

REVIEW

Mechanisms in cutaneous drug hypersensitivity reactions

P. S. Friedmann*, M.-S. Lee†, A. C. Friedmann† and R. St C. Barnetson†

*Dermatopharmacology Unit, Southampton General Hospital, Southampton, UK, and †Department of Dermatology, Royal Prince Alfred Hospital, Camperdown NSW 2050, Australia

Summary

Up to 3% of all hospital admissions are due to adverse drug reactions (ADRs), and between 10% and 20% of hospital inpatients develop ADRs. Individual susceptibility to becoming 'sensitized' or allergic to a drug is thought to result from altered metabolic handling of the drug. Reactive intermediate compounds form haptens, bind to proteins and induce immune responses. Depending on whether the immune system generates antibodies or sensitized T cells, different clinical patterns of hypersensitivity may result. At present, both *in vivo* or *in vitro* tests to identify the culprit drug or to confirm the presence of hypersensitivity are not widely used because they are either not generally robust or not readily accessible. *In vitro* tests require the true immunogen/antigen to detect antibodies or sensitized T cells. As the metabolic basis underlying susceptibility to adverse drug reactions is elucidated, the resolution of immunological mechanisms and development of reliable tests will ensue. This will also become of great value for prediction of individuals at risk of becoming sensitized by a particular drug.

Introduction

Adverse drug reactions (ADRs) are responsible for about 3% of all hospital admissions and between 10% and 20% of hospital inpatients develop ADRs. ADRs can have many manifestations and, in order to approach diagnosis and identification of the culprit drug, it is important to understand their classification [1].

- Type A (augmented) are the most common type. They are predictable from the primary and secondary pharmacology of the drug and are dose-related – hence dose reduction resolves the problem. Genetic factors are important in determining susceptibility to these drug effects.
- Type B (bizarre or idiosyncratic) are not predictable from the known pharmacology of the drug. The term reflects patient individuality rather than mechanism – which will be dealt with below.
- Type C (chemical) could be predicted from the structure of the drug or its metabolites. Examples include paracetamol that is bioactivated in the liver to a toxic quinone imine that is hepatotoxic and azathioprine, metabolized to 6-mercaptopurine that is myelotoxic and must be further converted by the enzyme thiopurine methyl transferase (TPMT).
- Type D (delayed) are late effects of drugs including teratogenicity and carcinogenicity.
- Type E (end of dose) are withdrawal reactions that follow discontinuation of the drug; e.g. seizures after the discontinuation of benzodiazepines.

In considering ADRs, it is important to distinguish between agents that are small molecular weight organic compounds (xenobiotics) and larger protein or glycoprotein agents; the former undergo metabolic processing, which is usually critical in determining the generation, and removal of metabolites, which are thought to be the cause of the ADR. Macromolecules are degraded in different ways, but proteolytic degradation may generate peptide fragments with other biological or immunogenic properties.

In considering the immune basis of type B reactions, the general principles of the immune system and its responses apply. It takes an initial exposure to an immunogen to prime the immune system and to activate the development of immunological memory – which, in humans takes about 7 days. It then requires further exposure to the immunogen to elicit the immune effector mechanisms. This may be a second course of the same drug at a later date, or the availability of the drug from a continuous/ongoing exposure. An observation that often confuses the picture is the development of an ADR after the patient has been taking the culprit drug for a longer period. Although the reasons for this are not known, it may be because something changes in the host's internal milieu altering the interaction with the drug, suddenly allowing generation of immunogenic metabolite(s). Alternatively, it may take a longer time for low levels of altered (immunogenic) proteins to activate immune recognition.

Type B ADRs can affect single or multiple organ systems, but the skin is one of the most commonly involved organs; cutaneous reactions to drugs affect 2–3% of hospitalized patients [2]. The visibility of the skin makes it the most sensitive clinical indicator of the presence of an ADR. There is evidence that immune hypersensitivity mechanisms are involved in many type B reactions; moreover, the clinical patterns of drug reactions can be related to immune

Correspondence: Professor Peter S. Friedmann, MD, FRCP, F. Med. Sci., Dermatopharmacology Unit, Level F South Block (825), Southampton General Hospital, Southampton, UK. E-mail: psf@soton.ac.uk

mechanisms. This review focuses on drug reactions caused by immunological mechanisms and gives a brief overview of current techniques available for investigating suspected drug allergy.

Mechanisms of immune sensitization

Drug disposition

The fundamental processes underlying activation of the acquired immune system, which generates specific immunological memory and humoral and cellular effector mechanisms, must start with the presentation of antigen to T lymphocytes. The classical view is that small molecules, such as drugs with a molecular weight less than 1000 Da, are not recognizable by T lymphocytes unless they become 'haptens' and are conjugated to a protein carrier (Fig. 1). Antigen-presenting cells (APCs) 'process' the hapten-modified carrier protein, degrading it to peptides that are complexed with the major histocompatibility complex (MHC) molecules and displayed on the cell surface to be recognized by T cells. In order for a drug molecule to become a hapten, it must bind to a protein carrier. Many native drug molecules are not intrinsically protein-reactive, although some including penicillins, cephalosporins and captopril can bind directly to serum or cellular proteins [1, 3]. It is thought that protein-reactive haptens are generated during the process of biodegradation and detoxication.

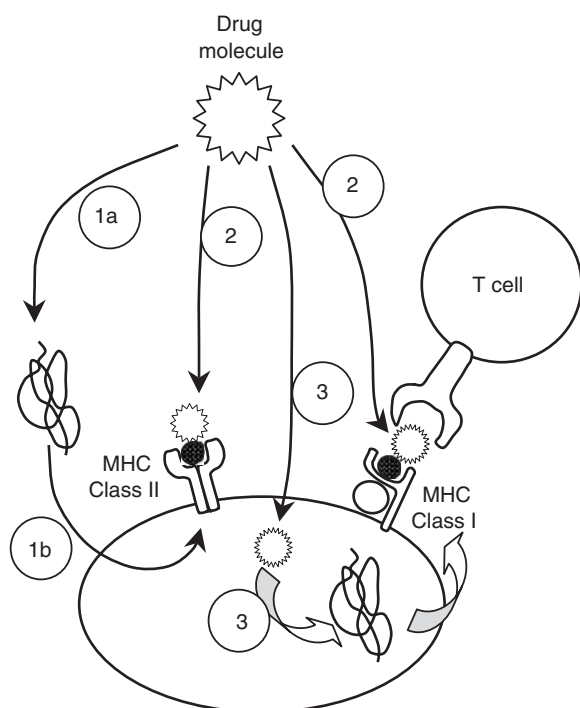


Fig. 1. Mechanisms of immune response to drug molecules. Reactive drug molecules can act as haptens, interacting directly with proteins (1a) or with MHC-bound peptides (2). If an exogenous protein is haptenated, it undergoes processing by antigen-presenting cells (1b), is complexed with MHC (presumably Class 2) and the haptenated peptides are presented to T cells. The drug molecule may only become a hapten after metabolic bioactivation (3). It can then bind to proteins that undergo processing for presentation, via MHC class 1 and 2, to T cells with receptors able to recognize the hapten-modified peptide.

Xenobiotics undergo metabolic processing in order to be 'detoxified' and excreted. The first phase of metabolism is performed by enzymes, the biggest and most important group of which are the cytochrome P450s (CYP450). These enzymes mainly perform oxygen addition reactions intended to detoxify the substrate. However, sometimes the products resulting from Phase 1 metabolism may be directly toxic – as for paracetamol and azathioprine. Also, they may be chemically unstable and reactive, able to bind to cellular proteins and other macromolecules. This may result in structural alterations leading to immune recognition. Hence, the metabolites generated by Phase 1 metabolism usually require immediate detoxication by Phase 2 enzymes that perform processes such as acetylation, sulphation, glucuronidation and glutathione conjugation. The main hypothesis to explain what is different about the individuals who develop ADRs (at least of types B and C) suggests that Phase 2 detoxication processes are deficient or fail, allowing persistence of higher levels of toxic or immunogenic metabolites [4]. So far, efforts to identify specific deficiencies of detoxicating enzymes have not met with success. However, there are a number of ways in which normal xenobiotic detoxication may become impaired with consequent generation of immunogenic haptens. A fairly common observation is that an individual is taking one drug without any problem, but when a second drug, which can interfere with or alter the metabolic handling of the first drug, is introduced, an adverse reaction develops to the first drug. A well-known example is the development of reactions to carbamazepine after the introduction of sodium valproate. This reflects the fact that the detoxication mechanisms might be only just coping with one drug, but when overloaded they cannot cope, so toxic or immunogenic metabolites persist to exert their effects.

This indicates a general way in which hapten formation may occur if drug-detoxicating reserves become depleted as a result of major oxidative stress. The glutathione-generating system is a major component of both general cellular anti-oxidant defences and also is an important Phase 2 drug-detoxifying mechanism. Many drug metabolites are detoxified by conjugation to glutathione, making less glutathione available, thus reducing detoxification reserves. A number of situations can be associated with depletion of anti-oxidant reserves and an increased tendency to develop drug hypersensitivities. HIV is the most common, causing significant reductions of glutathione peroxidase and glutathione transferase [5, 6]. Epstein-Barr virus and human herpesvirus 6 (HHV6) are also able to predispose to generation of drug hypersensitivities [7, 8]. All these viruses also modulate immune responses with changes in the ratios of CD4 and CD8 T cells.

While it has been presumed that the liver is the major site of drug metabolism, there is growing awareness that a variety of cell types may perform the metabolic processing of drugs and other xenobiotics. Keratinocytes have been shown to contain many of the CYP450 enzymes and to be able to metabolize dapsone to its hydroxylamine metabolite [9, 10]. This is likely to be important in the generation of immune hypersensitivity to topically applied drugs and xenobiotics, and may be involved in the cutaneous manifestations of systemic drug hypersensitivity reactions. Neutrophil leucocytes have been shown to generate immunogenic metabolites of several drugs

including procainamide and hydralazine involved in causing lupus syndromes [11] as well as carbamazepine [12] (Fig. 2) and propylthiouracil [13]. The myeloperoxidase enzyme system appears to be involved in these activities. Monocytes/macrophages and dendritic cells all possess the enzymatic machinery to perform drug metabolism and detoxication [14, 15].

Immune recognition

One area of ignorance is the nature of the protein/peptide carriers to which drug haptens become bound. However, it is clear that drug haptens can be presented via the MHC class 1 route to CD8⁺ T cells and via the MHC class 2 pathway to CD4⁺ T cells. There are several instances in which it has been shown that both CD4⁺ and CD8⁺ T cells are activated by a given drug – examples include aromatic anti-convulsants and sulphonamides [16–18]. It is presumed that to get into the MHC class 1 (endogenous protein) pathway, the reactive metabolite either enters the cell or is generated within the cell and becomes covalently bound to endogenous cellular proteins (Fig. 1). Lipophilic drugs will enter cells more easily. The hapten-conjugated protein is likely to be ubiquitinated, degraded by the proteasome complex and the peptides of suitable amino acid sequence and charge structure to be held stably in the groove of the MHC class 1 molecules, expressed on the cell surface. For entry into the class 2 pathway, it may be that the reactive metabolite haptens an extracellular carrier protein, or conceivably, the cell in which the hapten was generated may release its contents (through necrosis?) to be taken up by nearby APCs. The haptenated protein would then be processed down the exogenous pathway, haptenated peptides with an appropriate structure for binding to the MHC class 2 molecules being expressed on the cell surface.

An alternative mechanism for recognition of haptens has been identified for several drugs. This involves direct binding of hapten/metabolite molecules to the surface of APCs without the requirement for metabolic bioactivation or processing by the APC (Fig. 1). Thus, using T cell clones from patients with allergic sensitization to sulphamethoxazole, Pichler and colleagues showed that glutaraldehyde fixed, metabolically inactive APCs were still able to induce hapten-dependent activation of the T cell clones. It appeared that the hapten was bound to MHC/peptide complexes in a loose configuration that required its continuous presence. This presentation pathway is reviewed by Pichler [19, 20]. A similar processing-independent pathway has been characterized for the presentation of nickel to T cells. It was shown that nickel could bind to the histidine residues in peptides in MHC class grooves [21]. It is not clear whether these processing-independent routes are able to activate naïve T cells or whether they are only able to activate established memory T cells.

Evidence suggesting a possible general mechanism by which those drug molecules that require metabolic bioactivation might become protein bound, and hence recognizable by the immune system, comes principally from work in relation to halothane-induced hepatitis [22–25]. It has been shown that trifluoroacetyl halide, the critical metabolite of halothane, apparently becomes bound to the cytochrome P450 enzyme (CYP2E1) for which halothane is a substrate, altering the

structure of the enzyme. This has the effect of inactivating the enzyme and presumably, targeting it for proteolytic degradation. The immune response in patients with halothane hepatitis is detected in the form of specific antibodies against the complex of trifluoroacetyl/CYP450 [23, 26]. It has yet to be resolved whether the pathogenic immune effector mechanisms are antibody or T cell-mediated. Similarly, in patients who have had ADRs to carbamazepine and other anti-convulsants, antibodies are found against the rat CYP3A family responsible for metabolism of carbamazepine [27]. T lymphocytes reactive with carbamazepine or its metabolites can also be demonstrated in patients who have suffered ADRs from that drug [16, 28]. Thus, it appears that the reactive metabolite of many drugs, generated by the actions of CYP450 enzymes, can 'bite back' and become covalently bound to the enzyme. This complex of haptenic metabolite and protein carrier could be what is recognized by the immune system, as in the case of halothane/CYP2E1.

Type of immune response

As will be seen below, the immune responses that are generated to produce drug hypersensitivities can involve every type of immune effector mechanism. Thus, some reactions involve antibodies – any or all classes can be produced. T lymphocytes can be of CD4⁺ and/or CD8⁺ type. In some hypersensitivities both types can apparently produce cytokines of Th1 and Th2 profiles, while in some hypersensitivities both types can function as perforin and granzyme B-producing cytotoxic cells. There is little understanding of how different immune responses and clinical patterns can be generated by the same drug – for example, penicillin can induce all the four classical immune hypersensitivity reactions including IgE-mediated allergic reactivity, blood dyscrasias, vasculitis (presumably with IgG antibodies), pustular eruptions and maculo-papular eruptions with drug-specific T cells, in different individuals (Fig. 3) [29]. The differences are likely to reflect varying activation profiles of Th1 and Th2 cells. There is some evidence that the redox status and antioxidant balance mediated via glutathione levels in APCs can affect the generation of Th1 or Th2 cells [30]. Oxidative stress can activate transcription factors including NFκB and AP-1, which induce the expression of many cytokines.

Immunological mechanisms underlying drug hypersensitivity

The term hypersensitivity is applied when the immune response to an agent (immunogen) results in an increased or exaggerated response. Different types of immune effector mechanisms can produce diverse clinical patterns of hypersensitivity reaction – the mechanisms were originally classified into four types by Coombs and Gell (Table 1) [31]. It is a gross oversimplification to presume that there are no overlaps or combined involvement of different immune effector mechanisms in the different patterns of hypersensitivity.

Type I hypersensitivity (IgE-mediated) reactions

The reaction patterns that may involve the skin range from urticaria and angioedema to systemic anaphylaxis. Reactions

Table 1. Types of hypersensitivity reactions and the underlying immune effector mechanisms

Type of hypersensitivity	Effector mechanisms
Type 1	
Immediate or anaphylactic	IgE bound to the surface of mast cells or basophils. Antigen binding causes mast cell degranulation, release of histamine and other mediators.
Type 2	
Cytotoxic	Antigenic determinants on cell surfaces are the target of antibodies – may be IgG or IgM. The antibodies damage cells/tissues by activating complement, or by binding to cells through Fc γ receptors, they activate cytotoxic killing, e.g. by K cells.
Type 3	
Immune complex	Circulating immune complexes are deposited in vascular beds or on tissue surfaces. Complement is activated, neutrophils attracted and their products damage tissues.
Type 4	
Delayed	Effector T lymphocytes are activated after recognition of the 'antigen' for which they are specific. The antigen is 'presented' as peptide(s) in the groove of MHC molecules. CD4 ⁺ T cells are activated via MHC class 2, CD8 ⁺ T cells are activated via MHC class1. The cytokines released generate inflammation. The T cells (mainly CD8 ⁺ , but also CD4 ⁺) can act as cytotoxic cells, inflicting tissue damage.

Modified from [31].

after drug exposure may occur in about 15 min (immediate-phase) or 6–24 h later (late-phase). It is presumed that interaction of a drug or its immunogenic metabolite with specific IgE bound to the surface of mast cells and possibly basophils triggers the release of vasoactive mediators such as histamine, prostaglandin D₂, leukotriene C₄, eosinophil and neutrophil chemotactic factors, platelet activating factor and bradykinin.

The most common causes of IgE-mediated drug-induced hypersensitivity are antibiotics (especially the penicillins) and anaesthetic-related drugs, particularly muscle relaxants. Anaphylaxis occurs in one per 10 000 courses of penicillin and leads to death in one to five per 100 000 intramuscular courses [32]. The penicillins are low molecular weight compounds, which contain a 6-aminopenicillanic acid nucleus that includes a β -lactam ring. The β -lactam ring can open spontaneously under physiological conditions so that its carbonyl moiety forms an amide linkage with the amino groups of adjacent proteins (Fig. 2) [29]. Although this is the classically accepted mechanism by which antigenic penicilloyl groups are formed [32], logically, this might not be the mechanism in everyone, otherwise allergic hypersensitivity to penicillin would be much commoner than it is. Different patients produce IgEs that recognize different components of the penicilloyl structure [33]. Interestingly, the helper T cell recognizes the penicilloyl (or other β -lactam antibiotic) covalently bound to the peptide in the MHC groove [34, 35]. For other β -lactam ring-containing antibiotics, including cephalosporins and carbapenems, the hapten determinants are unknown, but there is significant cross-reactivity between penicillins and cephalosporins [36].

Other groups of drugs that are commonly associated with immediate hypersensitivity and anaphylaxis include drugs used in anaesthesia, the most important of which are muscle

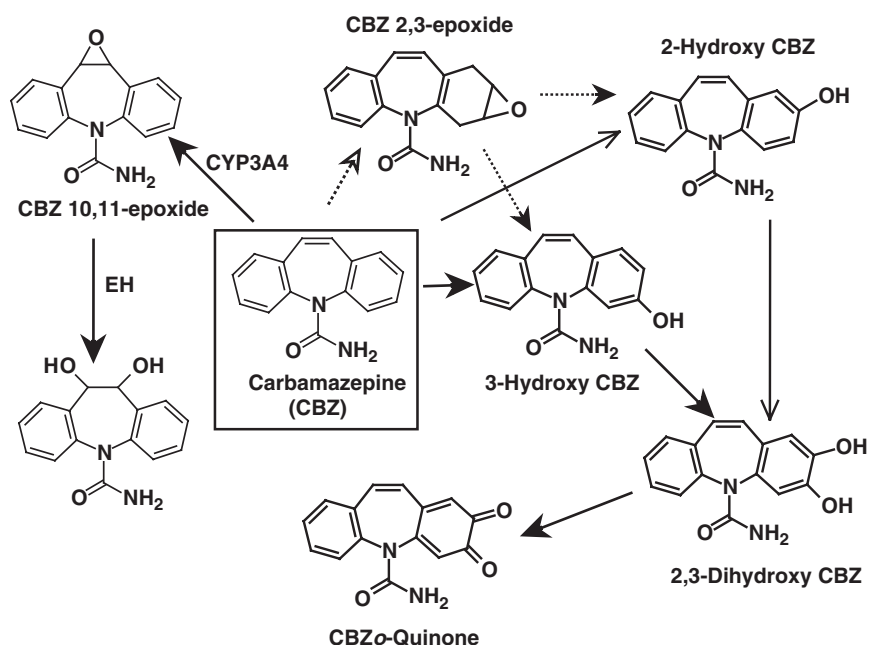


Fig. 2. Potential bioactivation of carbamazepine (CBZ). CBZ 10,11-epoxide is a major metabolite formed by CYP3A4. The epoxide is detoxified by epoxide hydrolase (EH). 3-Hydroxy CBZ is formed mainly by CYP2B6, lesser amounts of 2-hydroxy CBZ and CBZ 2,3-epoxide are formed. Various other potentially reactive metabolites can result. (Modified from [113] with permission.)

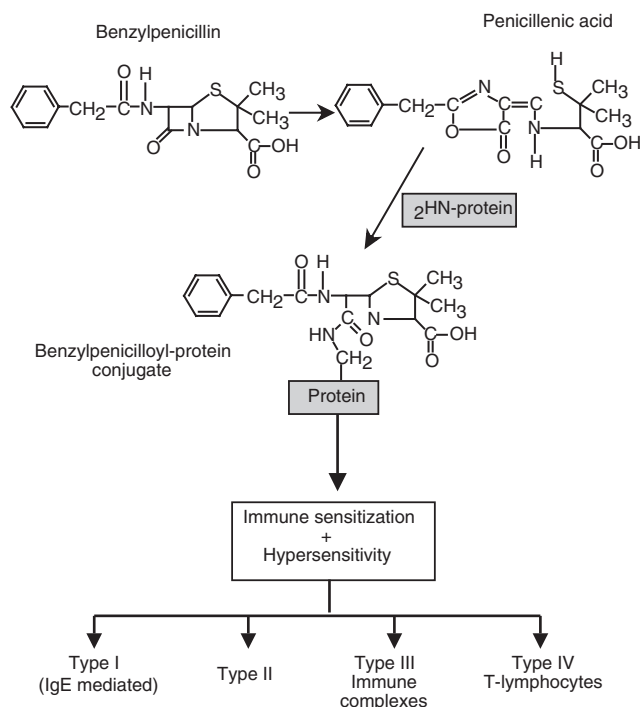


Fig. 3. Postulated mechanism by which benzylpenicillin becomes protein-bound. The protein-bound penicillin becomes immunogenic – the immune response may result in any of the principal forms of immune hypersensitivity. (Reproduced from [29] with permission.)

relaxants such as D-tubocurarine, suxamethonium, alcuronium, pancuronium and gallamine. The estimated incidence of peri-operative anaphylaxis ranges from 1:10 000 to 1:20 000. From two surveys of 1585 and 467 patients investigated in France, 52% had reactions of immunological origin, with muscle relaxants accounting for 70% in the first study and 69.2% in the second [37, 38]. Of the IgE-dependent reactions involving a muscle relaxant, suxamethonium was responsible for 43%, vecuronium for 37%, pancuronium for 13%, alcuronium for 7.6%, atracurium for 6.8% and gallamine for 5.6% [37, 38]. Induction agents implicated in causing anaphylaxis include propanidid, althesin, thiopentone, thiamylal and methohexitone [39]. Fisher and More [39] studied severe anaphylactic and anaphylactoid reactions during anaesthesia in 116 patients. They found that the majority of patients who reacted to induction agents had previous exposure to the drug, while the majority of patients (85%) who reacted to muscle relaxants had not [39]. The matter of how sensitization to muscle relaxants is induced, without prior exposure, is still unresolved.

It is important to distinguish between immune-mediated allergic reactions and pseudo-allergic (anaphylactoid) reactions in which direct pharmacological mast cell degranulation occurs. Also, certain individuals are intolerant of aspirin and salicylates, developing urticarial rashes, asthma or other features suggesting/mimicking atopic allergic processes.

Type II hypersensitivity reactions: cytotoxic/cytolytic mechanisms

In the skin, drug-induced type II immune hypersensitivity may manifest as pemphigus, bullous pemphigoid and linear

IgA disease. Drug-induced pemphigus can be divided into two main groups [40]:

- (1) *Drug-dependent pemphigus* – where exogenous factors such as thiol (–SH) containing drugs (e.g. D-penicillamine, captopril) cause pemphigus. The pemphigus usually regresses once the drug is discontinued. Thiol-containing drugs induce mainly pemphigus foliaceus and erythematosis – the pemphigus usually develops within the first 12–24 months of commencing the drug.
- (2) *True pemphigus* (drug-triggered) – where the patient has a genetic predisposition (DRB1*0402, DRB1*1402 and DRB1*0701) for pemphigus, but exogenous factors such as non-thiol drugs are required to trigger the disease. Non-thiol drugs (e.g. penicillins, cephalosporins, piroxicam) trigger pemphigus vulgaris, which explains the increased mucous membrane involvement seen in these patients compared to those with penicillamine-induced pemphigus. The onset of drug-triggered pemphigus is about 120 days after commencing the triggering drug [41, 42].

Several mechanisms have been postulated for drug-induced pemphigus:

- (1) Thiol drugs can form disulphide bonds with plakoglobin, a protein component in the hemidesmosome complex. The binding of drug alters the molecule, thus forming a neoantigen with the subsequent production of autoantibodies. These autoantibodies bind to the native molecule causing failure of intercellular attachment and acantholysis through activation of endogenous proteolytic mechanisms [43]. Immunoprecipitation studies have identified that the autoantibodies in drug-induced pemphigus have the same antigenic specificity as do autoantibodies from other pemphigus patients [44, 45].
- (2) Thiol drugs may directly interfere with the immune system. Penicillamine has a direct action on lymphocytes, promoting CD4⁺ numbers [46] and causing preferential activation of CD4⁺ Th2 cell function [47]. This mechanism may be related to the frequent detection of a range of organ/tissue-specific autoantibodies and the co-existence of other autoimmune disorders such as myasthenia gravis [48] in patients with thiol-induced pemphigus [43]. Sulphydryl groups and disulphide bonds are thought to play an important role in the development of specificity of γ -globulins. Therefore, another possible mechanism is that thiol drugs may modify the immunological specificity of some γ -globulins [49–52].
- (3) Thiol drugs are also able to provoke acantholysis (detachment of epidermal keratinocytes from each other) without antibody production (biochemical thiol acantholysis) [53–55].

Conceivably, drug-induced dissolution of desmosomal complexes may expose auto-antigens, which subsequently evoke an immune response.

Penicillamine is dimethylcysteine and is obtained by the hydrolytic breakdown of penicillin. Seven percent of patients who are administered penicillamine for more than 6 months develop pemphigus [49]. Captopril has a sulphydryl group in the same stereochemical position as penicillamine. Pyritinol, used in the treatment of rheumatoid arthritis, is a compound

obtained from two pyridoxine molecules linked by a disulphide bond: it is also chemically similar to the penicillamine molecule. Thiopronine, also used in the treatment of rheumatoid arthritis, is chemically similar to penicillamine with sulphhydryl and methyl groups. It is likely that pemphigus induced by penicillin is mediated by the hydrolytic breakdown of the molecule to penicillamine [49]. Pemphigus-like autoantibodies have been detected in the skin and serum of patients with penicillin-induced eruptions. Some non-thiol drugs known to induce pemphigus have an active amide group in their structure. Enalapril and captopril have been shown to be powerful acantholytic drugs *in vitro*. Enalapril is structurally related to captopril but does not contain a sulphhydryl group. However, both drugs contain an active amide group, thus indicating that the thiol group is not the sole determinant of acantholysis [56]. Other pemphigus-triggering drugs that possess an amide group include penicillins, cephalosporins, pyrazolone derivatives and rifampicin [56]. Pemphigus erythematosus can be induced by penicillamine, captopril, pyritinol, thiopronine, ceftazidime and propranolol [57]. Drug-induced pemphigus vegetans is rare, but has been recently reported to be induced by captopril [58] and enalapril [59]. Contact with some chemicals may also induce pemphigus: it can be induced by exposure to the pesticide baytan, phosphamide, chromium sulphate and feprazone suppositories [60]. The mechanisms of contact pemphigus are similar to drug-induced pemphigus: contact with the substance leads to alteration of the epidermal structure forming neoantigens and the subsequent production of autoantibodies or there is direct activation or inhibition of enzymes causing acantholysis [60].

Similar pathogenic mechanisms to drug-induced pemphigus have been proposed for drug-induced bullous pemphigoid. The drug may act as a hapten, binding to the lamina lucida forming a neoantigen, which induces autoantibody formation; alternatively, it may have a direct effect on immune-regulatory mechanisms enabling proliferation of 'forbidden' B cells to produce bullous pemphigoid autoantibodies [61]. Drugs known to induce bullous pemphigoid include penicillins and other lactams, enalapril, ibuprofen, frusemide, sulphasalazine, phenacetin, PUVA and IL-2 [61–63]. Bullous pemphigoid has been induced by topical fluorouracil. The mechanism for this reaction may be due to direct interaction with the basement membrane [64].

Linear IgA disease has been associated with administration of diclofenac, vancomycin, lithium carbonate and cefamandol naftate [65]. Antibodies in drug-induced pemphigus, bullous pemphigoid and linear IgA disease can be detected by the indirect immunofluorescence technique. However, the antibodies are indistinguishable from those found in the idiopathic forms of these conditions.

Type III hypersensitivity: immune complex-mediated vasculitis

Hypersensitivity vasculitis occurs when circulating immune complexes form between IgG or IgM antibodies and antigens from exogenous sources such as bacteria (streptococci), viruses or drugs. These immune complexes deposit within

the postcapillary venules causing vessel wall damage via activation of the complement cascade with subsequent neutrophil accumulation and release of vaso-active mediators and proteases. Drug-induced vasculitis develops in the skin and other organs approximately 8–10 days after exposure to the causative drug. Presumably, the immune response is directed at the combination of a drug-derived metabolite (hapten) conjugated to a self-protein. Vasculitis is a reaction pattern that may develop after exposure to a wide range of drugs including antibiotics, NSAIDs, cytotoxic drugs, to name but a few. There are so far no real clues as to how the drug or its metabolites are involved in the immune complexes. 'Drug' or therapeutic agents that are proteins, such as heparin or streptokinase, can cause vasculitis. Presumably, the protein is handled as a complete antigen in the same way as microbe-derived proteins that evoke 'allergic or hypersensitivity' vasculitis. It is generally perceived that the quality of the antibody response in terms of its binding affinity determines whether immune complexes are of the type easily cleared by the reticulo-endothelial system or are soluble and persist in the circulation.

In theory, the most useful test for identifying immune complex deposition in the skin would be by immunofluorescence with labelled antibody to the drug antigen. However, a battery of antibodies to common drugs causing immune complex-mediated disease has not yet been developed.

Type IV hypersensitivity: T lymphocyte-mediated reactions

There are two main types of T lymphocyte-mediated reactions: those from topical contact and those from systemic exposure:

(1) *Contact allergic drug hypersensitivity* is due to topical drug preparations such as antibiotics, antiseptics, local anaesthetics and corticosteroids. Much has been learnt about both the mechanisms of induction of immune sensitization and the immune effector mechanisms involved in hypersensitivity reactions in the skin using the model of contact hypersensitivity. In order to sensitize, the low molecular weight compound must penetrate the permeability barrier [66]. As for other drugs, organic contact sensitizers must either be intrinsically reactive or must be bio-activated to form a hapten capable of protein binding, to produce a complete antigen. Metals such as nickel and gold appear capable of interacting with APCs by processing-independent mechanisms [21, 67]. Nickel has been shown to bind directly to histidine residues in peptides lying in the groove of the MHC class 2 molecules [21]. Non-metal antigens are processed by Langerhans' cells that migrate to the lymph nodes and present the antigen to T lymphocytes [68] – as mentioned above, presentation may be via MHC class 1 or class 2. Once immunological memory is established, re-exposure to the agent elicits an eczematous reaction characterized by a mononuclear cell infiltrate accompanied by oedema of the epidermis with microvesicle formation, peaking at 48–72 h.

There are interesting data regarding the phenotype of nickel-reactive T cells both in individuals who express clinical

contact sensitivity and in clinically non-reactive people. Thus, Cavani et al. [69] have shown that nickel-responsive CD4⁺ T cells are present equally in both nickel-allergic and non-allergic individuals. Nickel-specific CD4⁺ T cells cloned from blood of non-allergic individuals produced significant amounts of IL-10 and lower amounts of IFN- γ than those from patients with clinical nickel allergy, and thus have the phenotype suggesting a regulatory or suppressive function. However, nickel-specific CD8⁺ T cells were found only in people with clinical allergy to nickel and these T cells produce high amounts of IFN- γ and no IL-4 [69].

One of the most commonly used topical drug groups is the corticosteroids. It seems paradoxical that corticosteroids can induce T cell sensitization when they are normally used to suppress delayed-type hypersensitivity (DTH) by impairing the function of both dendritic cells and lymphocytes. It has been suggested that the two effects occur at different concentrations – as reflected by the finding that when alcoholic solutions of the corticosteroid are applied on paper discs as patch test challenges, the inflammatory contact sensitivity response is often seen initially as a ring at the edge of the disc [70]. The authors argued that this is somehow different from the normal 'edge effect' seen with patch tests using liquid vehicles, where the solute accumulates in increased concentration at the edge [71]. They postulated that diffusion of the steroid into the skin at the edges, dilutes its anti-inflammatory effect. An alternative explanation would be that the immunogenic metabolite lacks immuno-suppressive/anti-inflammatory properties.

There are forms of systemic sensitivity that can be detected through topical patch test reactions. Examples include sensitization by intra-vesical administration of mitomycin C in the treatment of bladder carcinoma. It appears that Langerhans' cells in the bladder wall induce T cell activation which can subsequently be detected by patch tests with mitomycin C [72]. This observation indicates that T cells able to home to the skin, presumably through expression of the cutaneous lymphocyte antigen CLA, can be induced through non-percutaneous sensitization routes. Sensitization by gold aurothiomalate, used in the treatment of rheumatoid disease, can produce systemic symptoms and positive patch tests with gold salts [73, 74]. Moreover, in people with gold allergy, systemic administration of gold challenges can elicit a flare up of previous gold contact-induced skin reactions as well as inducing systemic symptoms of fever and malaise, indicating a systemic hypersensitivity reaction [74].

(2) *Systemic T cell-mediated drug hypersensitivity* is thought to occur in a variety of cutaneous drug reactions such as maculo-papular eruptions ('Ampicillin rash'), erythema multiforme (EM), toxic epidermal necrolysis (TEN), eczematous, lichenoid and toxic pustuloderma reactions. Many of the published studies have mixed findings from more than one type of reaction, but individual exanthemata have been discussed recently by Pichler et al. [75]. The involvement of T cells in the different patterns of drug-induced skin eruption will be considered separately in the following sections.

Maculo-papular eruptions

This is the most common pattern of drug-induced skin eruption and can be induced by a very wide range of drugs.

Some of the main drugs causing maculo-papular eruptions include antibiotics (sulphonamides, β -lactams and trimethoprim), anticonvulsants including carbamazepine and others, allopurinol and NSAIDs [76, 77]. Positive patch tests with the culprit drug can be demonstrated in significant numbers of these patients, e.g. in 6/23 in one study [78]. CD8⁺ T cells are found in the lesional epidermis and cells with specificity for penicillin have been cloned from the blood of patients with penicillin-induced skin eruptions [79, 80]. Mauri-Hellweg et al. [16] demonstrated that the relevant drug(s) could stimulate proliferative responses in both CD4⁺ and CD8⁺ T cells in peripheral blood from patients with maculo-papular eruptions. Moreover, these cells secreted the cytokine profile (high IL-5, low IFN- γ) characteristic of Th2 cells. Yawalkar and colleagues cloned T cells from the positive patch test reactions elicited in two patients with exanthematous reactions to antibiotics. They found that the drug-specific T cell clones were CD4⁺ and, mostly, produced high amounts of IL-5 and little or no IFN- γ [81], again confirming the Th2 phenotype of these cells. However, the same group showed, by immunohistochemical means, that significant numbers of the infiltrating T cells (both CD4⁺ and CD8⁺ T cells) also expressed perforin and granzyme B, markers of cytotoxic function [82]. Furthermore, drug-specific T cell clones were shown to kill drug-bearing autologous keratinocytes *in vitro* [83].

Erythema multiforme and toxic epidermal necrolysis (EM/TEN)

Positive patch and lymphocyte stimulation tests with the causal drugs indicate that type IV hypersensitivity is involved [16, 84–87]. Interestingly, it seems that both CD4⁺ and CD8⁺ T cells are involved in these reactions. Histological observation reveals predominantly CD4⁺ T cells in the dermal infiltrate, while CD8⁺ T cells and macrophages are found in the epidermal compartment [88, 89]. When EM becomes bullous, infiltrating CD4⁺ and CD8⁺ T cells have the phenotype of cytotoxic cells, expressing perforin and granzyme B. It seems likely that they are responsible for some of the keratinocyte apoptosis.

The immuno-pathogenesis of TEN appears to involve the activation of massive apoptosis of epidermal keratinocytes [90–92]. T cells from the bullous lesions of TEN have been shown to be CD8⁺, CD56⁺ and have NK-like features [92, 93]. Moreover, these cells recognize the native drug (sulphamethoxazole) or its nitroso metabolite in an MHC class I restricted manner, suggesting they may be activated by the drug in a non-covalent association with MHC/peptide complexes [93]. An additional trigger of such extensive apoptosis appears to result from induction of production of the active ligand for CD95 (fas death receptor) [91]. The CD95-ligand appears to be produced by keratinocytes themselves, probably in response to signals from drug-activated T cells or even macrophages.

These reaction patterns often involve other organ systems in addition to skin, as reflected by altered liver function tests or haematological indices. The drug hypersensitivity syndrome is characterized by rash, fever, lymphadenopathy, hepatitis and eosinophilia. It is believed that both type III and type IV hypersensitivity mechanisms may be responsible due to positive patch test results and finding of immune complex-

mediated vasculitis on direct immunofluorescence examination [94]. The presence of drug-specific T lymphocytes has been demonstrated *in vitro* [16]. Allopurinol hypersensitivity syndrome is characterized by a cutaneous eruption, hepatitis and interstitial nephritis. Studies have indicated that cell-mediated immunity is involved in the pathogenesis. Immunohistochemistry of the liver revealed a predominantly T lymphocytic infiltrate and lymphocyte stimulation tests conducted *in vitro* showed that allopurinol and its metabolite oxypurinol induced lymphocyte proliferation [95].

Toxic pustuloderma

A number of drug-induced reaction patterns involve the formation of pustules. Acute generalized exanthematous pustulosis (AGEP) is one variety in which progress has been made regarding pathogenesis. It comprises a widespread macular erythematous rash surmounted by large numbers of small sterile pustules usually accompanied by fever and blood leucocytosis. Positive patch tests can be elicited with the culprit drug in up to 50% of cases; the patch test reaction can mimic the natural disease with pustule formation [78, 96]. Microscopic examination shows intra-epidermal pustules surrounded by activated CD4⁺ and CD8⁺ T cells [96]. The epidermal keratinocytes and T cells in the epidermis as well as T cell clones, produce high levels of IL-8 [96]. This distinguishes this reaction pattern from other drug reactions.

In summary, different clinical reaction patterns are associated with a range of T cell phenotypes and functions. Both CD4⁺ and CD8⁺ T cells are involved in many reactions. They may produce either Th1 or Th2 cytokine patterns, and both CD4⁺ and CD8⁺ T cells can express markers characteristic of killer/cytotoxic function.

Why is skin involved in systemic drug hypersensitivity reactions?

There is no definite answer to this question, but there are a number of possibilities.

1. Native drugs can undoubtedly reach the skin through the systemic circulation [97] – as reflected by urticarial responses to antibiotics such as penicillin, in which the drug reacts with mast cell-bound IgE. Keratinocytes may contribute to extra-hepatic metabolism of drugs and hence could be a site for generation of immunogenic haptens. This is likely to be the case for contact sensitization to topically applied drugs. If reactive metabolites are generated by keratinocytes, some direct toxic effects resulting in necrosis and effective 'alerting' of the immune system, together with processing of the haptenated proteins by epidermal Langerhans' cells, would be one mechanism for induction of sensitization [19]. A second mechanism could be the generation of haptens either in keratinocytes or Langerhans' cells, with subsequent activation of T cells.
2. Immune effector mechanisms (T cells or antibodies) may localize to skin preferentially. One of the conundra relating to the causation of atopic eczema is the observation that in

infancy, allergic reactions to food-derived allergens often manifest as atopic eczema/dermatitis, which is thought to be mediated by antigen-specific T cells in the skin. Something has clearly caused the food-specific T cells (which have presumably encountered their antigen in the gut-associated lymphoid tissue), to express the cutaneous lymphocyte homing antigen (CLA) and hence, to come into the skin. By analogy, conceivably exposure to drugs or their metabolites of gut-lymphoid tissue associated T cells might somehow induce the expression of CLA. In many drug hypersensitivity reactions, T cells must express 'addressins' or homing molecules able to take them into a whole range of tissues. Whether drugs or their haptens induce an as yet undiscovered change in T cells that renders them capable of homing to many tissues, or whether drugs or their metabolites alter endothelial cells so they express adhesion molecules able to recruit activated T cells, are possibilities yet to be explored.

Investigations: the current position

Currently, drug hypersensitivity is diagnosed on clinical grounds, predominantly on the basis of a thorough history, paying particular attention to the timing of drug administration in relation to the adverse event. Clinical examination with histological examination of biopsy material is vital for defining the type of adverse hypersensitivity reaction which is relevant to what sort of tests may be used.

In vivo or *in vitro* tests may be carried out, but these are usually confined to research centres with an interest in drug allergy. The robustness and range of such tests is, at present, very limited mainly because for the majority of drugs, the correct antigenic molecule, hapten or metabolite is not known or available. The choice of test is important and depends on the suspected immune mechanism underlying the drug reaction. Skin testing, i.e. prick, intradermal or patch testing is usually performed 6 weeks–6 months after resolution of the ADR. It may, however, lead to relapse of symptoms and for this reason, low concentrations of the suspected agent are used initially.

Immediate (Type I) reactions

Diagnostic tests to demonstrate the presence of IgE-mediated allergy to a given drug are often needed in relation to suspected anaphylactic reactions, but in most cases they are not readily available. Skin prick tests (SPTs) are safe to perform (see below), and can be used for detection of penicillin allergy; both major (penicilloyl polylysine) and minor (benzylpenicilloate) determinants are readily available. Also, prick and intradermal tests have been used with considerable success in the field of anaesthetic-related anaphylaxis [98, 99]. Muscle relaxants are the cause of 62–70% of peri-operative episodes of anaphylaxis [99]; the specificity and sensitivity of SPTs and intradermal tests for this group of drugs is better than 90% [99]. For drugs that have direct mast cell-degranulating properties, positive skin tests do not distinguish between allergic and pharmacological (pseudo-allergic) mechanisms.

In vivo testing Skin tests should be performed in the sequence: patch test, prick test, intradermal test progressing to the next test only if the previous result is negative [100]. Immediate (15 min) and delayed (6 and 48 h) readings should be taken, and both major and minor determinant mixtures tested, e.g. benzylpenicilloyl polylysine (PPL) and benzylpenicilloate, respectively. Positive and negative controls should be included. It is important to bear in mind that immediate responses are likely in urticarial and anaphylactic reactions, whereas patch- and 48-h-positive reactions are unlikely to occur in these types of reactions, but are found in maculo-papular, eczematous and EM (delayed) reactions. Thus, Barbaud et al. [101] performed a range of skin tests in patients with different clinical patterns of drug rash. Fifty-nine percent of the patients in the study with maculo-papular rashes had positive patch test results compared with 11% of those with urticarias. For prick testing, the opposite trend was noted with urticaria being more likely to produce a positive result. If skin tests are negative, it may be appropriate to perform *in vitro* tests. Also, in patients with negative skin tests, cautious low-dose oral challenge has been attempted in several studies with mild adverse reactions reported in less than 5% of patients [102]. Oral challenge tests are contraindicated where there is a history of anaphylaxis.

In vitro testing *In vitro* tests have the advantage of being safer than *in vivo* tests. The *in vitro* test most commonly used in the diagnosis of immediate type hypersensitivity is the RAST. The RAST is available for penicillin and amoxicillin IgE antibody but not for minor determinant antigens, which limits its use in practice. Some studies of penicillin-allergic patients have shown that despite negative prick and intradermal tests to both major and minor determinants, a significant number had positive RAST to one of the β -lactam antibiotics (either amoxicillin or benzylpenicillin) ranging from 13% [103] to 42% [104]. Other *in vitro* tests currently available in research settings include the histamine release test, the basophil degranulation test and the flow cytometric detection of CD63 positive expression on activated basophils [105, 106].

Types II and III hypersensitivity

There are no reliable skin tests or *in vitro* tests in these types of drug hypersensitivity, mainly because of the unknown nature of the drug-derived antigens.

Type IV (T cell-mediated hypersensitivity)

Delayed skin reactions such as morbilliform, maculo-papular exanthemata and EM/Stevens-Johnson syndrome may need a combination of investigations [107].

In vivo testing Since these are delayed-type, lymphocyte-mediated reactions, prick tests are unlikely to be positive. However, patch tests can elicit positive responses in a high proportion of cases [101, 107, 108].

In vitro testing Two main *in vitro* tests have been applied to the diagnosis of delayed-type adverse drug reactions: the lymphocyte cytotoxicity test demonstrates that lymphocytes from patients are less able to detoxify metabolites of the

native drug, generated by activated liver microsomes [109, 110]. It is not widely available because of its specialized nature. The lymphocyte transformation test is much more available and has been applied with some success to a variety of drugs such as β -lactam antibiotics, sulphonamides and anticonvulsants [85, 87, 95, 111, 112]. It has been shown by Nyfeler and Pichler [85] to have a sensitivity of about 78% and a specificity of about 85% in determining the culprit agent in drug hypersensitivity. The probable reason for positive results being less than 100% is that the immunogenic metabolite or complete antigen is not necessarily present in the test system.

Conclusion

The awareness of the role of metabolism in generating the immunogenic haptens from native drugs together with the growing ability to analyse differences in metabolism in individuals who develop adverse drug reactions will lead to identification of the immunogens themselves. This will then provide the basis for tests both for diagnosis of established drug hypersensitivity, but more importantly, for identification of individuals at risk of developing such reactions.

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References

- 1 Park BK, Pirmohamed M, Kitteringham NR. Idiosyncratic drug reactions: a mechanistic evaluation of risk factors. *Br J Clin Pharmacol* 1992; 34:377–95.
- 2 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. *J Am Med Assoc* 1986; 256:3358–63.
- 3 Merk HF, Hertl M. Immunologic mechanisms of cutaneous drug reactions. *Semin Cutan Med Surg* 1996; 15:228–35.
- 4 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.
- 5 Buhl R, Jaffe HA, Holroyd KJ et al. Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. *Lancet* 1989; 2:1294–8.
- 6 Choi J, Liu RM, Kundu RK et al. Molecular mechanism of decreased glutathione content in human immunodeficiency virus type 1 Tat-transgenic mice. *J Biol Chem* 2000; 275:3693–8.
- 7 Descamps V, Bouscarat F, Laglenne S et al. Human herpesvirus 6 infection associated with anticonvulsant hypersensitivity syndrome and reactive haemophagocytic syndrome. *Br J Dermatol* 1997; 137:605–8.
- 8 Tohyama M, Yahata Y, Yasukawa M et al. Severe hypersensitivity syndrome due to sulfasalazine associated with reactivation of human herpesvirus 6. *Arch Dermatol* 1998; 134:1113–7.
- 9 Baron JM, Holler D, Schiffer R et al. Expression of multiple cytochrome p450 enzymes and multidrug resistance-associated transport proteins in human skin keratinocytes. *J Invest Dermatol* 2001; 116:541–8.
- 10 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.

- 11 Uetrecht J. Metabolism of drugs by activated leukocytes: implications for drug-induced lupus and other drug hypersensitivity reactions. *Adv exp Med Biol* 1991; 283:121–32.
- 12 Furst SM, Uetrecht JP. Carbamazepine metabolism to a reactive intermediate by the myeloperoxidase system of activated neutrophils. *Biochem Pharmacol* 1993; 45:1267–75.
- 13 von Schmiedeberg S, Hanten U, Goebel C, Schuppe HC, Uetrecht J, Gleichmann E. T cells ignore the parent drug propylthiouracil but are sensitized to a reactive metabolite generated *in vivo*. *Clin Immunol Immunopathol* 1996; 80:162–70.
- 14 Hodges VM, Molloy GY, Wickramasinghe SN. Demonstration of mRNA for five species of cytochrome P450 in human bone marrow, bone marrow-derived macrophages and human haemopoietic cell lines. *Br J Haematol* 2000; 108:151–6.
- 15 Saeki M, Saito Y, Nagano M, Teshima R, Ozawa S, Sawada J. mRNA expression of multiple cytochrome p450 isozymes in four types of cultured skin cells. *Int Arch Allergy Immunol* 2002; 127:333–6.
- 16 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.
- 17 Pichler WJ, Zanni M, vonGreysen S, Schnyder B, Maurihellweg D, Wendland T. High IL-5 production by human drug-specific cell clones. *Int Arch Allergy Immunol* 1997; 113:177–80.
- 18 Schnyder B, Maurihellweg D, Zanni M, Bettens F, Pichler WJ. Direct, MHC-dependent presentation of the drug sulfamethoxazole to human alpha beta T cell clones. *J Clin Invest* 1997; 100:136–41.
- 19 Pichler WJ. Deciphering the immune pathomechanism of cutaneous drug reactions. *Allergy* 2002; 57(Suppl. 72): 34–6.
- 20 Pichler WJ. Pharmacological interaction of drugs with antigen-specific immune receptors: the p-i concept. *Curr Opin Allergy Clin Immunol* 2002; 2:301–5.
- 21 Romagnoli P, Labhardt AM, Sinigaglia F. Selective interaction of Ni with an MHC-bound peptide. *EMBO J* 1991; 10:1303–6.
- 22 Satoh H, Gillette JR, Davies HW, Schulick RD, Pohl LR. Immunohistochemical evidence of trifluoroacetylated cytochrome P-450 in the liver of halothane-treated rats. *Mol Pharmacol* 1985; 28:468–74.
- 23 Kenna JG, Satoh H, Christ DD, Pohl LR. Metabolic basis for a drug hypersensitivity: antibodies in sera from patients with halothane hepatitis recognize liver neoantigens that contain the trifluoroacetyl group derived from halothane. *J Pharmacol Exp Ther* 1988; 245:1103–9.
- 24 Bourdi M, Chen W, Peter RM et al. Human cytochrome P450 2E1 is a major autoantigen associated with halothane hepatitis. *Chem Res Toxicol* 1996; 9:1159–66.
- 25 Obermayer-Straub P, Strassburg CP, Manns MP. Target proteins in human autoimmunity: cytochromes P450 and UDP-glucuronosyltransferases. *Can J Gastroenterol* 2000; 14:429–39.
- 26 Martin JL, Kenna JG, Pohl LR. Antibody assays for the detection of patients sensitized to halothane. *Anesth Analg* 1990; 70:154–9.
- 27 Leeder JS, Gaedigk A, Lu X, Cook VA. Epitope mapping studies with human anti-cytochrome P450 3A antibodies. *Mol Pharmacol* 1996; 49:234–43.
- 28 Strickland I, Fitzgerald D, Friedmann PS. Immune-responses to DNCB and carbamazepine are dependent on intermediate metabolism. *J Invest Dermatol* 1996; 107:308.
- 29 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.
- 30 Peterson JD, Herzenberg LA, Vasquez K, Waltenbaugh C. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc Natl Acad Sci USA* 1998; 95:3071–6.
- 31 Coombs RRA, Gell PGH. Classification of allergic reactions responsible for clinical hypersensitivity and disease. In: Gell PGH, Coombs RRA, eds. *Clinical aspects of immunology*. Oxford, UK: Oxford University Press, 1968:576–96.
- 32 Weiss ME, Adkinson NF. Immediate hypersensitivity reactions to penicillin and related antibiotics. *Clin Allergy* 1988; 18: 515–40.
- 33 Zhao Z, Baldo BA, Baumgart KW, Mallon DF. Fine structural recognition specificities of IgE antibodies distinguishing amoxicillin and amoxicillinyl determinants in allergic subjects. *J Mol Recognit* 2001; 14:300–7.
- 34 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.
- 35 Padovan E. T-cell response in penicillin allergy. *Clin Exp Allergy* 1998; 28(Suppl. 4):33–6.
- 36 Audicana M, Bernaola G, Urrutia I et al. Allergic reactions to betalactams: studies in a group of patients allergic to penicillin and evaluation of cross-reactivity with cephalosporin. *Allergy* 1994; 49:108–13.
- 37 Laxenaire MC. Drugs and other agents involved in anaphylactic shock occurring during anaesthesia. A French multicenter epidemiological inquiry. *Ann Fr Anesth Reanim* 1993; 12:91–6.
- 38 Laxenaire MC, Mertes PM. Anaphylaxis during anaesthesia. Results of a two-year survey in France. *Br J Anaesth* 2001; 87:549–58.
- 39 Fisher MM, More DG. The epidemiology and clinical features of anaphylactic reactions in anaesthesia. *Anaesth Intens Care* 1981; 9:226–34.
- 40 Brenner S, Wolf R, Ruocco V. Drug-induced pemphigus. I. A survey. *Clin Dermatol* 1993; 11:501–5.
- 41 Ruocco V, Gombos F, Lombardi ML. Drug-triggered pemphigus in a predisposed woman. *Acta Derm Venereol* 1992; 72:48–9.
- 42 Matzner Y, Erlich HA, Brautbar C et al. Identical HLA class II alleles predispose to drug-triggered and idiopathic pemphigus vulgaris. *Acta Dermato-Venereol* 1995; 75:12–4.
- 43 Ruocco V, De Angelis E, Lombardi ML. Drug-induced pemphigus. II. Pathomechanisms and experimental investigations. *Clin Dermatol* 1993; 11:507–13.
- 44 Korman NJ, Eyre RW, Zone J, Stanley JR. Drug-induced pemphigus: autoantibodies directed against the pemphigus antigen complexes are present in penicillamine and captopril-induced pemphigus. *J Invest Dermatol* 1991; 96:273–6.
- 45 Brenner S, Bialy-Golan A, Anhalt GJ. Recognition of pemphigus antigens in drug-induced pemphigus vulgaris and pemphigus foliaceus. *J Am Acad Dermatol* 1997; 36:919–23.
- 46 Rosada M, Fiocco U, De Silvestro G et al. Effect of D-penicillamine on the T cell phenotype in scleroderma. Comparison between treated and untreated patients. *Clin Exp Rheumatol* 1993; 11:143–8.
- 47 Hirohata S, Lipsky PE. Comparative inhibitory effects of bucillamine and D-penicillamine on the function of human B cells and T cells. *Arthritis Rheum* 1994; 37:942–50.
- 48 Jones E, Sobkowski WW, Murray SJ, Walsh NM. Concurrent pemphigus and myasthenia gravis as manifestations of penicillamine toxicity. *J Am Acad Dermatol* 1993; 28:655–6.
- 49 Mutasim DF, Pelc NJ, Anhalt GJ. Drug-induced pemphigus. *Dermatol Clin* 1993; 11:463–71.
- 50 Parodi A, Semino M, Gallo R, Rebora A. Bullous eruption with circulating pemphigus-like antibodies following interferon-alpha therapy. *Dermatology* 1993; 186:155–7.
- 51 Fryer EJ, Lebwohl M. Pemphigus vulgaris after initiation of psoralen and UVA therapy for psoriasis. *J Am Acad Dermatol* 1994; 30:651–3.
- 52 Niizeki H, Inamoto N, Nakamura K, Tsuchimoto K, Hashimoto T, Nishikawa T. A case of pemphigus foliaceus after interferon alpha-2a therapy. *Dermatology* 1994; 189(Suppl. 1):129–30.

- 53 Ruocco V, De Angelis E, Lombardi ML, Pisani M. *In vitro* acantholysis by captopril and thiopronine. *Dermatologica* 1988; 176:115–23.
- 54 Ruocco V, de Luca M, Pisani M, De Angelis E, Vitale O, Astarita C. Pemphigus provoked by D(–)penicillamine. An experimental approach using *in vitro* tissue cultures. *Dermatologica* 1982; 164:236–48.
- 55 Wolf R, Ruocco V. Gaining more insight into the pathomechanisms of thiol-induced acantholysis. *Med Hypotheses* 1997; 48:107–10.
- 56 Wolf R, Brenner S. An active amide group in the molecule of drugs that induce pemphigus: a casual or causal relationship? *Dermatology* 1994; 189:1–4.
- 57 Willemsen MJ, De Coninck AL, De Raeve LE, Roseeuw DI. Penicillamine-induced pemphigus erythematosus. *Int J Dermatol* 1990; 29:193–7.
- 58 Pinto GM, Lamarao P, Vale T. Captopril-induced pemphigus vegetans with Charcot–Leyden crystals. *J Am Acad Dermatol* 1992; 27:281–4.
- 59 Bastiaens MT, Zwan NV, Verschueren GL, Stoof TJ, Nieboer C. Three cases of pemphigus vegetans: induction by enalapril – association with internal malignancy. *Int J Dermatol* 1994; 33:168–71.
- 60 Brenner S, Wolf R, Ruocco V. Contact pemphigus: a subgroup of induced pemphigus. *Int J Dermatol* 1994; 33:843–5.
- 61 Fellner MJ. Drug-induced bullous pemphigoid. *Clin Dermatol* 1993; 11:515–20.
- 62 Fellner MJ, Katz JM. Occurrence of bullous pemphigoid after furosemide therapy. *Arch Dermatol* 1976; 112:75–7.
- 63 Ruocco V, Sacerdoti G. Pemphigus and bullous pemphigoid due to drugs. *Int J Dermatol* 1991; 30:307–12.
- 64 Bart BJ, Bean SF. Bullous pemphigoid following the topical use of fluorouracil. *Arch Dermatol* 1970; 102:457–60.
- 65 Hepburn DJ, Davis AL, Pigeon GB, Begg EJ. Linear IgA dermatosis with severe arthralgia. *Australas J Dermatol* 1991; 32:133–6.
- 66 Heylings JR, Clowes HM, Cumberbatch M et al. Sensitization to 2,4-dinitrochlorobenzene: Influence of vehicle on absorption and lymph node activation. *Toxicology* 1996; 109:57–65.
- 67 Romagnoli P, Spinass GA, Sinigaglia F. Gold-specific T-cells in rheumatoid-arthritis patients treated with gold. *J Clin Invest* 1992; 89:254–8.
- 68 Kimber I, Dearman RJ, Cumberbatch M, Huby RJD. Langerhans cells and chemical allergy. *Curr Opin Immunol* 1998; 10:614–9.
- 69 Cavani A, Mei D, Guerra E et al. Patients with allergic contact dermatitis to nickel and nonallergic individuals display different nickel-specific T cell responses- Evidence for the presence of effector CD8(+) and regulatory CD4(+) T cells. *J Invest Dermatol* 1998; 111:621–8.
- 70 Matura M, Goossens A. Contact allergy to corticosteroids. *Allergy* 2000; 55:698–704.
- 71 Fyad A, Masmoudi ML, Lachapelle JM. The 'edge effect' with patch test materials. *Contact Dermatitis* 1987; 16: 147–51.
- 72 Colver GB, Inglis JA, McVittie E, Spencer MJ, Tolley DA, Hunter JA. Dermatitis due to intravesical mitomycin C: a delayed-type hypersensitivity reaction? *Br J Dermatol* 1990; 122:217–24.
- 73 Larsson A, Moller H, Bjorkner B, Bruze M. Morphology of endogenous flare-up reactions in contact allergy to gold. *Acta Dermatol-Venereol* 1997; 77:474–9.
- 74 Moller H. Clinical response to gold as a circulating contact allergen. *Acta Dermatol-Venereol* 2000; 80: 111–3.
- 75 Pichler W, Yawalkar N, Schmid S, Helbling A. Pathogenesis of drug-induced exanthems. *Allergy* 2002; 57:884–93.
- 76 Chan HL, Stern RS, Arndt KA et al. The incidence of erythema multiforme, Stevens–Johnson syndrome, and toxic epidermal necrolysis. A population-based study with particular reference to reactions caused by drugs among outpatients. *Arch Dermatol* 1990; 126:43–7.
- 77 Calkin JM, Maibach HI. Delayed hypersensitivity drug reactions diagnosed by patch testing. *Contact Dermatitis* 1993; 29:223–33.
- 78 Wolkenstein P, Chosidow O, Flechet 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.
- 79 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.
- 80 Hertl M, Merk HF. Lymphocyte activation in cutaneous drug reactions. *J Invest Dermatol* 1995; 105:95S–8S.
- 81 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.
- 82 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.
- 83 Schnyder B, Frutig K, Mauriellweg D, Limat A, Yawalkar N, Pichler WJ. T-cell-mediated cytotoxicity against keratinocytes in sulfamethoxazol-induced skin reaction. *Clin Exp Allergy* 1998; 28:1412–7.
- 84 Zanni MP, vonGreylz S, Schnyder B et al. T cell reactions in patients showing adverse immune reactions to drugs. *Inflamm Res* 1996; 45:S79–S84.
- 85 Nyfeler B, Pichler WJ. The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. *Clin Exp Allergy* 1997; 27:175–81.
- 86 Barbaud A. Drug patch tests in cutaneous adverse drug reactions. *Rev Fr Allergol* 1998; 38:374–8.
- 87 Hari Y, Frutig-Schnyder K, Hurni M et al. T cell involvement in cutaneous drug eruptions. *Clin Exp Allergy* 2001; 31:1398–408.
- 88 Roujeau JC, Dubertret L, Moritz S et al. Involvement of macrophages in the pathology of toxic epidermal necrolysis. *Br J Dermatol* 1985; 113:425–30.
- 89 Friedmann PS, Strickland I, Pirmohamed M, Park BK. Investigation of mechanisms in toxic epidermal necrolysis induced by carbamazepine. *Arch Dermatol* 1994; 130:598–604.
- 90 Paul C, Wolkenstein P, Adle H et al. Apoptosis as a mechanism of keratinocyte death in toxic epidermal necrolysis. *Br J Dermatol* 1996; 134:710–4.
- 91 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.
- 92 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.
- 93 Nassif A, Bensussan A, Dorothee G et al. Drug specific cytotoxic T-cells in the skin lesions of a patient with toxic epidermal necrolysis. *J Invest Dermatol* 2002; 118:728–33.
- 94 Wolkenstein P, Charue D, Laurent P, Revuz J, Roujeau JC, Bagot M. Metabolic predisposition to cutaneous adverse drug reactions. Role in toxic epidermal necrolysis caused by sulfonamides and anticonvulsants. *Arch Dermatol* 1995; 131:544–51.
- 95 Braden GL, Warzynski MJ, Golightly M, Ballow M. Cell-mediated immunity in allopurinol-induced hypersensitivity. *Clin Immunol Immunopathol* 1994; 70:145–51.
- 96 Britschgi M, Pichler WJ. Acute generalized exanthematous pustulosis, a clue to neutrophil-mediated inflammatory processes orchestrated by T cells. *Curr Opin Allergy Clin Immunol* 2002; 2:325–31.

- 97 Morgan CJ, Renwick AG, Friedmann PS. Dermal levels of penciclovir following oral administration of famciclovir measured by microdialysis. *Br J Dermatol* 2002; 147(Suppl. 62):35–36.
- 98 Pepys J, Pepys EO, Baldo BA, Whitwam JG. Anaphylactic/anaphylactoid reactions to anaesthetic and associated agents. Skin prick tests in aetiological diagnosis. *Anaesthesia* 1994; 49:470–5.
- 99 Moneret-Vautrin DA, Kanny G. Anaphylaxis to muscle relaxants: rationale for skin tests. *Allerg Immunol (Paris)* 2002; 34:233–40.
- 100 Barbaud A, Goncalo M, Bruynzeel D, Bircher A. Guidelines for performing skin tests with drugs in the investigation of cutaneous adverse drug reactions. *Contact Dermatitis* 2001; 45:321–8.
- 101 Barbaud A, Reichert Penetrat S, Trechot P et al. The use of skin testing in the investigation of cutaneous adverse drug reactions. *Br J Dermatol* 1998; 139:49–58.
- 102 Pichichero ME, Pichichero DM. Diagnosis of penicillin, amoxicillin, and cephalosporin allergy: reliability of examination assessed by skin testing and oral challenge. *J Pediatr* 1998; 132:137–43.
- 103 Torres MJ, Romano A, Mayorga C, Moya MC, Guzman AE, Reche M, Juarez C, Blanca M. Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. *Allergy* 2001; 56:850–6.
- 104 Blanca M, Mayorga C, Torres MJ et al. Clinical evaluation of Pharmacia CAP System RAST FEIA amoxicilloyl and benzylpenicilloyl in patients with penicillin allergy. *Allergy* 2001; 56:862–70.
- 105 Monneret G, Benoit Y, Debard AL, Gutowski MC, Topenot I, Bienvenu J. Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol* 2002; 102:192–9.
- 106 Sanz ML, Gamboa PM, Antepara I et al. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy* 2002; 32:277–86.
- 107 Schnyder B, Pichler WJ. Skin and laboratory tests in amoxicillin- and penicillin-induced morbilliform skin eruption. *Clin Exp Allergy* 2000; 30:590–5.
- 108 Osawa J, Naito S, Aihara M, Kitamura K, Ikezawa Z, Nakajima H. Evaluation of skin test reactions in patients with non-immediate type drug eruptions. *J Dermatol* 1990; 17: 235–9.
- 109 Shear NH, Spielberg SP. An *in vitro* lymphocytotoxicity assay for studying adverse reactions to sulphonamides. *Br J Dermatol* 1985; 113(Suppl. 28):112–3.
- 110 Shear NH, Spielberg SP. Anticonvulsant hypersensitivity syndrome. *In vitro* assessment of risk. *J Clin Invest* 1988; 82: 1826–32.
- 111 Troost RJ, Van Parys JA, Hooijkaas H, van Joost T, Benner R, Prens EP. Allergy to carbamazepine: parallel *in vivo* and *in vitro* detection. *Epilepsia* 1996; 37:1093–9.
- 112 Zakrzewska JM, Ivanyi L. *In vitro* lymphocyte proliferation by carbamazepine, carbamazepine-10, 11-epoxide, and oxcarbazepine in the diagnosis of drug-induced hypersensitivity. *J Allergy Clin Immunol* 1988; 82:110–5.
- 113 Pearce RE, Vakkalagadda GR, Leeder Pathways of carbamazepine bioactivation *In vitro* I- Characterization of human cytochromes P450 responsible for the formation of 2- and 3-hydroxylated metabolites. *Drug Metab Dispos* 2002; 30: 1170–9.