated diseases, which are always directed to suppress or enhance the overall immune system in an unspecific way, the p-i concept opens the theoretical possibility of suppressing or enhancing a specific immune response by a certain T cell in a highly focused way (actually similar to a vaccination). Drugs could be designed to interact with a certain target cell by binding to its unique T-cell receptor. What we need to achieve this rather futuristic goal is a better knowledge of the structural interplay of the drug with the T-cell receptor, and an understanding of the role of MHC molecules in these reactions and the relationship of T-cell receptor structure to its peptide specificity. Eventually, the study of drug hypersensitivity may open a new promising area of research, combining immunology with pharmacology in an unexpected way.

Summary

Immune reactions to drugs can elicit many different diseases involving multiple organ systems. These hypersensitivity reactions involve drug-specific CD4+ and CD8+ T cells and antibodies and different effector cells. Drug-specific T cells can be detected in the circulation and in the affected tissue. They recognize drugs via their β -T-cell receptors in a major histocompatibility complex (MHC)-dependent way. Drugs are stimulatory for T cells if they bind covalently

to peptides (haptens), or if the drug has structural features allowing it to interact with certain T-cell receptors directly. The later concept is new and has been termed *pharmacologic interaction with immune receptors* (p-i concept). It leads to an exclusive T-cell reaction.

Immunohistochemistry and functional in vitro studies of drug-reactive T cells from patients with distinct forms of exanthema reveal that these cells exert distinct functions that lead to different clinical phenotypes. In maculopapular exanthema, perforin and granzyme B-positive, CD4+ T cells kill activated keratinocytes, whereas a large number of cytotoxic CD8+ T cells in the epidermis is associated with the formation of vesicles and bullae. Drug-specific T cells also orchestrate inflammatory skin reactions through the release of various cytokines (eg, interleukin [IL]-5, interferon- γ , and chemokines [eg, IL-8]). Depending on the particular function of the drug-activated T cells, a specific clinical presentation may evolve (eg, bullous or pustular exanthema). Taken together, these data allow further subclassification of delayed type hypersensitivity reactions (type IV according to Gell and Coombs) into T-cell reactions, which, through the release of certain cytokines and chemokines, preferentially activate and recruit monocytes (IVa), eosinophils (IVb), or neutrophils (IVd). Moreover, cytotoxic functions by CD4+ or CD8+ T cells (type IVc) appear to be involved in all type IV reactions.

References

- [1] Naisbitt DJ, Gordon SF, Pirmohamed M, Park BK. Immunological principles of adverse drug reactions: the initiation and propagation of immune responses elicited by drug treatment. *Drug Saf* 2000;23:483–507.
- [2] Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA* 1998;279:1200–4.
- [3] Hunziker T, Bruppacher R, Kuenzi UP, et al. Classification of ADRs—a proposal for harmonization and differentiation based on the experience of the comprehensive hospital drug monitoring Bern/St. Gallen, 1974–1993. *Pharmacoepidemiology and Drug Safety* 2002;11:159–63.
- [4] Pouyanne P, Haramburu F, Imbs JL, Begaud B. Admissions to hospital caused by adverse drug reactions: cross-sectional incidence study. French Pharmacovigilance Centres. *BMJ* 2000; 320:1036.
- [5] Roujeau JC, Stern RS. Severe adverse cutaneous reactions to drugs. *N Engl J Med* 1994;331: 1272–85.
- [6] Bigby M, Jick S, Jick H, Arndt K. Drug-induced cutaneous reactions: a report from the Boston Collaborative Drug Surveillance Program on 15,438 consecutive inpatients, 1975 to 1982. *JAMA* 1986;256:3358–63.
- [7] Swanbeck G, Dahlberg E. Cutaneous drug reactions: an attempt to quantitative estimation. *Arch Dermatol Res* 1992;284:215–8.
- [8] Hunziker T, Kunzi UP, Braunschweig S, Zehnder D, Hoigné R. Comprehensive hospital drug monitoring (CHDM): adverse skin reactions, a 20-year survey. *Allergy* 1997;52:388–93.
- [9] Coombs PRA, Gell PGH. Classification of allergic reactions responsible for clinical hypersensitivity and disease. In: Gell RRA, editor. *Clinical aspects of immunology*. Oxford: Oxford University Press; 1968. p. 575–96.
- [10] Vercelli D. Immunoglobulin E and its regulators. *Curr Opin Allergy Clin Immunol* 2001;1:61–5.
- [11] Romagnani S. The Th1/Th2 paradigm. *Immunol Today* 1997;18:263–6.

- [12] Janeway CA, Travers P, Walport M, Shlochik M. Immunobiology. New York: Garland Publishing; 2001.
- [13] Pichler WJ, Schnyder B, Zanni M, Hari Y, von Greyerz S. Role of T-cells in drug allergies. *Allergy* 1998;53:225–32.
- [14] Adkinson Jr NF, Essayan D, Gruchalla R, Haggerty H, Kawabata T, Sandler JD, et al. Health and Environmental Sciences Institute Task Force. Task force report: future research needs for the prevention and management of immune-mediated drug hypersensitivity reactions. *J Allergy Clin Immunol* 2002;109(3):461–78.
- [15] Pichler WJ. Lessons from drug allergy: against dogmata. *Curr Allergy Asthma Rep* 2003; 3(1):1–3.
- [16] Landsteiner K, Jacobs J. Studies on the sensitization of animals with simple chemical compounds. *J Exp Med* 1935;61:643–56.
- [17] Schneider CH, de Weck AL. A new chemical aspect of penicillin allergy: the direct reaction of penicillin with epsilon-amino-groups. *Nature* 1965;208:57–9.
- [18] Pirmohamed M, Kitteringham NR, Park BK. The role of active metabolites in drug toxicity. *Drug Saf* 1994;11:114–44.
- [19] Knowles SR, Uetrecht J, Shear NH. Idiosyncratic drug reactions: the reactive metabolite syndromes. *Lancet* 2000;356:1587–91.
- [20] Padovan E, Bauer T, Tongio MM, Kalbacher H, Weltzien HU. Penicilloyl peptides are recognized as T-cell antigenic determinants in penicillin allergy. *Eur J Immunol* 1997;27:1303–7.
- [21] Park BK, Pirmohamed M, Kitteringham NR. Role of drug disposition in drug hypersensitivity: a chemical, molecular, and clinical perspective. *Chem Res Toxicol* 1998;11:969–88.
- [22] Mauri-Hellweg D, Zanni M, Frei E, et al. Cross-reactivity of T-cell lines and clones to beta-lactam antibiotics. *J Immunol* 1996;157:1071–9.
- [23] Weltzien HU, Moulin C, Martin S, Padovan E, Hartmann U, Kohler J. T-cell immune response to haptens: structural models for allergic and autoimmune reactions. *Toxicology* 1996;107:141–51.
- [24] Pichler WJ. Delayed drug hypersensitivity reactions. *Ann Intern Med* 2003;139:683–90.
- [25] Griem P, Wulferink M, Sachs B, Gonzalez JB, Gleichmann E. Allergic and autoimmune reactions to xenobiotics: how do they arise? *Immunol Today* 1998;19:133–41.
- [26] Cribb AE, Spielberg SP. Sulfamethoxazole is metabolized to the hydroxylamine in humans. *Clin Pharmacol Ther* 1992;51:522–6.
- [27] Reilly TP, Lash LH, Doll MA, Hein DW, Woster PM, Svensson CK. A role for bioactivation and covalent binding within epidermal keratinocytes in sulfonamide-induced cutaneous drug reactions. *J Invest Dermatol* 2000;114:1164–73.
- [28] Naisbitt DJ, Gordon SF, Pirmohamed M, et al. Antigenicity and immunogenicity of sulfamethoxazole: demonstration of metabolism-dependent haptenation and T-cell proliferation in vivo. *Br J Pharmacol* 2001;139:295–305.
- [29] Dansette PM, Bonierbale E, Minoletti C, Beaune PH, Pessayre D, Mansuy D. Drug-induced immunotoxicity. *Eur J Drug Metab Pharmacokinet* 1998;23:443–51.
- [30] Schnyder B, Mauri-Hellweg D, Zanni M, Bettens F, Pichler WJ. Direct, MHC-dependent presentation of the drug sulfamethoxazole to human T-cell clones. *J Clin Invest* 1997;100:136–41.
- [31] Zanni MP, von Greyerz S, Schnyder B, et al. HLA-restricted, processing- and metabolism-independent pathway of drug recognition by human $\alpha\beta$ T lymphocytes. *J Clin Invest* 1998; 102:1591–8.
- [32] Zanni MP, von Greyerz S, Schnyder B, Wendland T, Pichler WJ. HLA-unrestricted presentation of lidocaine by HLA-DR molecules to specific β + T-cell clones. *Int Immunol* 1998;10:507–15.
- [33] von Greyerz S, Zanni M, Frutig K, Schnyder B, Pichler WJ. Interaction of sulfonamide derivatives with the T-cell receptor of sulfamethoxazole-specific β + T-cell clones. *J Immunol* 1999;162: 595–602.
- [34] Schnyder B, Burkhart C, Schnyder-Frutig K, et al. Recognition of sulfamethoxazole and its reactive metabolites by drug specific T-cells from allergic individuals. *J Immunol* 2000;164: 6647–54.
- [35] Burkhart C, von Greyerz S, Depta JPH, Naisbitt DJ, et al. Influence of reduced glutathione on the

- proliferative response of sulfamethoxazole-specific and sulfamethoxazole-metabolite specific human CD4+ T-cells. *Br J Pharmacol* 2001;132:623–30.
- [36] Pichler WJ. Pharmacological interaction of drugs with antigen-specific immune receptors: the p-i concept. *Curr Opin Allergy Clin Immunol* 2002;2:301–5.
- [37] Naisbitt DJ, Farrell J, Wong G, Depta JPH, Dodd CC, Hopkins J, et al. Characterization of skin homing lamotrigine-specific T-cells from hypersensitive patients. *J Allergy Clin Immunol* 2003;111:1393–403.
- [38] Naisbitt DJ, Britschgi M, Wong G, Farrell J, Depta JPH, Chadwick DW, et al. Hypersensitivity reactions to carbamazepine: characterization of the specificity, phenotype, and cytokine profile of drug-specific T-cell clones. *Mol Pharmacol* 2003;63:732–41.
- [39] Sieben S, Kawabuko Y, Al Masaoudi T, Merk HF, Bloemeke B. Delayed-type hypersensitivity reaction to paraphenylenediamine is mediated by 2 different pathways of antigen recognition by specific alphabeta human T-cell clones. *J Allergy Clin Immunol* 2002;109:1005–11.
- [40] Britschgi M, Steiner U, Schmid S, et al. T-cell involvement in drug-induced acute generalized exanthematous pustulosis. *J Clin Invest* 2001;11:1433–41.
- [41] von Greyl S, Burkhart C, Pichler WJ. Molecular basis of drug recognition by specific T-cell receptors. *Int Arch Allergy Immunol* 1999;119:173–80.
- [42] von Greyl S, Bültemann G, Schnyder K, et al. Degeneracy and additional alloreactivity of drug-specific human $\alpha\beta$ + T-cell clones. *Int Immunol* 2001;7:877–85.
- [43] Depta J, Altnauer F, Gerner K, Burkhart CH, Weltzien HU, Pichler WJ. Drug interaction with T-cell receptors: T-cell receptor-density determines degree of cross-reactivity. *J Allergy Clin Immunol* 2004;113:519–22.
- [44] Pichler WJ. Predictive drug allergy testing, an alternative viewpoint. *Toxicology* 2001;158: 31–41.
- [45] Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ. Activation of drug specific CD4+ and CD8+ T-cells in individuals allergic to sulfonamides, phenytoin and carbamazepine. *J Immunol* 1995;155:462–72.
- [46] Aster RH. Drug-induced immune thrombocytopenia: an overview of pathogenesis. *Semin Hematol* 1999;36(1 Suppl):2–6.
- [47] Sullivan JR, Shear NH. The drug hypersensitivity syndrome: what is the pathogenesis? *Arch Dermatol* 2001;137:357–64.
- [48] Bourezane Y, Salard D, Hoen B, Vandel S, Drobacheff C, Laurent R. DRESS (drug rash with eosinophilia and systemic symptoms) syndrome associated with nevirapine therapy. *Clin Infect Dis* 1998;27:1321–2.
- [49] Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002;359:727–32.
- [50] Ching W-H, Hing S-I, Hong H-S, Hsieh H-S, Yang L-C, Ho H-C, et al. A marker for Stevens-Johnson syndrome. *Nature* 2004;428:486.
- [51] Pirmohamed M, Park BK. Genetic susceptibility to adverse drug reactions. *Trends Pharmacol Sci* 2001;22:298–305.
- [52] Hertl M, Geisel J, Boecker C, Merk HF. Selective generation of CD8+ T-cell clones from the peripheral blood of patients with cutaneous reactions to beta-lactam antibiotics. *Br J Dermatol* 1993;128:619–26.
- [53] Brander C, Mauri-Hellweg D, Bettens F, Rolli HP, Goldman M, Pichler WJ. Heterogeneous T-cell responses to β -lactam-modified self-structures are observed in penicillin-allergic individuals. *J Immunol* 1995;155:2670–8.
- [54] Renn CN, Straff W, Dorfmueller A, Al-Masaoudi T, Merk HF, Sachs B. Amoxicillin-induced exanthema in young adults with infectious mononucleosis: demonstration of drug-specific lymphocyte reactivity. *Br J Dermatol* 2002;147(6):1166–70.
- [55] FDA Advisory Committee Meeting, March 4, 2003. Factive (gemifloxacin). Available at: <http://www.fda.gov/ohrms/dockets/ac/03/slides>. Accessed 2003.
- [56] Pichler WJ. Multiple drug hypersensitivity. In: Marone G, editor. *Proceedings of the 21st EAACI Congress*, 2002. Naples: JGC editions; 2003. p. 193–8.

- [57] Sullivan TJ, Remedios C, Ong MD, Gilliam LK. Studies of the multiple drug allergy syndrome. *J Allergy Clin Immunol* 1989;83:270.
- [58] Moseley EK, Sullivan TJ. Allergic reactions to antimicrobial drugs in patients with a history of prior drug allergy. *J Allergy Clin Immunol* 1991;87:226.
- [59] Zanni MP, von Greyerz S, Hari Y, Schnyder B, Pichler WJ. Recognition of local anaesthetics by β + T-cells. *J Invest Dermatol* 1999;112:197–204.
- [60] Blanca M. The contribution of the side chain of penicillins in the induction of allergic reactions. *J Allergy Clin Immunol* 1994;94:562–3.
- [61] Pichler WJ. Drug-induced autoimmunity. *Curr Opin Allergy Clin Immunol* 2003;3:249–53.
- [62] Medzhitov R, Janeway Jr C. Innate immunity. *N Engl J Med* 2000;343:338–44.
- [63] Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Curr Opin Immunol* 2001; 13:114–9.
- [64] Fellner MJ, Prutkin L. Morbilliform eruptions caused by penicillin: a study by electron microscopy and immunologic tests. *J Invest Dermatol* 1970;55:390–5.
- [65] Yawalkar N, Shrikhande M, Hari Y, Nievergelt H, Braathen LR, Pichler WJ. Evidence for a role for IL-5 and eotaxin in activating and recruiting eosinophils in drug-induced cutaneous eruptions. *J Allergy Clin Immunol* 2000;106:1171–6.
- [66] Hari Y, Frutig K, Humi M, et al. T-cell involvement in cutaneous drug eruptions. *Clin Exp Allergy* 2001;31:1398–408.
- [67] Yawalkar N, Egli F, Hari Y, Nievergelt H, Braathen LR, Pichler WJ. Infiltration of cytotoxic T-cells in drug-induced cutaneous eruptions. *Clin Exp Allergy* 2000;30:847–55.
- [68] Barbaud AM, Bene MC, Reichert-Penetrat S, Jacquin-Petit MA, Schmutz JL, Faure GC. Immunocompetent cells and adhesion molecules in 14 cases of cutaneous drug reactions induced with the use of antibiotics. *Arch Dermatol* 1998;134:1040–1.
- [69] Barbaud AM, Bene MC, Schmutz JL, Ehlinger A, Weber M, Faure GC. Role of delayed cellular hypersensitivity and adhesion molecules in amoxicillin-induced morbilliform rashes. *Arch Dermatol* 1997;133:481–6.
- [70] Stepp SE, Mathew PA, Bennett M, de Ssainat Basile G, Kumar V. Perforin: more than just an effector molecule. *Immunol Today* 2000;21:254–6.
- [71] Schnyder B, Frutig K, Mauri-Hellweg D, Limat A, Pichler WJ. T-cell-mediated cytotoxicity against keratinocytes in sulfamethoxazole-induced skin reaction. *Clin Exp Allergy* 1998;28: 1412–7.
- [72] Behrendt C, Gollnick H, Bonnekoh B. Up-regulated perforin expression of CD8+ blood lymphocytes in generalized non-anaphylactic drug eruptions and exacerbated psoriasis. *Eur J Dermatol* 2000;10:365–9.
- [73] Yawalkar N, Hari Y, Frutig K, et al. T-cells isolated from positive epicutaneous test reactions to amoxicillin and ceftriaxone are drug specific and cytotoxic. *J Invest Dermatol* 2000;115: 647–52.
- [74] Yawalkar N, Pichler WJ. Immunohistology of drug induced exanthemas: clues to its pathogenesis. *Curr Opin Allergy Clin Immunol* 2001;1:299–303.
- [75] Viard I, Wehrli P, Bullani R, et al. Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. *Science* 1998;282:490–3.
- [76] Sharma K, Wang RX, Zhang LY, et al. Death the Fas way: regulation and pathophysiology of CD95 and its ligand. *Pharmacol Ther* 2000;88:333–47.
- [77] Hertl M, Bohlen H, Jugert F, Boecker C, Knaup R, Merk HF. Predominance of epidermal CD8+ T lymphocytes in bullous cutaneous reactions caused by beta-lactam antibiotics. *J Invest Dermatol* 1993;101:794–9.
- [78] Pichler WJ, Zanni M, von Greyerz S, Schnyder B, Mauri-Hellweg D, Wendland T. High IL-5 production by drug specific T-cell clones. *Int Arch Allergy Immunol* 1997;113:177–80.
- [79] Hari Y, Urwyler A, Humi M, et al. Distinct serum cytokine levels in drug or measles induced exanthema. *Int Arch Allergy Immunol* 1999;120:225–9.
- [80] Choquet-Kastylevsky G, Intrator L, Chenal C, Bocquet H, Revuz J, Roujeau JC. Increased levels of interleukin 5 are associated with the generation of eosinophilia in drug-induced hypersensitivity syndrome. *Br J Dermatol* 1998;139:1026–32.

- [81] Gerber BO, Zanni MP, Ugucioni M, et al. Functional expression of the eotaxin receptor, CCR3, in T lymphocytes co-localizing with eosinophils. *Curr Biol* 1997;7:836–43.
- [82] Le Cleach L, Delaire S, Boumsell L, et al. Blister fluid T lymphocytes during toxic epidermal necrolysis are functional cytotoxic cells which express human natural killer (NK) inhibitory receptors. *Clin Exp Immunol* 2000;119:225–30.
- [83] Nassif A, Bensussan A, Dorothée G, et al. Drug-specific cytotoxic T lymphocytes in the skin lesions of a patient with toxic epidermal necrolysis. *J Invest Dermatol* 2002;118:728–33.
- [84] Bachot N, Roujeau JC. Intravenous immunoglobulins in the treatment of severe drug eruptions. *Curr Opin Allergy Clin Immunol* 2003;3:269–74.
- [85] Knowles SR, Shapiro LE, Shear NH. Anticonvulsant hypersensitivity syndrome: incidence, prevention and management. *Drug Saf* 1999;21:489–501.
- [86] Descamps V, Valance A, Edlinger C, et al. Association of human herpes virus 6 infection with drug reaction with eosinophilia and systemic symptoms. *Arch Dermatol* 2001;137:301–4.
- [87] Schaub N, Bircher AJ. Severe hypersensitivity syndrome to lamotrigine confirmed by lymphocyte stimulation in vitro. *Allergy* 2000;55:191–3.
- [88] Hashimoto K, Yasukawa M, Tohyama M. HHV-6 and drug allergy. *Curr Opin Allergy Clin Immunol* 2003;3:255–60.
- [89] Arakawa M, Kakuto Y, Ichikawa K, Chiba J, Tabata N, Sasaki Y. Allopurinol hypersensitivity syndrome associated with systemic cytomegalovirus infection and systemic bacteremia. *Intern Med* 2001;40:331–5.
- [90] Wolkenstein P, Chosidow O, Fléchet ML, et al. Patch-testing in severe cutaneous adverse drug reactions including Stevens-Johnson syndrome and toxic epidermal necrolysis. *Contact Dermatitis* 1996;35:234–6.
- [91] Beylot C, Bioulac P, Doutre MS. Pustuloses exanthématiques aiguës généralisées: a propos de 4 cas. *Ann Dermatol Venerol* 1980;107:37–48.
- [92] Roujeau JC, Bioulac-Sage P, Bourseau C. Acute generalized exanthematous pustulosis: analysis of 63 cases. *Arch Dermatol* 1991;127:1333–8.
- [93] Schmid S, Kuechler PC, Britschgi M, Steiner UC, Yawalkar N, Limat A, et al. Acute generalized exanthematous pustulosis: role of cytotoxic T-cells in pustule formation. *Am J Pathol* 2002;161:2079–86.
- [94] Mochizuki M, Morita E, Yamamoto S, Yamana S. Characteristics of T-cell lines established from skin lesions of Behçet's disease. *J Dermatol Sci* 1997;15:9–13.
- [95] Neukomm C, Yawalkar N, Helbling A, Pichler WJ. T-cell reactions to drugs in distinct clinical manifestations of drug allergy. *J Invest Allergy Clin Immunol* 2001;11:275–84.