Noncovalent drug presentation leads to the activation of drug-specific T cells. In some patients with hypersensitivity, such a response occurs within hours even upon the first exposure to the drug. Thus, the reaction to the drug might not be due to a classical, primary response, but rather mediated by existing, preactivated T cells that are cross specific for the drug, and have an additional (peptide) specificity as well.

**Hapten and prohapten concepts**

How do drugs stimulate T-cells? An answer to this question is required to understand the development of side effects, and perhaps open new avenues to predict these reactions based on the characteristics of the drug. For a long time, the ‘immunological dogma’ postulated that small, low-molecular weight compounds per se are not capable of eliciting an immune response; in order for this to occur, antigen-presenting cells have to take up and process complex and large antigens, and subsequently present these to T cells. However, small compounds such as drugs or metal ions are able to trigger an immune response, and the hapten and pro-hapten models are currently the accepted explanations for these observations: chemically reactive, small compounds (haptens) bind to proteins or peptides and modify them [10,11,12,13]. These are then processed and presented as hapten-modified peptides (‘altered self’) to T cells, which can react to them. Pro-haptens need an intermediate metabolism step to become chemically reactive [4,5,14,15].

**The p-i concept**

Our group recently proposed a third model for drug–T cell interactions, which supplements the hapten and pro-hapten concepts. We have termed it the p-i concept, which stands for ‘direct pharmacological interaction of drugs with immune receptors’ [16]. It states that certain drugs bind to some of the highly variable antigen-specific T cell receptors (TCRs) and MHC molecules in a direct way. This proposed interaction is similar to drug interactions with other, non-immunological receptors, and would suffice to stimulate the T cell if an MHC interaction is also provided. Furthermore, it is metabolism- and processing-independent, and stimulation depends on the structural features of the drug that enable it to fit into the TCR. Drug allergy would thus reflect the fact that a particular drug leads to a TCR-dependent T cell stimulation and an ensuing inflammatory immune response.
We have developed this model to account for several observations that cannot be explained by the hapten or pro-hapten models, including both certain peculiar features of the drug-specific T cell clones (TCCs) and certain immunological observations. These peculiar characteristics have become apparent during years of work with T cells from drug allergic patients, showing that drug-reactive TCCs differ in many respects from protein-specific TCCs, as discussed in detail below. A summary of these differences is shown in Table 1, whereas Figure 1 presents them schematically.

### Immunological characteristics of drug reactive T cell clones

Different groups have shown that chemically inert drugs are able to stimulate T cells via the TCR in an MHC-dependent way. The following drugs were investigated: carbamazepine [8], lamotrigine [9], sulfamethoxazol (SMX) [17,18], mepivacaine [18], lidocaine [18,19], p-phenylendiamine [20] and radio contrast media (RCM) [21]. Most work has been done with SMX; as a reactive metabolite, SMX-nitroso, (SMX-NO), acting as a typical hapten, was available for comparison [5,22]. The interaction of these drugs with the TCR is of sufficient affinity to cause cytokine secretion, proliferation or cytotoxicity in the reactive TCCs [17,18,23,24]. In one case, even blister-fluid-derived CD8+DR+CLA+CD56+ T lymphocytes from a patient with toxic epidermal necrolysis reacted with the chemically inert SMX [25]. Of note, only a minority of TCCs derived from SMX-allergic patients reacted with the chemically reactive metabolites [26]. Some drugs, such as RCM [21] or lidocaine [19,24], have no known metabolism resulting in a reactive metabolite, and the p-i concept offers the only plausible explanation for their ability to induce delayed-type, T cell mediated reactions.

T cells react quasi immediately after encountering SMX in the presence of antigen-presenting cells (APCs), as revealed by a rapid and sustained intracellular Ca2+ increase. It is difficult to reconcile this timing with an intermediate metabolism and processing step, which might need >60 minutes to occur [24]. The kinetics of TCR downregulation on drug reactive TCCs after encountering the inert drug are similar to the recognition of pre-processed, immunogenic peptides (occurring within the first 30 minutes), clearly differing from the recognition of proteins [24]. For several drugs, specific TCCs reacted even if the APCs were fixed by glutaraldehyde, suggesting that neither processing nor intracellular metabolism is involved [8,9,17,18,24].

Upon pulsing of APCs (incubation of APCs with the drug for 1 hour followed by two washing steps), which obviously removes the drug, no T cell stimulation was observed, whereas the hapten SMX-NO, which is able to covalently modify the MHC–peptide complex, was still able to stimulate hapten-reactive T-cells [17].

### Transfection of drug specific T cell receptors

We recently established a method for transfecting drug-specific human TCR into the mouse T cell hybridoma cell line 54[17 according to the method described by Vollmer et al. [27]. These transfectants expressed a drug-specific TCR on the cell surface, and the transfected cells could be stimulated in a specific way in the presence of APCs, which resulted in IL-2 secretion. The transfection of such TCR-expressing hybridomas is the formal proof that drugs interact with specific TCRs, and this technique will greatly facilitate the detailed analysis of such TCRs. So far, we have transfected SMX- and SMX-NO-specific TCRs [28]. Key findings with these transfectants were that the drug can be washed away (which is in contrast to hapten covalently bound to carrier molecules), that the presence of APCs (and MHCs) is required for IL-2 production, and that fixed APCs are still able to present the drug.

Hypersensitivity to the drug SMX is thought to be a consequence of bioactivation in response to the
hydroxylamine metabolite (SMX-NHOH) and further oxidation to the ultimate, reactive metabolite SMX-NO. The antioxidant glutathione (GSH) is known to protect cells from reactive metabolites by conjugation and subsequent dissociation to SMX-NHOH and/or SMX [5]. Most surprisingly, the addition of GSH to peripheral blood mononuclear cells enhanced, rather than reduced, proliferation in response to SMX metabolites [29], presumably by transforming SMX-NO back to the ‘original’ antigen, SMX. The response of SMX-NO-specific TCCs was abrogated when GSH was present during the covalent modification of APCs. These data support the concept that, in allergic individuals, T cells recognize the non-covalently bound parent drug SMX rather than APCs covalently modified by SMX-NO [26,29].

**Peculiar features of drug reactive T cell clones**

Drug-reactive TCCs generally differ from peptide-specific TCCs as they show some features that are more reminiscent of superantigen stimulation [16]:

1. MHC allele-independent drug recognition. Although some drug-specific TCCs are MHC allele restricted, quite a few are able to recognize the drug in association with other MHC alleles [30].
2. Elution of MHC-bound peptides by lowering the pH to pH2 for a short period of time and blocking neosynthesis did not affect TCC reactivity to the drug. This suggests that the type of peptide is irrelevant for drug recognition and raises doubts if the presence of a peptide within the MHC groove is required at all [31].
3. Simultaneous HLA-DQ or HLA-DR restriction. In the same individual, SMX-specific TCCs could be detected that were either HLA-DR or -DQ restricted, and both CD4+ and CD8+ cells reacted with the same, SMX ‘antigen’ [28].
4. We repeatedly observed CD8+ TCCs that were clearly MHC class II dependent, as shown by antibody-blocking studies [17,19].
5. High frequency of alloreactivity of drug-specific TCCs. Previous studies revealed a high heterogeneity

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**Figure 1**

A schematic comparison of the p-i concept with the hapten model. According to the p-i concept, the non-covalent, reversible binding of a drug to a matching TCR together with co-stimulatory signalling by MHC molecules would suffice to drive the activation and expansion of cross-reactive, peptide-specific memory T cells. In this way, the induction of a primary immune response can be ‘circumvented’, which would explain the strong allergic reactions observed in some patients without previous exposure to certain drugs. In contrast, the hapten-specific immune response relies on ‘normal’ priming of naïve T cells with the involvement and activation of APCs, such as dendritic cells.
and cross-reactivity of drug-specific TCCs in individual patients [18,23,32]. We analyzed the MHC-restriction pattern of a panel of 100 drug-specific TCCs isolated from different drug-allergic donors. 28 of the tested clones showed an MHC allele-unrestricted drug recognition. Most of these clones were, at the same time, highly drug specific and could only be stimulated by the original drug and not by any drug derivatives. By contrast, TCCs with the ability to interact with different drug derivatives clearly displayed an MHC allele-restricted drug recognition. Moreover, we tested all clones for additional allo-reactivity and found that 27 out of 100 clones could be stimulated by a self-MHC–peptide drug complex as well as by a non-self-MHC–peptide complex [33]. This cross-reactivity with allogeneic MHC molecules was substantially higher in drug-specific TCCs in comparison to tetanus toxoid-specific clones from the same donors, indicating that drug-specific TCRs might have an additional peptide specificity [33].

**Genetic factors**

The unpredictable nature of drug hypersensitivity reactions prompted an intensive search for genetic factors to explain their occurrence in a small subset of treated persons (reviewed in [34]). Major emphasis was put on the search for pharmacogenetic factors. An altered metabolism would be a good explanation for the appearance of immunological side effects, if it resulted in the generation of a more reactive intermediate able to modify autologous proteins. However, the possible association of drug hypersensitivity reactions with a particular pharmacological genotype remained often vague and controversial [35]. A slow acetylator phenotype seemed to enhance the occurrence of side effects to SMX [36], whereas a moderate association with more severe symptomatology was described for some TNF-α promoter polymorphisms [37]. Recent data, however, revealed a surprisingly clear association of certain drug hypersensitivity reactions with HLA-class I alleles: abacavir causes a severe hypersensitivity reaction affecting multiple organs in approximately 5% of treated patients. The majority of these patients carried the HLA-B57 allele, and this association was strongest in Caucasians [38]. Even more striking is the association of carbamazepine treatment and appearance of Stevens-Johnson syndrome in Han-Chinese carrying the HLA-B 1502 allele [39**], which is stronger than any other association described so far. These two findings attribute a decisive role to the presenting HLA molecule for the ability to elicit a strong immune response. They are also in agreement with recent data on the structure of a NiSO₄-reactive TCR and the involved MHC molecule, which seems to be HLA-DR52c restricted, with a histidine in position 81, suggesting a role for this amino acid in Ni²⁺ binding to MHC [13**].

**Drug reactivity as a ‘side effect’ of peptide specificity**

It is well known that drug-induced, T-cell-mediated skin reactions can occur within a few hours after administration without previous exposure to the drug, as documented for RCM [21]. These kinetics, although compatible with some of the in vitro findings discussed above and summarized in Table 1, are much too fast to be explained by the induction of a classical, primary response. Rather, drugs might happen to stimulate peptide-specific, pre-activated T cells already present in the circulation and tissue (note that the vast majority of drug-specific TCCs bear the αβ TCR, which usually recognizes peptides, and that a general stimulation of T cells as in Epstein-Barr virus infection during HIV infection is an important risk factor for drug hypersensitivity). As long as a sufficient stimulation by the drug occurs, these preactivated T cells could then be stimulated ‘accidentally’. Of note, it has recently been demonstrated in vitro that individuals who have never been exposed to SMX nevertheless harbour SMX- and SMX-NO-specific cells in their T cell repertoire [40**].

Such a mechanism might not be as far-fetched as it first appears. After all, αβ TCRs are peptide receptors, and it has been known for thirty years that drugs can activate receptors that have peptides or proteins as endogenous receptors, the classical example being the opiate alkaloids. More recently, non-peptide agonists have been found not only for other serpentine receptors but also for tyrosine kinases, as well as growth factor and cytokine receptors [41]. The fact that the overwhelming majority of low molecular weight ‘drug-like’ compounds known to bind to differing receptor classes act as antagonists implies that drugs might be identified that not only activate but also block (drug-specific) TCRs. Indeed, drugs such as the gold compound ‘sodium-aurothiomalate’ as well as carbamazepine have been described as inducers of immunoglobulin deficiency [42]. This hypothesis, if correct, would imply that it may be feasible to modulate T cell-mediated responses in an antigen-specific way using drugs. The generation and characterization of TCCs with double specificity for a peptide and a drug would thus constitute an important first step on the road towards drug-modulated, antigen-specific immunomodulation, which might be of clinical relevance as well.

**Conclusions**

Recent studies indicate that drug-induced hypersensitivity reactions can (or must) be explained by different mechanisms, the range of which has expanded considerably. Genetic factors have been identified that show a clear correlation with certain hypersensitivity reactions. Also, evidence is accumulating that certain drugs are able to activate T cells in ways that differ from established concepts in immunology, including — but not limited to — the hapten concept. As far as drugs are concerned, it
might, in fact, prove useful to draw inspiration from concepts of classical pharmacology, which have been established for greatly differing receptor classes. The potential reward might not only be a better understanding of drug-induced hypersensitivity reactions, but also the identification of novel means for immunomodulatory therapies.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


A detailed analysis of the specific T cell immune response in patients with carbamazepine hypersensitivity. The clones analyzed in detail secreted high amounts of IFN-γ but low amounts of IL-5, which distinguishes them from clones isolated from patients with cutaneous symptoms. Interestingly, all clones expressed the skin-homing receptor cutaneous lymphocyte antigen (CLA) and secreted substantial amounts of IFN-γ, IL-2, IL-4, and IL-6.


A detailed analysis of the specific T cell immune response in patients with systemic lamotrigine hypersensitivity with hepatic involvement. Interestingly, all clones expressed the skin-homing receptor cutaneous lymphocyte antigen (CLA) and secreted substantial amounts of IFN-γ, IL-2, IL-4, and several chemokines. Moreover, the parent compound was recognized by the TCCs.


This is a very detailed and comprehensive structure/function analysis of a Ni-specific TCC, which identifies three Ni coordination sites, two on the TCR and one in the MHC. The direct linkage between TCR and MHC is similar to superantigen stimulation even though idiotypic residues are required for stimulation.


This is another detailed and comprehensive structure/function analysis of a Ni-specific TCC, which requires a preformed complex of Ni2+, a specific peptide, the DRS2c allele, and a histidine at position 81 of the MHC β2 chain.


This is a comprehensive analysis of hybridoma cells transfected with SMX-specific TCR to investigate the influence of receptor density, specificity and cross-specificity on various cellular responses. A higher density led to a broader cross-reactivity, closely mimicking the phenotype of the original TCCs.


This is the second demonstration of an HLA association with a drug hypersensitivity reaction, and the strongest known association between an HLA allele and any disease to date.


This paper describes for the first time the induction of a primary T cell immune response against a chemically inert drug in healthy individuals who have not been exposed to the drug previously.


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