

Drug-induced autoimmunity

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Purpose of review

Presentation of different mechanism of drug-induced autoimmunity and highlighting of new developments.

Recent findings

Drugs can induce autoimmune diseases by their pharmacological properties. Injection of certain drugs into the thymus can alter positive selection in the thymus and elicit autoimmune reactions. Peripheral tolerance can be broken by increasing the expression of LFA-1 adhesion molecule on T cells. This can be related to the inhibition of intracellular kinases. Alternatively, a drug specific immune response might elicit autoimmunity by cross-reactivity: the drug reactive T cells might be cross-reactive with certain peptide antigens and possibly autoantigens.

Summary

Drug-specific immune responses are well described. They have a great tendency to be cross-reactive with peptide antigens. This 'immunological' cause of autoimmunity elicited by drugs may occur more frequently than thought. It connects the field of drug hypersensitivity with drug-induced autoimmunity.

Keywords

drug hypersensitivity, drug-induced autoimmunity, cross-reactivity, autoimmunity

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Abbreviations

APC	antigen-presenting cell
ERK	extracellular signal-regulated kinase
LFA-1	lymphocyte function-associated antigen-1
MHC	major histocompatibility complex
PAHA	procainamide-hydroxylamine
SLE	systemic lupus erythematosus
TCC	T-cell clone
TCR	T-cell receptor

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Introduction

Drugs can interfere with the specific immune system and induce allergic and autoimmune diseases. In allergic reactions, the drug itself is the target structure by specific immunoglobulins or T cells, while in autoimmune reactions the drug elicits an immune response to certain auto-antigens. The symptoms of drug-induced autoimmunity can resemble typical systemic autoimmune diseases like systemic lupus erythematosus or are more organ specific autoimmune reactions like haemolytic anaemia, pemphigus vulgaris, and so on. Depending on the type of reactions, patients may develop auto-antibodies to nuclear antigens, in particular histones (H2A-2B), to erythrocytes or to other protein antigens similar to other autoimmune diseases [1,2]. These auto-antibodies normally disappear after stopping drug therapy, and symptoms regress in most instances rapidly. Patients with drug-induced autoimmunity may also have auto-reactive T cells, however these are less well investigated as the potential auto-antigen or auto-antigenic epitopes for T cells are often unknown.

Drug-induced autoimmune diseases are relatively rare (an estimated 10% of systemic lupus erythematosus (SLE) cases in the USA were attributed to a drug-induced mechanism) and represent potentially dangerous side effects, although most cases of drug-induced lupus are relatively mild [1,2] with rare central nervous system or renal involvement. The study of drug-induced autoimmune diseases in humans is therefore quite limited, as the respective drug is either removed from the market or used very cautiously to avoid such side effects. There are, however, some animal and in-vitro models that help us to better understand drug-induced autoimmunity.

Many different drug-related mechanisms may lead to a disturbance of the immune system, affecting both B and T lymphocytes [2]. Drug-induced lupus is probably mediated by reactive drug metabolites, not the ingested medications, and susceptibility to neutrophil-mediated oxidative transformation is a property of 10 lupus-inducing drugs reported so far [2]. Of particular interest is the effect of drugs on the function and antigen recognition by T cells, as these cells appear to have a key role in the tolerance mechanism to auto-antigens. Moreover, the decisive role of T cells in recognizing drugs in different drug allergic diseases has been well established during the last few years and the better understanding of T-cell receptor (TCR)–drug (hapten) interaction in drug allergy may be a clue to better

understanding some forms of drug-induced autoimmunity [3–5,6•].

In this short review I will focus on recent developments in the analysis of the structural basis of drug recognition, particularly on TCR–drug–peptide–major histocompatibility complex (MHC) interactions, and the impact on our understanding of autoimmunity.

Principal mechanism of drug-induced autoimmunity

The immune system has developed different barriers to prevent autoimmunity and it is likely that drug-induced autoimmunity can arise due to different mechanisms [7]. Thus, the pharmacological properties of a drug or its structure might give rise to changes of the immune reactivity. Consequently, one could postulate that drug-induced autoimmunity is due to

- (1) interference with central tolerance;
- (2) interference with peripheral tolerance;
- (3) crossreactivity, which overcomes tolerance.

Interference with central tolerance

The thymus provides tolerance by deleting auto-reactive T cells during thymus maturation. Negative selection first eliminates the large part of TCR positive cells, followed by positive selection. Positive selection in the thymus requires the engagement of thymocytes with autologous peptides on cortical epithelial cells. Rubin *et al.* [8–10] evaluated to what extent injection of procainamide-hydroxylamine (PAHA) – a metabolite of procainamide, which is a lupus-inducing drug – into the thymus of normal adult mice alters the positive selection. Intrathymic injection of PAHA in C57Bl/6 mice led to autoimmunity with an SLE-like clinical picture and production of anti-H2A-H2B auto-antibodies. Thus, in the presence of PAHA, positive selection is hampered and thymocytes fail to develop unresponsiveness to low-affinity self-antigens. As a result, the mice develop systemic autoimmunity [10,11].

Interference with peripheral tolerance

The deletion of auto-reactive T cells in the thymus is never complete and auto-reactive T cells are still present in the circulation [7]. These cells might be activated and are a potential source for either transient or long lasting autoimmune reactions. Therefore, further tolerance mechanisms in the periphery are important, which are based on different principles [7]. Immunological ignorance refers to a barrier between the antigen-presenting cell (APC) and the T cell, abolishing interaction. An important tolerance mechanism is surely the fact that most auto-antigens are presented in a nonimmunogenic way. The lack of involvement of the innate immune system due to the lack of inflammatory signals blocks the

induction of a primary immune response to self-antigens and no inflammatory reaction develops [12]. Finally, inhibitory mechanisms via cytokines like IL-10 or transforming growth factor- β , or by contact with regulatory T cells (CD4/CD25 positive) with suppressive activity may prevent the development of an immune reaction [13,14].

Different possibilities have been proposed as to how drugs might overcome peripheral tolerance. Some drugs seem to break tolerance due to their pharmacological activity: PAHA and hydralazine are inhibitors of DNA methylation and were found to induce lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18) overexpression in T cells [15]. LFA-1 is the ligand for intercellular adhesion molecule type 1 on APCs. Overexpression may enhance immune reactivity even to antigens with low-affinity binding to TCRs by stabilizing T cell–APC interaction. Indeed, these LFA-1 high T cells show auto-reactivity *in vitro* and transfer of these auto-reactive, LFA-1+ T cells was found to induce a SLE-like disease in mice (with the development of anti-DNA autoantibodies [15]). Recently, the mechanism underlying this LFA-1 upregulation by hydralazine was clarified in more detail [16••]. Following earlier observations that T cells from patients with active SLE have hypomethylated DNA [17], these authors evaluated whether hydralazine decreases DNA methyltransferase expression. This could lead to LFA-1 overexpression and autoimmunity. *In vitro*, hydralazine did not directly inhibit DNA methyltransferase activity but instead inhibited extracellular signal-regulated kinase (ERK) pathway signalling. This decreased DNA methyltransferase 1 and DNA methyltransferase 3a mRNA expression. Hydralazine thus reproduces the lupus ERK pathway signalling abnormality and its effects on DNA methyltransferase expression, and inhibiting this pathway induces autoimmunity.

A completely different mechanism of drug-induced autoimmunity was postulated by the group of Gleichmann *et al.* [18]. Certain drugs, like gold, may interfere with antigen processing: Au(III) binds to a protein (in their model bovine ribonuclease A). This covalent binding alters the normal processing, and a hidden (cryptic) epitope – to which no tolerance exists – is newly presented and can elicit an immune response. This mechanism would explain certain drug-induced autoimmune-mediated side effects by generating neo-antigenic epitopes. This mechanism, however, still needs to be demonstrated in a real auto-antigen.

Cross-reactivity

A more ‘immunological’ approach to understanding autoimmunity relies on the fact that drugs *per se* might represent antigens as well, possibly causing the genera-

tion of cross-reactive T cells: T cells do recognize haptens like penicillin, which are covalently bound to peptides or proteins (which are processed to peptides) [5,19]. In addition, T cells are able to be triggered by drugs directly [20,21]. The p-i concept implies that drugs might interact with certain 'fitting' TCRs, whereby for full activation of the T cell a further interaction with a presenting MHC molecule seems to be required [6•]. This concept has been shown to be relevant for sulfamethoxazole, lidocaine, quinolones, carbamazepin and lamotrigine [20–23,24•], and even some classical hapten-like compounds with well known hapten-like metabolites (p-phenylenediamine [25•]). In most instances the T cell recognizes the drug in either hapten-like form (e.g. sulfamethoxazole–nitric oxide, which is covalently bound to a carrier molecule) or the inert substance sulfamethoxazole itself. Only a few T-cell clones (TCCs) cross-react with the hapten and the inert drug [26].

Drug-induced autoimmunity: relationship to drug hypersensitivity

T cells can be stimulated by drugs and be cloned. The detailed analysis of such drug/hapten specific T cell clones revealed some interesting aspects with regard to cross-reactivity.

Cross-reactivity between haptenized and nonhaptenized peptides

The decisive step forward to a better understanding of T-cell immune responses to haptens was the finding that MHC-restricted hapten-specific TCRs in their majority do not react to covalently modified MHC molecules, but to haptenized peptides associated with the MHC peptide-binding groove [5,19,27,28]. This finding allowed the introduction of synthetic hapten–peptide conjugates, the MHC specificity of which may be predetermined by allele-specific peptide sequence motifs. Depending on the localization of the hapten modification of the 9-amino acid long peptides, two major types of hapten-specific TCRs were identified. One type reacted to the hapten without being affected by the chemical composition of the carrier peptide; alterations of the amino acids in the rest of the peptide were tolerated. The other model of TCR interaction included contact with the hapten and the peptide by two apparently independent contact sites. This has consequences for hapten-specific autoimmunity, as certain TCCs, which are raised to the haptenized peptide and are reactive with the hapten bound to the peptide, could also react with the unmodified peptide alone [28].

Drug reactive T-cell receptors: cross-reactivity with peptides

TCRs react with certain peptides, but also have the ability to recognize structurally related peptides. Even

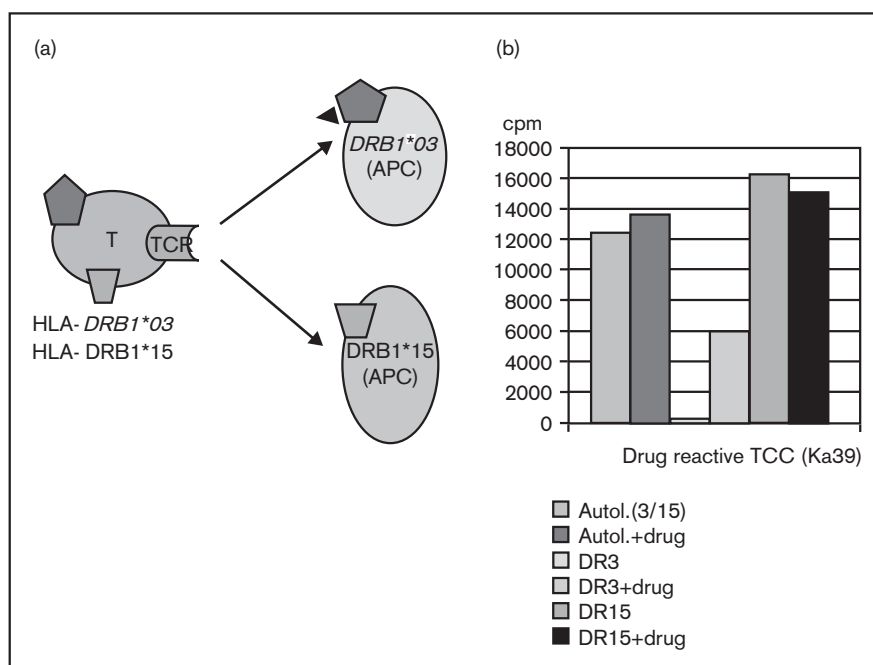
completely different peptides together with unrelated MHC molecules can be recognized by the same TCR (allo-reactivity) [29]. A peculiar feature of drug reactive T cells is the finding that such T cells have a high heterogeneity and cross-reactivity in individual patients, which is compatible with a high degeneracy of drug-reactive TCRs. Of a panel of 100 drug-specific TCCs isolated from different drug-allergic donors, 28 of the tested clones showed a MHC allele-unrestricted drug recognition [30,31]. They recognized the drug in association with different HLA-DR alleles. Most of these 'promiscuous' clones were at the same time highly drug-specific, that is they could only be stimulated by the original drug and not by any drug derivatives. It was concluded that they interact perfectly with the drug as small alterations of the drug are not tolerated, but that the presenting MHC structure is of minor importance. In contrast, TCCs with the ability to interact with different drug derivatives clearly displayed a MHC allele-restricted drug recognition, meaning that the drug was only recognized with the restricting MHC allele. This probably happened because the TCR interaction with the drug was less stimulatory and an additional interaction with a fitting MHC molecule was required [30].

A further peculiar feature of drug-reactive TCRs is a high incidence of allo-reactivity: if drug-specific TCCs were also tested for additional allo-reactivity, 27 of 100 TCCs could be stimulated by a self-MHC–peptide–drug complex as well as by a non-self-MHC–peptide complex (without drug! [29]). This cross-reactivity with allogeneic MHC molecules was substantially higher in drug-specific TCCs compared with tetanus toxoid-specific clones from the same donors (5/100). The biological functions of bi-specific TCCs were not influenced by the nature of the stimulating ligand. Both drug and allogeneic stimulation led to similar reaction patterns in the analysed TCCs. The cross-reactivity between drug and alloantigens suggests that for a drug-specific TCR, non-self-MHC–peptide complexes have a high incidence of mimicking the 'original' self-MHC–peptide–drug complex. Since alloantigen-specific T cells are thought to represent T cells which are able to recognize self-MHC/peptide complexes as well, this observation may indicate that drug-specific T cells could also react with auto-antigens.

The above described observation of high alloreactivity of drug-reactive TCCs paved the way for an interesting hypothesis (Fig. 1): could drug-induced T-cell stimulation also lead to an 'allo-reactivity' to the other alleles of the same individual? In other words, in, for example, an HLA-DRB1*03/DRB1*15 heterozygous individual, could a T cell, stimulated by HLA-DRB1*03 plus peptide and drug also react with HLA DRB1*15 plus peptide but without drug? To analyse this question,

Figure 1. A patient (HLA-DRB1*03/15) with amoxicillin hypersensitivity (exanthema and interstitial pneumopathy) was found to show a vigorous proliferative response to amoxicillin *in vitro*

The cells were expanded by adding the drug to the culture and T-cell clones (TCCs) were raised with specificity to amoxicillin or to nonmodified autologous antigen-presenting cells (APCs). All TCCs were found to be HLA-DR restricted (by antibody inhibition experiments). The autoreactive TCCs were further analysed for drug specificity, if amoxicillin was presented by one of the two HLA-DR alleles. (a) The scheme of potential (and found) reactivity; (b) the proliferative response (3H thymidine incorporation after stimulation, counts per minute, SE < 7%) of the TCCs Ra39 to autologous APCs ($\pm$ drug) or to HLA-matched APCs bearing one (HLA-DRB1*03) or the other HLA-DRB1 allele (HLA-DRB1*15). Note that the TCC was bispecific: it failed to react to HLA-DRB1*03 but reacted to HLA-DRB1*03 plus drug; it also reacted to HLA-DRB1*15 without drug, indicating a cross-reactivity between the autologous HLA-DRB1*15 and DRB1*03 plus drug. The peptide specificity of the TCC is not known. TCR, T-cell receptor.



peripheral blood mononuclear cells of drug-allergic individuals were stimulated with the drug, and both autoreactive and drug-specific T cells were cloned after this initial drug stimulation. As shown in Fig. 1, certain TCCs were found, which indeed reacted not only with the autologous allele and the drug, but also with the second autologous allele without the drug. These TCCs were thus auto-reactive and drug-reactive. The clinical consequences of this observation are still unclear but might be far reaching, as some severe forms of drug hypersensitivity reactions are not well explained by drug-specific reactions alone. This new concept for drug-induced auto-reactivity needs to be further analysed with regard to peptide specificity, but would be an explanation for certain, probably more organ-specific reactions, which persist weeks to months after cessation of drug therapy [32,33]. In addition, some severe and extensive hypersensitivity reactions with defined HLA-association, as recently described for abacavir hypersensitivity [34**], might be at least partly due to an autoimmune reaction.

Conclusion

Drug-induced autoimmunity can be explained by different mechanisms. Thereby, both pharmacological interference with tolerance mechanisms, for example with the ERK-signalling cascade, as well as more immunological mechanisms like cross-reactivity in the frame of a drug hypersensitivity reaction might be clues for different forms of drug-induced autoimmune dis-

eases. More systemic autoimmune reactions like SLE elicited by drugs might be better explained by a pharmacological mechanism of the drug, for example by abolishing a general tolerance mechanism against auto-antigens. In contrast, more limited, organ-specific reactions could be due to immunological cross-reactivity; these are connected to a drug hypersensitivity reaction and the generation of a drug-specific immune reaction. A closer analysis of such drug-TCR interactions in drug hypersensitivity reactions might help to better understand such mechanisms and explain why certain drugs elicit a certain pattern of disease (e.g. abacavir hypersensitivity with involvement of internal organs). Moreover, an accompanying, probably transient, autoimmune reaction may be a more common feature of so-called drug hypersensitivity reactions than originally thought.

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