doi: 10.1111/j.1365-2222.2007.02742.x
Clinical and Experimental Allergy, **37**, 989–999
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REVIEW

Delayed drug hypersensitivity reactions – new concepts

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Clinical and Experimental Allergy

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Summary
Immune reactions to small molecular compounds such as drugs can cause a variety of diseases mainly involving skin, but also liver, kidney, lungs and other organs. In addition to the well-known immediate, IgE-mediated reactions to drugs, many drug-induced hypersensitivity reactions appear delayed. Recent data have shown that in these delayed reactions drug-specific $CD4^+$ and $CD8^+$ T cells recognize drugs through their T cell receptors (TCR) in an MHC-dependent way. Immunohistochemical and functional studies of drug-reactive T cells in patients with distinct forms of exanthems revealed that distinct T cell functions lead to different clinical phenotypes. Taken together, these data allow delayed hypersensitivity reactions (type IV) to be further subclassified into T cell reactions, which by releasing certain cytokines and chemokines preferentially activate and recruit monocytes (type IVa), eosinophils (type IVb), or neutrophils (type IVd). Moreover, cytotoxic functions by either $CD4^+$ or $CD8^+$ T cells (type IVc) seem to participate in all type IV reactions. Drugs are not only immunogenic because of their chemical reactivity, but also because they may bind in a labile way to available TCRs and possibly MHC-molecules. This seems to be sufficient to stimulate certain, probably preactivated T cells. The drug seems to bind first to the fitting TCR, which already exerts some activation. For full activation, an additional interaction of the TCR with the MHC molecules is needed. The drug binding to the receptor structures is reminiscent of a *p*harmacological interaction between a drug and its (*i*mmune) receptor and was thus termed the p–i concept. In some patients with drug hypersensitivity, such a response occurs within hours even upon the first exposure to the drug. The T cell reaction to the drug might thus not be due to a classical, primary response, but is due to peptide-specific T cells which happen to be stimulated by a drug. This new concept has major implications for understanding clinical and immunological features of drug hypersensitivity and a model to explain the frequent skin symptoms in drug hypersensitivity is proposed.

Introduction

Adverse drug reactions (ADRs) are grouped into two broad categories, those that are predictable, common, and related to the pharmacologic actions of the drug (type A reactions) and those that are unpredictable, uncommon and usually not related to the pharmacologic actions of the drug (type B reactions) [1]. Approximately 80% of ADRs fall into the first category, and typical examples are drug-induced toxicity, gastrointestinal bleeding after treatment with NSAID, or bradycardia with β blocker treatments, etc. Immune-mediated or allergic drug reactions belong to type B reactions. They account for only 15% of all ADR [2]. Unlike type A reactions, type B reactions often do not appear until a drug is marketed and more widely used. Moreover, their development appears to depend on both genetic and environmental factors. Reactions in this category include drug intolerance (an undesired drug effect produced by the drug at therapeutic or subtherapeutic doses), idiosyncratic reactions (reactions that are not explicable in terms of the known pharmacologic actions of the drug, but probably due to some genetic preponderance of the individual), and allergic or hypersensitivity reactions (reactions that are dependent on one or more immunological mechanisms). The distinction between idiosyncratic and immune-mediated side-effects is justified, although some immune-mediated side-effects have clearly a genetic basis as well: but in many drug-allergic reactions the stimulation of the immune system by, e.g. viruses (EBV or HIV) lead to reaction in $>50\%$ of the infected individuals without clear correlation to a genetic basis.

Allergic reactions can be classified according to Coombs and Gell [3] into immediate-type hypersensitivity reactions (mediated by drug-specific IgE antibodies), cytotoxic and immune complex reactions (mediated by drug-specific IgG or IgM antibodies), and delayed-type hypersensitivity reactions (mediated by drug-specific T lymphocytes). This classification system has proven to be helpful in clinical practice and can guide diagnostic decisions. However, the term *delayed hypersensitivity reactions*, originally coined to describe T cell reactions to tuberculin, became an umbrella term for various T cell-mediated immune mechanisms leading to clinically distinct diseases. Indeed, T cells have been found to differ in the cytokines they produce, which results in distinct types of immune defence. T-helper type 1 (Th1) T cells activate macrophages by secreting large amounts of interferon γ (IFN-γ), drive the production of complement-fixing antibody isotypes, and costimulate proinflammatory and $CD8^+$ T cell responses. T-helper type 2 (Th2) T cells secrete the cytokines IL-4 and IL-5 [4], 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. Further, T cells can exert cytotoxic functions either by releasing perforin/granzyme or by secreting FasL.

This review relies on new data and concepts of the role of T cells in drug-induced hypersensitivity, which evolved from the *in vitro* study of drug-specific T cells derived from blood and tissues of the patients suffering from various drug-induced hypersensitivity diseases. These T cells were analysed functionally and compared with the T cells observed in the immunohistology of the organs affected by the drug allergy. The following new results were obtained: (a) a novel mechanism of T cells by drug stimulation could be defined, extending the hapten concept [5–7], (b) the type IV reactions were further subclassified into four subtypes based on the clinical, immunohistochemical and functional heterogeneity of certain drug allergies [8, 9]. In this review, we combine these new concepts and we propose a concept on how and why T cells cause skin-related symptoms so frequently.

Subclassifying drug reactions

The old Gell and Coombs classification was established before a detailed analysis of T cell subsets and functions was available [3]. In the meantime immunological research has revealed that all four types of hypersensitivity reactions (types I–IV) require an involvement of T cells, which produce cytokines that aid immunoglobulin-isotype switching. Moreover, T cells can orchestrate different forms of inflammations. Therefore T cell-meditated type IV reactions were further subclassified in IVa–IVd reactions as proposed in Table 1 [9].

The following type IV reactions were defined:

Type IVa reactions correspond to Th1 immune reactions: Th1 type T cells activate macrophages by secreting large amounts of IFN-γ, drive the production of complement fixing antibody isotypes (IgG1, IgG3), act as costimulators for proinflammatory responses (TNF, IL-12) and $CD8^+$ T cell responses. An *in vivo* correlate would be on one hand a monocyte activation, e.g. in skin tests to tuberkulin or even granuloma formation as seen in sarcoidosis. On the other hand, these Th1 cells are known to activate CD8 cells, which might explain the common combination of IVa (high IFN-γ values) and IVc reactions (e.g. in contact dermatitis).

Type IVb corresponds to the Th2 type immune response. Th2 Tcells secrete IL-4, IL-13 and IL-5 cytokines, which promote B cell production of IgE and IgG4, macrophage deactivation and mast cell and eosinophil responses. The high production of the Th2 cytokine IL-5 leads to an eosinophilic inflammation, which is the characteristic inflammatory cell type in many drug hypersensitivity reactions [9, 10]. In addition, there is a link to type I reactions, as Th2 cells boost IgE production by IL-4/IL-13 secretion. An *in vivo* correlate might be an eosinophil-rich maculopapular exanthema, but also presence of nematodes infestations or bronchial or nasal inflammation (asthma and rhinitis, respectively).

Type IVc: T cells can also act as effector cells. they emigrate to the tissue and can kill tissue cells, such as hepatocytes or keratinocytes in a perforin/granzyme B and/or FasL-dependent manner [11, 12]. Such reactions are occurring in most drug-induced delayed hypersensitivity reactions, mostly together with other type IV reactions involving monocyte, eosinophil or PMN recruitment and activation. Cytotoxic T cells thus play a role in maculopapular or bullous skin diseases, as well as in neutrophilic inflammations and in contact dermatitis. Type IVc reactions appear to be predominant in bullous skin reactions, like Stevens Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), where activated $CD8^+$ T cells kill keratinocytes [9, 12, 13], but may also be the dominant cell type in hepatitis or nephritis [14, 15]. In general, a cytotoxic skin reaction mediated by $CD8^+$ T cells seems to be more dangerous than $CD4^+$ T cell-mediated cytotoxicity, as all keratinocytes etc. are possible target cells for $CD8^+$ T cells as they all express MHC class I, while CD4 reactions may be directed to few cells only, namely exclusively activated, MHC class II positive cells. Consequently, the more severe reactions like SJS and TEN are characterized by massive $CD8^+$ cell presence, which are found mainly in the blister fluid of SJS/TEN patients.

Type IVd: Rather neglected was the concept that T cells driven by an antigen can coordinate (sterile) neutrophilic inflammations as well. typical examples would be sterile neutrophilic inflammations of the skin, in particular acute-generalized exanthematous pustulosis (AGEP). In

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Table 1. Revised type IV reactions

	T Cell type	Immune reactant	Effector mechanism	Clinical symptoms (example)
Type IVa	Th1	IFN-γ, TNF-α	Monocyte/macrophage activation	Tuberculin skin test, participation in contact dermatitis, bullous exanthema . . .
Type IVb	Th2	IL-5, IL-4, IL-13, eotaxin	T cells driving eosinophilic inflammation	Maculopapular and bullous exanthema, etc.
Type IVc	Cytotoxic T cells	perforin, granzyme B, FasL	$CD4^+/CD8^+$ mediated T cell killing	Contact dermatitis maculopapular, pustular and bullous exanthema, etc.
Type IVd	T cells	CXCL-8, GM-CSF	T cell leading to recruitment and activation of neutrophils	Pustular exanthema

Includes the cytotoxic activity of both CD4 and CD8 T cells (IVc); and it emphasizes the participation of different effector cells like monocytes (IVa), eosinophils (IVb), or neutrophils (IVd), which are the cells causing the inflammation and tissue damage. This subclassification is in principle based on the effector cells involved (monocytes, eosinophils, T cells themselves, and PMN), and thus clinically applicable without need to determine the cytokine pattern. One should, however, be aware, that in most reactions different type IV reactions may occur together, as e.g. cytotoxic T cells and T cells recruiting PMN contribute together to the clinical phenotype of AGEP and contact dermatitis is due to type IVc reaction, but often with some IVa involvement as well, etc.

this disease CXCL8 and GM-CSF producing T cells recruit neutrophilic leucocytes via CXCL8 release and prevent their apoptosis via GM-CSF release [16]. Besides AGEP, such T cell reactions are also found in Behçet disease and pustular psoriasis [17].

Drug recognition by T cells

The hapten and pro-hapten concept

An immune reaction starts by the involvement of the innate immune system. The antigen (bacterium, virus, etc.) stimulates this system via pattern recognition receptors, e.g. Toll-like receptors (TLR) on dendritic cell (DC)s and thereby sets an initial alarm signal. The activated DCs function as antigen-presenting cells (APC) as they take up and process complex and larger antigens, which subsequently are presented as peptides to T cells in a suitable environment, namely the secondary lymphoid organs, such as the lymph nodes. The ensuing immune response is variable and efficient, as different antigens soluble or cell-bound viral antigens can elicit a distinct immune response capable of eliminating the infectious agent.

Because of their macromolecular form, some drugs, such as mouse antibodies, are intrinsically immunogenic. Many drugs, however, have a molecular mass of $<$1000 Da and are considered incapable of inducing an immune response in their native state. For these agents to become effective immunogens, they must bind covalently to high-molecular weight proteins, thereby forming a hapten–carrier complex, which then undergoes processing and presentation. The explanation for an immune response to drug antigens is based primarily on this hapten hypothesis [18], which is well documented for certain chemicals. Some drugs, such as penicillin, are directly chemically reactive as their molecular structure allows spontaneous covalent binding to other molecules. Other drugs, however, must be metabolized or bioactivated to a chemically reactive form before they are able to covalently bind to proteins and induce an immune response against the carrier hapten complex (Fig. 1). Although bioactivation (transformation of a pro-hapten into a hapten) is typically mediated by cytochrome P450 enzymes in liver hepatocytes, it may, albeit in a very reduced amount, also occur at other sites, such as skin keratinocytes. On the other hand, oxidation may frequently also occur at extrahepatic sites, e.g. by enzymes-like myeloperoxidase in neutrophilic leucocytes and macrophages. Bioactivation is usually followed by a bioinactivating process. In some cases, however, genetic or environmental factors may perturb the balance between these two processes, leading to increased formation or decreased elimination of reactive drug metabolites.

Once formed, these reactive species may affect the cells in various ways. (1) they may bind to macromolecules and cause direct cellular damage, (2) they may bind to nucleic acids to produce an altered gene product, or (3) they may bind covalently to larger macromolecular targets, form an immunogenic complex and induce an immune response. The hapten and pro-hapten model is currently the accepted explanation for these observations. Haptens thereby have two essential features: they can namely activate innate (immunogenicity) and adaptive immunity (antigenicity).

Hapten stimulation of the innate immune system. This could occur by covalently binding to cellular proteins or receptors such as TLR [19]. The chemical modifications might thereby transmit a danger signal, which results in stimulation of cells of the innate immune system, for example, by up-regulating CD40 on DCs [19, 20]. Alternatively, the uptake and intracellular processing may already activate the cell – and if it is an APC, it might up-regulate costimulatory molecules like CD40.

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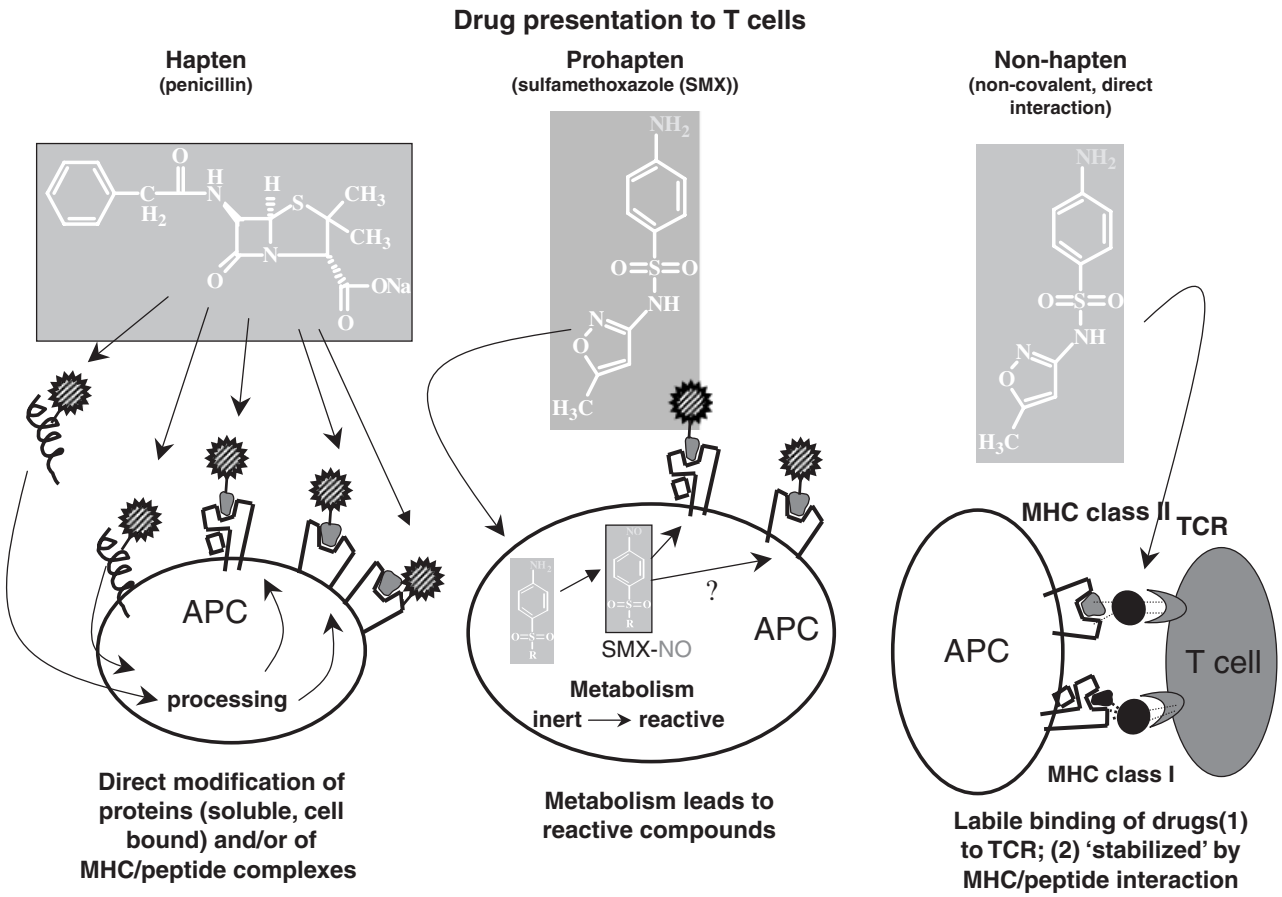


Fig. 1. Drug presentation to T cells. Hapten-like drugs (penicillins) can bind covalently to both soluble or cell-bound molecules. They can even bind directly to the immunogenic MHC–peptide complex on antigen-presenting cells (APC), either the embedded peptide or the MHC molecule itself. Thus, the chemical reactivity of haptens leads to the formation of many distinct antigenic epitopes, which can elicit both humoral and cellular immune responses. Other drugs are pro-haptens, which require metabolism to become haptens (that is, chemically reactive). The metabolism occurs mainly inside cells [for example, from sulphamethoxazole to the chemically reactive form, sulphamethoxazole-nitroso (SMX–NO)]. Metabolism may lead to modification of cell-bound or soluble proteins by the chemically reactive metabolite. A third, nonhapten pathway (pharmacologic interaction with immune receptors, also known as the 'p–i concept') does not require covalent association of the drug with the MHC molecule. The chemically inert drug seems to bind directly to the T cell receptor (labile interaction with TCR). Full T cell stimulation requires an interaction with the MHC molecule. This type of drug stimulation is restricted to certain drugs that fit into TCRs and results in an exclusive T cell stimulation.

Stimulation of the specific immune system. The hapten–protein complex represents a neo-antigen, which is processed and presented as hapten-modified peptides to T cells. Relatively many T and B cells are able to bind to hapten–carrier complexes [21], and a vigorous immune response may arise. A predominant humoral immune response may occur, if the hapten-modification affect soluble and cell bound proteins, but exclusive T cell responses can be seen if the hapten binds directly to the MHC–peptide complexes themselves. Thus, the type and target structure of the chemical modification of proteins determines already which type of immune reaction might develop.

Pro-haptens are per se not chemically reactive, and are thus unable to form a covalent bridge to peptides. They need to be converted into a hapten by being metabolized into a compound that is chemically more reactive [21–24]. Their metabolism may occur in the liver, where it may induce tolerance [25]. On the other hand, the reactive compound may escape the tolerogenic environment of the liver and be further metabolized outside the liver. It may then cause an immune response to the compound in lymph nodes and a systemic drug hypersensitivity syndrome like DRESS, drug rash with eosinophilia and systemic symptoms may develop [26].

The hapten and pro-hapten concept elegantly circumvent the (presumed) blindness of the immune system for low molecular weight compounds by postulating chemical reactivity and subsequent coupling to a macromolecular carrier is an absolute necessity. As a consequence, drugs and other substances that are incapable of such conjugation with a carrier would not be antigens and could not induce hypersensitivity reactions. However, this concept

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has been challenged recently by the development of the p–i concept.

The p–i concept

During the last years our group elaborated a new possibility for drug stimulation of T cells namely a *p*harmacological interaction of drugs with *i*mmune receptors, termed the p–i-concept [27, 28]. Chemically inert drugs, unable of covalently binding to peptides or proteins, can nevertheless associate with some of the $>10^{12}$ different T cell receptors (TCRs) (Fig. 3) (second step). This interaction is reminiscent of other drug–receptor cross-talks and does not rely on a covalent drug–peptide complex being presented by MHC molecules. The interface with the TCR is labile, which is confirmed when the drug binding is reversed by simple washing, while the covalent hapten binding to MHC peptides is not. In pharmacological terms, this means that the binding of the drug to the TCR has a low affinity, probably in the millimolar to micromolar range.

Drug allergy is the example of the drug–receptor interaction, where the consequence of the interaction results in T cell activation and expansion: this stimulation is dependent on an additional MHC interaction for full activation of the reactive T cell, which results in cytokine synthesis, proliferation and probably clinical symptoms. As it mimicks an immunological response, it is interpreted as an immune mechanism – but it is actually a drug-driven T cell expansion. The stimulated T cells have an additional peptide specificity, which is unknown.

In considering all these aspects, the hapten concept is still the only explanation that small compounds stimulate the immune system to develop hapten-specific immune response. However, a nonimmunological, namely pharmacological activation of the immune system is also possible by drugs. This stimulation resembles an immune response to a hapten and may be an explanation for the sometimes bizarre nature of the clinical symptoms of drug hypersensitivity.

Evidence for the p–i concept. Most evidence for this p–i model has been generated for sulphamethoxazole, lidocaine, mepivacain, celecoxib, carbamazepin, lamotrigine and ciprofloxacin [27–32]. It is relevant for maculopapular drug eruption, erythema exsudativum multiforme, acute-generalized exanthematous pustulosis, DRESS, SJS/TEN, and possibly more diseases.

Clinical data

The chronology of the clinical reaction. Some drugs can stimulate an apparently specific immune response to a drug at the first encounter, without chance of a sensitization and before a new, hapten-specific immune response had time to evolve. Other reactions seem to occur in less than three days. This time interval seems to be too short to mount a specific immune response and to elicit an inflammatory process.

Skin tests. Many chemically inert drugs, unable to form hapten–carrier complexes in the skin, are nevertheless able to cause positive skin tests with lymphocyte infiltration [33]. The transport of the locally applied drugs to the liver and the return of their metabolite to the skin, which then may cause a local reaction does not seem to be a plausible explanation in most instances.

Immunological data. The analysis of drug – T cell stimulations through the generation of drug-specific T cell clones (TCC) showed that several observations argue against necessity for drug processing or protein covalent binding.

Specific TCC reacted even if the *APC were fixed by glutaraldehyde*, excluding processing or intracellular metabolism as being involved [27–31]. Many different chemically inert drugs were found to be able to stimulate TCCs via the TCR in an MHC-dependent way, such as lamotrigine [30], carbamazepine [31], sulphamethoxazol (SMX) [27, 28], mepivaccaine [34] lidocaine [29], p-phenylendiamine [35], ciproxin or moxifloxacin [32, 36] and radio contrast media (RCM) [33, 37]. Actually, some studies addressed the antigenicity of metabolites quite extensively: consistently the parent drug stimulated more T cells than the metabolite [27, 28, 38].

Covalent binding is not necessary. Upon pulsing of APC (= incubation and washing steps, which removes a labile bound drug), no T cell stimulation was observed for lidocain, lamotrigine, carbamazepine, ciproxin and SMX [28, 30–32, 36]. In contrast, the hapten sulphamethoxazole-nitroso (SMX–NO), capable of covalently modifying the MHC–peptide complex, was already able to stimulate hapten-reactive
T cells after 1 h incubation [27].

The *kinetics of T cell activation* are too fast for any involvement of antigen processing. In the presence of APC, lidocaine and SMX activate T cells quasi immediately as revealed by a rapid and sustained intracellular Ca^{2+} increase [28]. This timing does not fit to an intermediate metabolism and processing step, which needs 60 min or longer. Also, the kinetics of TCR down-regulation on the surface of drug reactive TCC after encountering the inert drug are similar to the recognition of preprocessed, immunogenic peptides (occurring within the first 30 min), and clearly differ from the recognition of proteins, which requires several hours [28].

The *metabolized form of the drug is less antigenic for T cells in sensitized individuals.* Hypersensitivity to the drug SMX was thought to be a consequence of bioactivation to

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the hydroxylamine metabolite (SMX–NHOH) and further oxidation to the ultimate, reactive metabolite SMX–NO. The antioxidant glutathione is known to protect cells from reactive metabolites like SMX–NO by conjugation and subsequent dissociation to SMX–NHOH and/or SMX [39]. Addition of glutathione to peripheral blood mononuclear cells enhanced rather than reduced the proliferation to SMX-metabolites [39], presumably by transforming SMX–NO back to the 'original' antigen, SMX. The response of SMX–NO-specific TCC was abrogated when glutathione was present during the covalent modification of APC. Collectively, these experiments support the concept that T cells in allergic individuals preferentially recognize the non-covalently bound parent drug SMX rather than APC covalently modified by SMX-NO [39].

MHC restriction. Some drug specific TCCs show some peculiar features, mimic superantigen stimulations not seen with classical peptide antigens: many drug-specific TCC were found to be MHC allele-unrestricted [40, 41], and the frequency of alloreactive TCC is much higher among drug- than peptide-specific TCC from the same donor [41]. The MHC-bound peptide seems to be irrelevant for SMX-specific T cell activation [42]. Lastly, drugs simultaneously elicit a $CD4^+$ and $CD8^+$ T cell responses to the same compound.

Drug hypersensitivity in the skin

The liver is quantitatively the primary site of bioactivation for most drugs, but hypersensitivity reactions in the liver are rather rare. The reason for this is unknown. One might speculate that the liver is a rather tolerogenic organ, and the generation of metabolites in the liver may either not lead to an immunogenic presentation, or the presentation may result in tolerance [25].

Many drug hypersensitivity reactions become manifested in the skin. The hapten/pro-hapten concept has to postulate that drugs are, or can easily become haptens, for example by simple oxidation, which may be the case for many contact allergens eliciting a local reaction. For other, orally, or parentally applied drugs the p–i concept postulates that the drugs distribute to the skin either as the parent (inert) compound, which are metabolized locally in the skin to a reactive hapten, or they arrive the skin already as metabolites, which are locally further oxidized to more reactive compounds (e.g. SMX–NHOH → SMX–NO). This hapten concept is supported by the finding that indeed a survey of drugs associated with DTH reactions in the skin revealed that all have been found to be either metabolized or degraded to reactive species [43]. Moreover, relevant drug metabolizing enzymes have been found to be present in the skin, including cytochromes P-450 [44], flavin monooxygenases [45], cyclooxygenases [46] and *N*-acetyltransferase [47]. Epidermal keratinocytes and dermal fibroblasts [48] have been demonstrated to have some moderate ability to bioactivate sulphamethoxazole and/or dapsone, which might give rise to intracellular haptenation.

In a recent review by Roychowdhury and Svensson [48], the immune reaction to hapten or pro-hapten were outlined: In the skin a drug deriving from the outside (like in contact dermatitis) or possibly also from the circulation would be metabolized by keratinocytes and possibly some DCs and LC. The reactive form would then covalently bind to either intracellular and extracellular cutaneous proteins forming drug–protein adducts, which may subsequently be processed and presented by DC/LC as neo-antigens (steps 1–4, in Fig. 2). Simultaneously, either the drug processing to a reactive compound or the binding of the chemically reactive compound would stimulate the immature DC or resident LC to start migration to draining lymph nodes and to up-regulate adhesion molecules (step 5, Fig. 2). During their journey to draining lymph nodes, DC/LC undergo a maturation process [49]. Various changes have been reported to occur in LCs as a result of antigen exposure, including internalization of surface – MHC-II molecules via endocytosis [50–52], induction of tyrosine phosphorylation [53], modulation of various cell surface markers [54, 55] and cytokine expression [54, 56]. Processing and presentation of the hapten-carrier molecule by DC/LC in the lymph nodes (step 6, Fig. 2) may then lead to the local stimulation and expansion of hapten-specific T cells, which then home to the skin and develop a cytotoxic response (steps 7–9, Fig. 2). What determines the nature of the immune response is rather unclear.

While this concept is well documented and plausible for contact allergens – it has some unexplained features considering *systemic* drug hypersensitivity. (a) The metabolism in the skin is very moderate and restricted to some enzymes: it is controversial whether a complex metabolism, as required for some drugs, can occur in the skin, e.g. the data with SMX have been achieved with very high concentrations of SMX only. (b) If the metabolism occurs in the liver, why would the generated metabolites preferentially go to the skin, when they could form hapten–carrier complexes all over the body.

The p–i concept and skin symptoms

We would like to propose a novel, different concept to explain the skin symptoms in systemic drug hypersensitivity reactions. It takes into account (a) that drugs are ubiquitously distributed in the body, (b) the p–i concept and the lack of potent metabolism in the skin and (c) the well-described sentinel function of some resident T cells in the skin (Fig. 3) [57].

(a) It is well known, that the oral or parenteral uptake of drugs leads rapidly to a distribution throughout the body: This is particularly well documented for the skin,

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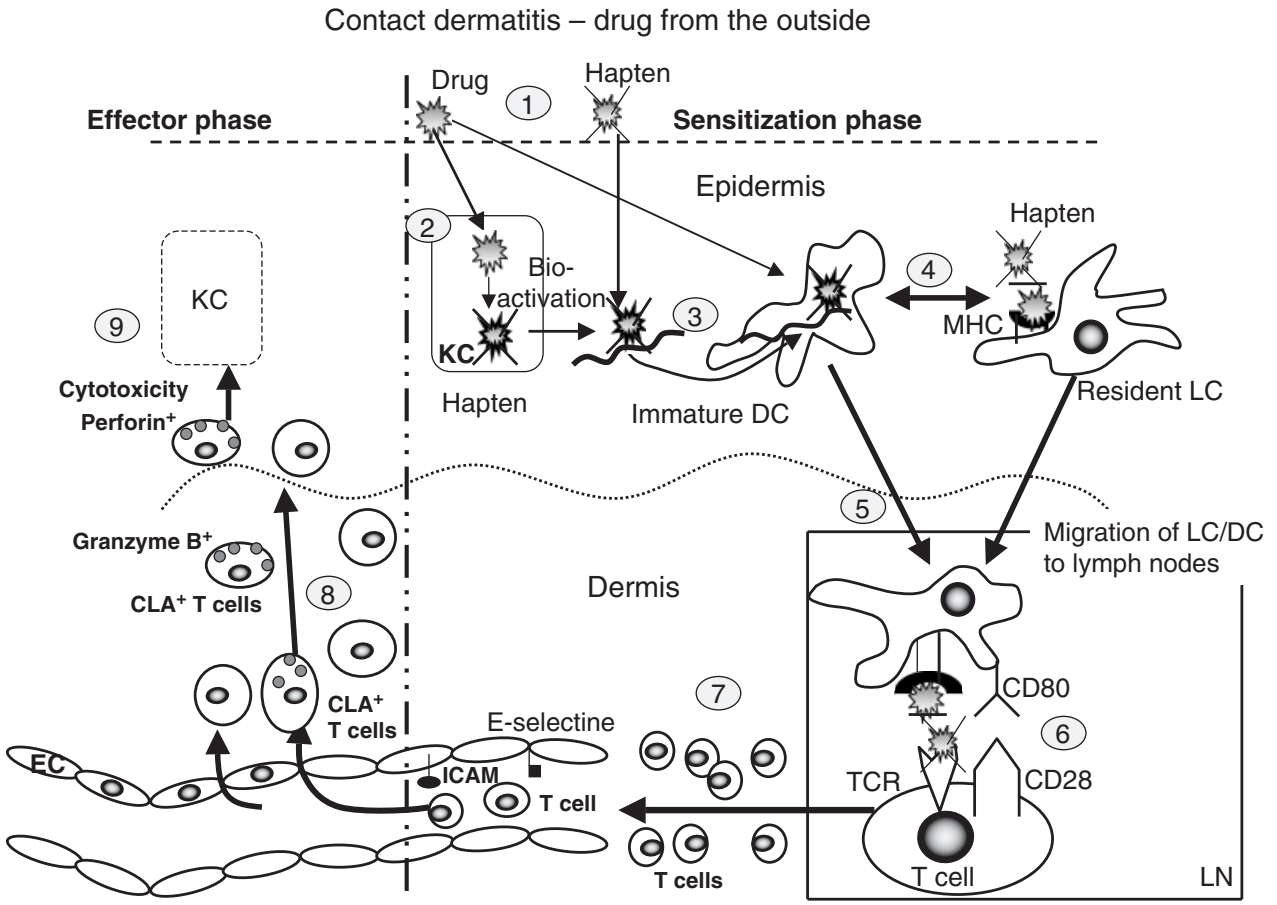


Fig. 2. Skin symptoms according to hapten/pro-hapten concept. Schematic representation of critical events involved in *hapten-induced immune reactions in skin*. Drug or chemicals, metals enter the epidermis through the stratum corneum (step 1). Alternatively, drug or metabolite may enter via the systemic circulation. The pro-hapten may undergo bioactivation in keratinocytes (KC) (step 2), but also in dendritic/Langerhans cells (DC/LC), followed by intracellular protein haptenation (step 3). Haptens or reactive metabolites released from KC may haptenate extracellular proteins. These events may provide some local stimulation (danger signal), and in particular stimulate DC/LC (step 4), which process and present the hapten–protein complexes and emigrate to the lymphnodes (step 5). There, the engagement of the T cell receptor (TCR) of hapten-specific, naive T cells in the presence of costimulatory signals (CD80/CD28) (step 6) results in the generation of a hapten-specific immune response and clonal expansion of hapten reactive T effector cells (step 7). The activated T cells express skin homing receptors, which allows them to be recruited to the skin, where the local inflammation induces chemokine expression able to attract these T cells (step 8). Activation of hapten-reactive T cells by local hapten presentation results in cell killing and inflammation (step 9). This concept is well accepted for contact dermatitis.

where for example an antihistamine given orally can reach tissue levels within 45–60 min, which can block H1 receptors. In addition, these levels have been documented by biopsies and analytical methods to be in the nanomolar range [58].

(b) Drug levels may not only allow an interaction with an appropriate but also with TCRs on specific T cells (p–i concept). Recent data suggest that drugs may interact with a great variety of TCR, albeit not always leading to a complete stimulation (B. Gerber et al., in preparation). The stimulatory potential of this drug–TCR interaction may decisively depend on the *readiness of the T cell to react to a 'minor' signal like a drug*. This may be related to an acute or previous activation, as e.g. (memory) effector T cells have a lower threshold of activation then naive T cells. No metabolism is needed.

(c) The skin is a border region where the immune system is geared to react with a strong immune response, while in the gastrointestinal tract tolerance mechanisms are important as well. It consequently contains an enormously dense network of antigen presenting and T cells, ready to mount an immune response. There are some controversies how best characterize by using surface markers the homing of various T cells (resident and recruited from lymphoid organs) to the skin during an inflammation. Here, we follow the concept of Schaerli et al. [57], but adaptation with regard to the optimal characterization of these skin 'resident' sentinel T cells and skin *immigrating* T cells with respect to surface molecules (CLA, CCR4, CCR6, CCR8, CCR9, CCR10), is still possible.

The resident T cells would be CCL-1 responders (CCR8) and the immigrating T cells are CCL20 responders (CCR6).

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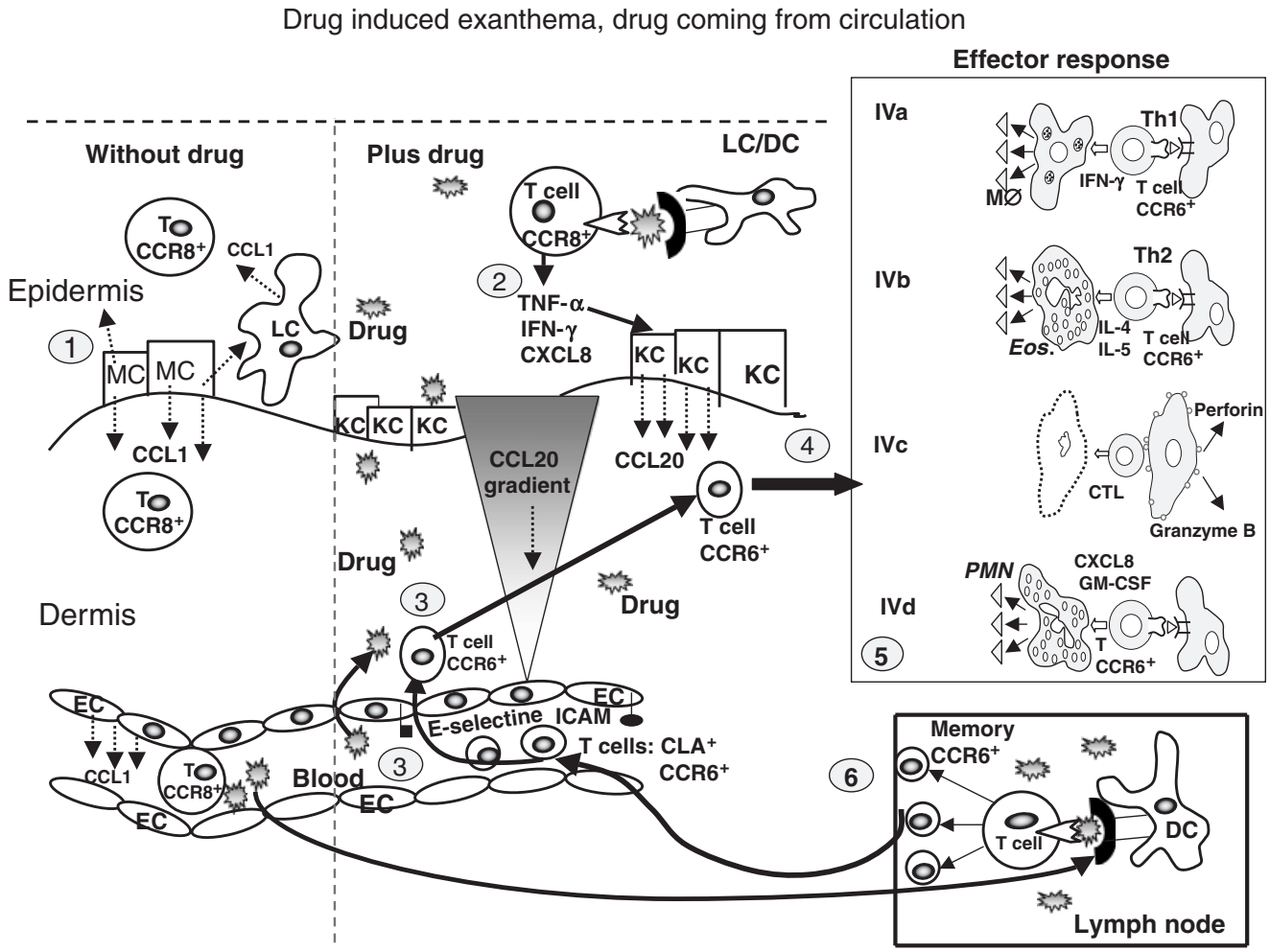


Fig. 3. Skin symptoms according to p–i concept. Schematic representation of critical events involved in the frequent manifestation of drug hypersensitivity in the skin based on the p–i concept. CCR8$^+$ T cells reside in the epidermis and dermis and may have an immune surveillance function, In this scenario (without drug), one finds in the skin a constitutive production of CCL1 by noninflamed skin endothelium (melanocytes and Langerhans cells (LC) as well as dermal microvasculature), which might mediate the steady-state extravasation of CCR8$^+$ blood precursors of skin-tropic T cells (step 1). Skin resident CCR8$^+$, CD4$^+$, TCRαβ$^+$ sentinel T cells are stimulated by the drug directly (p–i mechanism), as the drug is present in sufficient amount in the tissue (step 2). This drug-induced activation of the local T cells is facilitated by the close contact of T cells to dendritic cells (DC)/LC in the skin. This initial T cell stimulation provides signals to local epidermal and endothelial cells and thus sets an alarm signal. Simultaneously, T cells with a low activation threshold may be activated by the drug in the lymph nodes (step 6), where they expand and them emigrate to the skin (step 3). The local CCR8$^+$, but mainly the recruited T cells (CCR6$^+$) would evoke an *generalized* inflammatory skin response, as it is typical for exanthems (step 4). The cytokine pattern released by the T cells leads to different forms of the exanthema, which can be subclassified into IVa–IVd type reactions (step 5).

In this scenario, one finds in the skin a constitutive production of CCL1 (step 1, Fig. 3) by non-inflamed skin endothelium (melanocytes and LCs as well as dermal microvasculature), which might mediate the steady-state extravasation of CCR8$^+$ blood precursors of skin-tropic T cells. Such CCR8$^+$ T cells reside in the epidermis and dermis and may have an immune surveillance function, as they have a high degree of reactivity and produce high amounts of TNF-α, interferon-γ and possibly also CXCL8 upon moderate stimulation. Moreover, CCL1 is also produced by LCs and may thus allow a co-localization of the CCR8$^+$ T cells with the epidermal APCs.

We propose that the frequent manifestation of drug hypersensitivity in the skin is due to readiness of such skin resident CCR8$^+$/CD4$^+$/TCRαβ$^+$ T cells to be stimulated by drugs via the p–i mechanism. This initial activation provides stimulatory signals to local epidermal and endothelial cells and thus steers the immune reaction to the skin (step 2, Fig. 3).

The possible steps of this new concept are outlined in Fig. 3: In drug hypersensitivity, the local concentration of drugs in the skin may lead to a successful TCR engagement by drugs directly interacting with the TCR (p–i concept), supplemented by appropriate co-stimulation

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(through the close contact to the abundantly available epidermal APCs (DC and LC). The drug-stimulated $CCR8^+$ T cells initiate an immune defence programme by secretion of TNF-α, IFN-γ, etc., which leads to an acute phase response via induction of inflammatory chemokines like CXCL8 or CCL20 (=chemokine for $CCR6^+$ T cells) in keratinocytes, up-regulation of vascular adhesion receptors, and consequently recruitment of leucocytes of the innate immune system (steps 1 and 2, Fig. 3).

Simultaneously to these local events in the skin, T cells in the lymph nodes may be activated as well by the drugs: again, drugs are ubiquitously present in the body and do not need transport by the lymph or within DC/LC to the lymph nodes. These T cells in the lymph nodes may be particularly responsive to the drug stimulation, if they have been activated, e.g. by an infection. They will enter the circulation and may express CCR6 and other skin homing markers (e.g. CLA). Upon arrival in the skin vasculature, they may adhere to the locally activated endothelium (due to the above-described T cell stimulation in the skin) and emigrate from the vasculature to the skin, following the CCL20 gradient (steps 3 and 6 of Fig. 3).

Finally, the local $CCR8^+$ together with the recruited $CCR6^+$ T cells (which represent the majority of T cells in inflammed areas) would evoke an inflammatory skin response. Its type might be determined by the profile of cytokines produced by the $CCR6^+$ T cells, whereby the different responses (IVa–IVd) described above might evolve. Why the immune response leads to a more IVa or IVb etc. response is at present unknown (steps 4 and 5, Fig. 3).

Figure 3 demonstrates these events in a simplified scheme. Of note is the fact, that this concept does not postulate a drug metabolism in the skin and does not involve the hypothetical generation of an own immune response, but stimulates and expands pre-existing, effector or memory T cells, which already reside in the skin and lymph nodes. These T cells have actually a peptide specificity as well. It is possible that a certain drug preferentially activates T cells with a particular peptide specificity, which might explain the puzzling finding that certain drugs elicit rather consistent similar clinical pictures.

This concept would explain:

(a) *the preferential localization to the skin*: other organs do not have such highly reactive sentinel T cells – and therefore, the tissue is not alarmed, and activated T cells cannot home there so easily.
(b) *The dose dependency of many drug hypersensitivity reactions*: higher concentrations of drugs are more often associated with delayed drug hypersensitivity [36], as higher doses would better stimulate the T cells via TCR.
(c) *The observation that generalized viral diseases like HIV or Herpes virus infections are risk factors for drug hypersensitivity*: in such diseases lymphocytes (and DC/LC) are already activated, which may lower the threshold of activation by drugs.

Why are drug hypersensitivity reactions elicited by this concept not more frequent? One could attribute it (a) to a lack of fitting TCR in the available T cell repertoire, as drugs which would react frequently are eliminated in the development process because of side-effects; (b) the threshold of immune cells to react to a drug according to the p–i concept might actually be quite high; (c) the interaction may not lead to cell stimulation; and (d) other, not well-defined factors may play a role, e.g. gemifloxacin could elicit a mild exanthema in ca. 30% of the female population of childbearing age, while only ca. 4% of males can react to this particular quinolones [59].

Conclusion

Drug hypersensitivity reactions are termed type B reactions, whereby B is often interpreted as bizarre (=non-dose related and having a clinical picture, which is not related to the action of the drug). Their indeed often unpredictable clinical features might well be due to an unusual stimulation of the immune system, bypassing many rules: this situation is reminiscent to transplantation medicine, another 'radical' intervention of modern medicine, where the allo-response caused unforeseen immunological problems. In both situations, the immune system is faced with a situation it was not prepared to handle during the evolution – as neither transplantation nor the extensive use of xenobiotics had previously occurred. Recognizing and understanding these peculiar, man-made iatrogenic diseases may be the first step to reduce the size of this problem and possibly prevent it.

Acknowledgements

Dr Sinforiano J. Posadas is a Postdoctoral fellow supported by MEC/FullBright program, Ministry of Science, Spain.

We thank Drs Monika Keller, Basil Gerber, Andreas Beeler, Anna Zawodniak and Nikhil Yawalkar for helpful discussions and Franzisca Mitton for her help in the elaboration of the Figs 2 and 3.

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