Allergic reactions to drugs: involvement of T cells

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Drug side effects are common. They are usually classified as type A reactions if they are related to the pharmacological activity of the drug, and type B reactions if unrelated to the pharmacological activity. Immune mediated side effects are type B reactions and account for about one seventh of all drug related side effects.[1] Their frequency is, however, highly dependent on the type of drug. Some drugs are notorious for their allergic side effects—for example, some antibiotics and antiepileptics—while other drugs are seldom related to allergies.

The diagnosis of drug allergy is difficult for various reasons. Firstly, the clinical manifestations of drug allergies are very heterogeneous. Drug allergic reactions imitate diseases, causing symptoms similar to infectious, autoimmune, or superantigen triggered diseases. If no skin symptoms are present, drug induced allergic reactions such as hepatitis or interstitial lung diseases are likely to be underdiagnosed. Moreover, viral infections such as HIV or EBV might be crucial cofactors to elicit symptoms.[2 3]

Secondly, there are no reliable tests generally available to diagnose drug allergy and to pinpoint the relevant drug. The large number of different drugs able to elicit side effects limits the possibility of having standardised tests prepared for each compound, even if one neglects metabolites. Skin test systems have been systematically developed for penicillin hypersensitivity only, but they are tailored to detect IgE mediated allergies and are not standardised to detect other types of drug induced immunological side effects. Moreover, the presence of minor or more general cross reactivity limits the relevance of these tests.[4]

Finally, the pathophysiology of most drug related side effects is unknown. Only a small proportion of drug allergies is IgE mediated. How sensitisation to the drug occurs, to what extent T cells are involved, and how the different pathologies are related to the symptoms of drug allergy are still unknown. The classification of drug allergies based on the scheme of Gell and Coombs[5] is helpful for some drug related side effects, but the most frequent side effect of drugs—the exanthema (rash)—as well as some of the most severe side reactions—toxic epidermal necrolysis, Stevens-Johnson's syndrome, and hypersensitivity reactions involving internal organs—are not explained by this classification.

For the practical approach to a patient with suspected drug allergy, a schematic differentiation between different forms of drug interactions with the immune system is advisable (table 1). This subdivision into "real" drug allergies, pseudoallergies, autoimmune diseases, and pharmacological interference by drugs with immune cell functions is useful for diagnostic procedures and helpful for advising the patient to avoid further symptoms. Thus, in real drug allergies care must be taken that structurally similar compounds are avoided because of a possible immunological cross reactivity. In contrast, in pseudoallergic reactions pharmacological activities such as inhibition of prostaglandin synthesis, but not structural similarities, have to be taken into account.

Immunogenicity of drugs for T cells

The involvement of drug specific antibodies in some drug allergies such as anaphylaxis, urticaria, angio-oedema, and haemolytic anaemia is well known. In contrast, the role of drug specific T cells has long been controversial. There are two possible explanations for this neglect of T cells in drug allergy: (1) T cells were thought to recognise peptides only, and not haptens or drugs. According to the hapten carrier model, T cells are responsible for the recognition of the carrier molecule but are unable to recognise haptens themselves while antibodies are specific for the hapten.[6 7] (2) Although there is ample evidence that T cell infiltrates are present in various allergic skin lesions, the function of these lesional T cells was not understood.[8]

However, clinical experience and laboratory data have indicated that T cells participate in various drug allergic reactions (table 2). Moreover, recent data have shown convincingly that naturally occurring or synthesised non-peptidic, low molecular weight substances are recognised by T cells.[9] Both αβ+ and γδ+ T cells are involved in the recognition of these so-called "non-peptide antigens" which are presented by MHC or MHC-like (CD1) molecules.[9–11] These non-peptide antigens can be subclassified according to their structure into lipids, prenyl-pyrophosphates, sugars, metals, or drugs. It is thought that the recognition of some of these non-peptide antigens is important for the natural immune reaction to

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Table 1 Interaction of drugs with the immune system

	Examples
Specific interactions	
Immune reaction to the drug itself ("real" drug allergy)	Penicillins, sulphonamides
Induction of an autoimmune response	D-penicillamine, procainamide
Non-specific interactions	
IgE (type 1)-like symptoms without evidence for sensitisation (= pseudoallergy)	Acetylsalicylic acid
Interference with immune cell activation, signal transduction, cytokine production	Cyclosporin A, thalidomide

Table 2 Evidence for T cell participation in drug allergy

Clinical symptoms such as contact dermatitis, exanthema, hepatitis, etc with no evidence of formation of drug specific antibodies
Positive lymphocyte transformation and skin patch tests
Formation of T cell dependent anti-drug antibodies (IgE, IgG)
Drug specific T cell lines and clones

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pathogens since, for example, prenyl-pyrophosphates produced by mycobacteria and recognised by γδ+ T cells might steer the immune response in the direction of a Th1 like phenotype.[12]

The direct recognition of haptens by T cells has been convincingly shown by the work of Weltzien and collaborators.[13 14] They directly coupled trinitrophenyl (TNP) to immunogenic peptides which are presented by MHC class I or II molecules. These hapten modified peptides (but not the unmodified peptides) were recognised by TNP specific T cells obtained from mice sensitised to TNP. The immunogenicity of the hapten relied on its position within the immunogenetic peptide: T cell clones which recognised the hapten in a central position within the immunogenic peptide (that is, position 5 in a peptide with nine amino acids) were rather hapten specific, and the sequence of the immunogenic peptide was irrelevant for T cell recognition. However, if the hapten is bound to an amino acid at position 1, 2, or 9 of the peptide with nine amino acids, the T cell receptor (TCR) co-recognised the amino acid of the peptide. In most, but not all, instances hapten recognition was MHC restricted.[15 16]

Basic concepts of drug recognition by T cells

Our group has intensively studied the role of T cells in drug allergies. We were able to clone drug specific T cells from the peripheral blood of allergic patients sensitised to penicillins, cephalosporins, sulphamethoxazole, lidocaine (lignocaine), and mepivacaine. These clones proved to be highly valuable tools for studying the way in which T cells recognise drugs and the function exerted by drug specific T cells in vivo on drug specific stimulation. Four main findings could be elaborated[17–22]:

(1) Drug specific T cell clones are TCR αβ+ and CD4+ or CD8+. Of over 400 T cell clones, only two were TCR γδ+.[20] All drug specific T cell clones are MHC restricted but about 5–10% of the clones are able to recognise the drug in an MHC allele unrestricted way[23]—that is, a T cell clone from a patient with HLA DRB1*04 could recognise the drug also with HLA DRB1*07, 01, 15, etc (but not with HLA DP or HLA DQ). This allele unrestricted recognition could contribute to enhanced presentation of the drug.

(2) Chemically reactive drugs like β-lactam antibiotics are able to modify proteins and peptides directly in a covalent way and can also change the MHC embedded peptides. This hapten modification leads to the generation of new immunogenic determinants and thus enables immunogenicity of an autologous peptide. Most drugs are, however, chemically inert. They are thought to gain immunogenicity by metabolism, as this might generate reactive intermediates which are then able to function as haptens. However, chemically inert drugs such as lidocaine or sulphamethoxazole do not seem to require processing to a reactive metabolite before binding to proteins or peptides, as fixed antigen presenting cells (APC) can present the sulphamethoxazole or lidocaine, and as the drug specific clones start to react almost immediately after encountering the drug and APC, which is hard to reconcile with an intermediate processing step (fig 1). We concluded that certain drugs are able to bind directly, in a non-covalent way, to the MHC-peptide complex and (fitting) TCR, and that this rather labile binding (washing removes the drug) is sufficient to stimulate T cell clones.[21 22]

(3) Most drug specific clones express the CD4 phenotype. A relatively high proportion secrete high levels of interleukin (IL)-5 and IL-4, but some CD4+ and, particularly, the few CD8+ T cell clones have a clear Th1 like cytokine pattern (high interferon (IFN)γ, low IL-4/IL-5). The high levels of IL-5 could explain the frequent eosinophilia in both the circulation and the tissue of drug allergic patients.[20 24 25]

(4) Quite a few drug specific clones are cytotoxic. Autologous Epstein-Barr virus (EBV) transformed B cell lines incubated with the drug are killed in an MHC restricted way. Interestingly, not only CD8 but also drug specific CD4 cells were able to kill, and the cytolysis was mediated by perforin as revealed by inhibition experiments with concanamycin A and positive perforin staining of the T cell clones. Further investigations revealed that CD4+ T cell clones were also killing autologous keratinocytes, but only after preincubation of the keratinocytes with IFNγ which was required to upregulate MHC class II molecules. The drug had to be present during the incubation period of the cytotoxicity assay. This finding suggested that drug specific CD4+ T cells might be relevant for drug induced exanthema by killing keratinocytes.[21 26]

How do these in vitro elaborated mechanisms apply to the in vivo situation? To address this question we performed a series of immunohistochemical stainings of frozen skin biopsy specimens of patients with drug induced exanthema. The patients had mainly morbilliforme like maculopapular drug eruptions, but some patients suffered also from bullous skin disease. The relevant drug was pinpointed in all patients by a clear history,

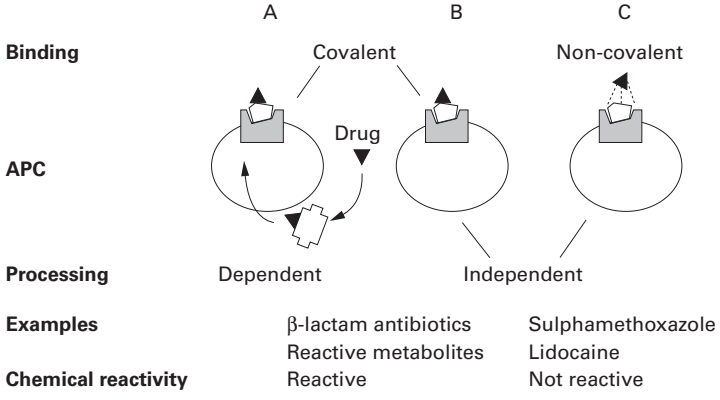

Figure 1 Schematic representation of the way in which T cells might recognise drugs. Hapten-like drugs (▲) undergo covalent binding to soluble proteins (□, A) or to the cell surface and might modify the MHC-peptide complex directly (B). Most drugs are not chemically reactive, however, and appear to interact directly with the MHC-peptide complex and stimulate T cells (C).

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positive patch, and/or in vitro tests (lymphocyte transformation tests). Control biopsy specimens were obtained from operation specimens. In this report we will review the data of the maculopapular drug eruption only.

Histology of the maculopapular exanthema

Hypersensitivity reactions to drugs can cause different types of skin disorders, most frequently maculopapular eruptions. The mechanism of these and other drug induced skin diseases is still not well understood, in contrast to the well known mechanism of IgE mediated urticarial and angio-oedematous skin diseases or anaphylaxis. The lack of a profound understanding of these most frequent drug induced side effects—namely, the drug elicited maculopapular exanthema—has a major impact on the correct diagnosis, prevention, and treatment of these and related allergic diseases. It is not only highly relevant that diagnostic procedures in a suspected drug allergy are directed to detect drug specific IgE as these IgE tailored tests are not suitable for identifying a T cell mediated mechanism. Moreover, since exanthema is often associated with more severe internal diseases such as hepatitis, interstitial nephritis, or interstitial lung disease, clarification of this mechanism is also relevant for the prevention of these more life threatening allergic diseases.

The characteristics of the histological appearance of maculopapular exanthema is a mononuclear cell infiltrate of variable intensity, the frequent presence of eosinophils, and an interphase dermatitis. The interphase dermatitis is characterised by hydropic degeneration of keratinocytes, particularly of those close to the infiltrating lymphocytes.

Immunohistochemistry revealed that most infiltrating lymphocytes in maculopapular drug eruptions are CD4+.[27] They are present around the vessels in the dermis, but partly migrate to the epidermis and some even penetrate into the epidermis. CD8+ T cells are also found in most histological specimens but are present in lower numbers in the maculopapular exanthema. One patient had almost no CD8+ T cells in the skin, suggesting that CD4+ T cells are sufficient to cause the pathology of a morbilliforme like drug exanthema.

The infiltrating T cells are activated as revealed by IL-2R (CD25) or MHC class II expression. MHC class II molecules were also found on cells of the epidermis, both on the residual dendritic cells (Langerhans' cells) and on keratinocytes. Thus, keratinocytes are stimulated in exanthema and, like the APC, are also able to present antigens to CD4+ T cells. This additional APC function of keratinocytes is further supported by the expression of adhesion molecules such as ICAM-1.

The infiltrating T cells, both from the dermis and the epidermis, express perforin and granzyme, molecules characteristic of cells with cytotoxic potential. Double staining for cytotoxic molecules and CD4 or CD8 indicated that both cell types might have cytotoxic activity. It is probable that a distinct CD4 subset expresses eotaxin and IL-5. Thus, histochemical analysis confirmed the in vitro data and ruled out the expression of perforin or granzyme as an artifact of T cell cloning, generated in the long term cultures.

Figure 2 summarises schematically the possible events leading to maculopapular drug eruptions. Both chemically reactive and non-reactive drugs are presented by dendritic cells and eventually other cells in the dermis/epidermis. The former bind covalently, while the latter do not need to be metabolised to be presented and can bind from the outside in a non-covalent way to MHC class I and II molecules. In the presence of sufficiently large numbers of specific T cells generated in the lymph nodes, a cell infiltrate will develop supported by the production of chemotactic chemokines by some resident and immigrating cells. The infiltrating T cells will be composed of CD4 and CD8 cells, the ratio of which may determine the type of exanthema. If the drug presentation leads to a substantial expansion of CD8+ T cells a bullous reaction is likely, but if the infiltrate is composed of CD4 cells a morbilliforme like rash might develop. The infiltrating cells may recruit eosinophils by IL-5 and eotaxin expression. In addition, the cells seem to be cytotoxic and able to kill MHC class II molecules and ICAM-1 expressing keratinocytes. The hydropic degeneration of keratinocytes is a correlate of this T cell mediated cytotoxicity.

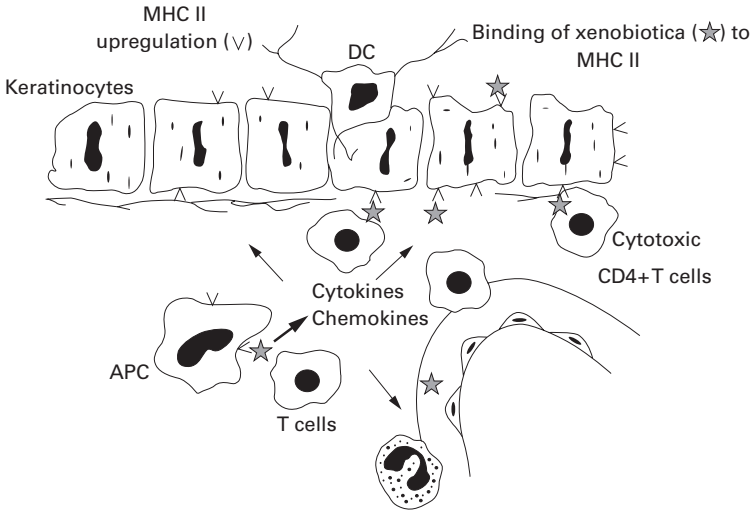

Figure 2 Phases of drug induced maculopapular exanthema. A schematic representation of the events occurring in the skin is shown. Xenobiotica bind to dendritic cells (DC); keratinocytes express MHC class II (see text). Both cells present the drug which is either bound covalently or non-covalently to MHC-peptide complexes. CD4+ T cells are recruited and are either cytotoxic or express eotaxin/IL-5, recruiting more eosinophils. Cytotoxic T cells kill keratinocytes and lead to the typical histological features of dying keratinocytes (see text). Killing of keratinocytes occurs via perforin and granzyme B which is synthesised and released by the cytotoxic CD4+ T cells upon encounter with the antigen (= MHC class II and drug). Keratinocytes might additionally express ICAM-1 molecules which bind to LFA-1 on T cells and thereby stabilise the interaction between T cells and keratinocytes.

Implications for cutaneous manifestation and viral enhancement of drug allergies

The data obtained give a rather homogeneous picture which agrees with both in vitro and in vivo findings. Nevertheless, they also raise some questions which at present can only be answered by hypotheses.

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WHY DO MOST DRUG ALLERGIES MANIFEST THEMSELVES IN THE SKIN?

We speculate that allergies manifest themselves in the skin because the skin is a border region where the immune system is in a constantly low level of activation as it requires constant effort to defend the body's integrity. This constant level of activation provides the "danger" signal which facilitates the immune response and may allow the initiation of an inflammatory response. Other regions of the body may lack this essential pro-inflammatory signal and are therefore more rarely the targets of an allergic immune response. However, if the incriminated drug also has a toxic effect on certain tissues—for example, on certain cells of the kidney—then this organ might also become involved because the cell damage and subsequent necrosis may elicit a danger/pro-inflammatory signal.

This hypothesis would explain the manifestation of certain drug allergies in other tissues as well as the skin—for example, non-steroidal anti-inflammatory drug (NSAID) related allergies in the kidney as NSAIDs tend to damage kidney cells. If the drug affects alveolar cells an interstitial lung disease might develop. Additional factors such as interference by the drug itself with the production of pro-inflammatory cytokines might also be relevant.

WHY DO GENERALISED VIRAL INFECTIONS ENHANCE THE OCCURRENCE OF DRUG ALLERGIES?

It is well known that EBV or HIV infections enhance the occurrence of drug allergies. The use of amoxicillin in infectious mononucleosis is frequently associated with exanthemas and is considered to be an almost obligatory pathognomonic event. In HIV infection the use of sulphamethoxazole also frequently causes allergic skin diseases, some of which may be rather severe. After remission of the EBV infection or change of the immune status of HIV patients, the same drug can be applied without problems.

Both diseases are accompanied by a massive stimulation of the immune system and high levels of IFNγ. This may lead to upregulation of MHC class II molecules (and other costimulatory/adhesion molecules) on professional and non-professional APCs (such as keratinocytes) and thus facilitate drug presentation. After cessation of the acute viral infection the level of activating cytokines (particularly IFNγ) decreases and consequently the conditions for drug presentation again become worse. Further exposure to the same drug is tolerated in a non-activated organism; one reason for this is that the conditions of drug presentation are suboptimal.

This concept implies that most people have circulating T cells which are able to react with haptenised peptides (in the case of amoxicillin) or sulphamethoxazole directly. This agrees with our observation that the immune response to sulphamethoxazole can be heterogeneous and is composed of T cells bearing many different TCR-Vβ. The affinity of most of these drug specific T cells may, however, be too low to become damaging under normal conditions with low cytokine values and a low level of drug presentation.

Conclusions

T cells are important and relevant in drug allergy, in particular in exanthema and associated diseases. They are able to recognise the drug directly without covalent hapten modification of proteins or peptides. This peculiar feature might explain the manifestation of drug allergies in organs without well documented metabolic activity and the high frequency of positive lymphocyte transformation tests to drugs using peripheral mononuclear cell cultures without addition of metabolic enzymes.

In the most common form of drug allergy, the maculopapular exanthema, studies of circulating T cells, of T cells eluted from the skin, and in situ staining suggest a dominant role for cytotoxic CD4+ T cells. These cells infiltrate the skin (dermis and epidermis) in maculopapular rashes and probably cause the typical morphological changes seen in this common form of drug allergy—namely, hydropic degeneration and keratinocyte apoptosis—as the keratinocytes express MHC class II molecules and are thus able to present the drug to the T cells. In addition, the same or other cells secrete large amounts of IL-5, thus contributing to the eosinophilia, another typical characteristic of drug allergy.

This new concept has important implications for predictive allergy testing—namely, determination of the allergenic potential of a drug in the process of its development—as severe allergic side effects can occur even with chemically inert drugs. It has implications for the testing of a patient with suspected drug allergies and emphasises the urgent need for standardised tests for T cell reactivity (patch and lymphocyte transformation tests).

The European Network of Drug Allergy (ENDA), which is the EAACI interest group on drug hypersensitivity/allergy, is attempting to do this by collecting skin test and in vitro data of well defined patients.

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