Predicting Drug Hypersensitivity by In Vitro Tests

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Summary
Recently it was found that drugs causing drug hypersensitivities do not only rely on the formation of hapten-carrier conjugates but can stimulate T cells directly via their T cell receptors for antigen. This new mechanism was termed pharmacological interaction of drugs with immune receptors (p-i concept). It is frequent in systemic drug hypersensitivity reactions and has major implications for predicting them. First experiments to identify such drugs able to interact with T cells are presented.

Keywords: T cell, allergy, hypersensitivity, TCR

T cell immune response
Certain chemicals (including biological agents and drugs) are immunotoxic, which means they can stimulate or suppress the human immune system. The adverse side-effects of a stimulated immune system can be sensitisation or autoimmunity with an inflammatory reaction. In rather rare cases, sensitised individuals can experience an allergic reaction to a second exposure of the stimulus (antigen, allergen) and become hypersensitive. Well known are compounds which, when given on the skin, cause inflammation of skin areas (allergic contact dermatitis). The side effects can be even more severe when an immune stimulant enters the body and reaches the blood system (e.g. after oral ingestion) inducing a T cell-mediated immune response.

T cells are a subpopulation of lymphocytes and are involved in most allergic reactions (hypersensitivity). T cells are able to recognise peptides, lipids, metals and complexes consisting of chemicals and peptides as antigens. The immunogenicity of chemicals (but not of biological agents = proteins) is generated by the previous covalent binding of the hapten to a protein, which is then processed to a peptide and presented on MHC-structures (= HLA-molecules). Such hapten-peptide complexes are recognised by some T cells with the fitting T cell receptors (TCR) for the particular antigen.

However, WJ Pichler argues that this hapten model is not a sufficient explanation for many drug-induced side effects. He and his group showed that a direct non-covalent binding of drugs to the TCR is possible and under certain circumstances stimulatory for T cells (p-i concept). Binding of the drug to the TCR activates T cells, whereby MHC interaction (regardless of the enclosed peptide!) with the TCR supports this drug mediated signal. The T cells start to divide and organise an inflammatory response in the body by secreting cytokines and killing other cells. These immune-mediated inflammatory responses may cause mild symptoms such as maculopapular exanthema, but a substantial fraction of these reactions are severe, causing Stevens-Johnson-Syndrome, toxic epidermal necrolysis, hepatitis, pancreatitis, fever, vasculitis, eosinophilia, and even death.

Prediction of systemically applied compounds
There is an urgent need to improve the prediction of immune-mediated side effects of drugs, biological agents and chemicals. Available animal and in vitro tests (e.g. skin sensitisations and lymph node assays) are mainly positive with haptens or prohapten, which rapidly become hapten. Nevertheless, these tests lack a reliable prediction of generalised forms of drug hypersensitivity (Bala et al., 2005). Furthermore, immunemediated side effects appear only in a minority of patients which might have a special genetic predisposition (Chung et al., 2004) which is not present in animal models. Finally, new concepts of drug hypersensitivity, such as the presented p-i concept, are hardly covered by animal experiments or in vitro studies using animal cells (Pichler, 2001).

Consequently, one might favor in vitro testing with human material. For the safety assessment of chemicals or compounds which are applied topically and sensitise via skin or lung, in vitro tests seem to be promising (www.sens-it-iv.eu). However, they focus exclusively on haptens or prohapten and the pathway via dendritic cells. For testing the safety of systematically (orally or parentally) applied drugs inducing allergic reactions via the p-i concept, in vitro tests require human material, since these reactions are exquisitely specific. Already small alterations of the T-cell receptor (TCR), of the structure of the drug, of the MHC molecule, which interact with the TCR, alter the reactivity of T cells dramatically. Moreover, animals may have a greater resistance to the immune stimulation via the p-i concept.
The p-i concept

This concept postulates that drugs can bind to the TCR like to other receptors – and that this interaction might stimulate T cells directly. It does not require a previous hapten-carrier formation (Fig. 1). The consequence is a reaction mimicking an immune reaction, however not triggered by a classical antigen, but by a drug. It does not (!) require the generation of an own immune response to the hapten (as the drug is no hapten) or the involvement of the innate immune system. This additional (alternative) pathway is called pharmacological interaction with immune receptors (p-i concept) (Pichler, 2003). Considering this pathway might be highly relevant for detecting the potential hypersensitive (allergic) activity of a given compound and provides a good explanation for a number of open questions in drug hypersensitivity reactions (Pichler, 2003).

Initiating an immune response in vitro

The p-i concept was originally established based on comparing the immune response of patients allergic to sulfamethoxazole (SMX) to the parent, non-hapten like compound and to its main metabolite, the hapten Sulfamethoxazole-Nitroso (SMX-NO). SMX-NO binds covalently to proteins or peptides.

Hapten-like drugs and chemicals can easily induce an in vitro immune response by culturing peripheral blood mononuclear cells (PBMC) of non-sensitised individuals with the hapten for a prolonged time period. We could confirm this finding (Engler et al., 2004) and tested ten healthy non-sensitised individuals and found nine responders to SMX-NO.

Initiating an immune response via the p-i pathway

But what happens if not the hapten SMX-NO, but the parent compound SMX is added to the cell culture? According to the p-i-concept, T cells are the main target cells for the reaction (instead of dendritic cells). We were able to show that the metabolism of SMX to SMX-NO does not occur in vitro using peripheral blood mononuclear cells (PBMC). The addition of SMX to PBMC of non-sensitised individuals (previously never exposed to SMX) did not induce a proliferative response in PBMCs. However, by extending the cell culture period to 4-6 weeks and the repetitive addition of PBMCs as antigen presenting cells (APC) and IL-2, we could detect some reactivity in three of ten healthy non-sensitised volunteers (Fig. 2). The immune response was clearly directed to the non-reactive parent compound (SMX) itself and not to SMX-NO (Engler et al., 2004). One of these volunteers was tested several times, always giving the same positive response. We even cloned SMX-reactive T cells from this non-sensitised individual, which were specific for SMX (but not for SMX-NO) and had the same characteristics as SMX-specific T cells obtained from SMX-sensitised individuals. Thus, under certain culture conditions, SMX stimulates T cells via their TCR. However, only certain individuals seem to react in this assay, and a massive co-stimulation of the T cells seems to be required (Engler et al., 2004).

Long lasting immunological memory

It is well known that a previous drug hypersensitivity reaction poses a risk for a new one. This implies the existence of an immunological memory. But how many cells are actually involved? By analysing the precursor frequency to five different drugs in five different patients with different forms of drug hypersensitivity reactions (sulfamethoxazole, carbamazepine, phenytoin, vancomycin and amoxicillin) (Beeler et al., 2006) we were able to detect specific T cells in patients which had reacted 12 years to 4 months before the analysis was performed.
**High precursor frequency of drug-specific T cells**

The frequency of drug-reactive T cells was measured with two assays and compared to the frequency of T cells reactive with tetanus toxoid, which is a common recall antigen in Switzerland since the whole population is regularly vaccinated. CSFE labeling of peripheral blood lymphocytes allowed to measure the proliferation, as this CSFE fluorescein cell stain is halved at each cell division, allowing a precise calculation of how many cells have divided in a certain time period. In the ELISPOT analysis the cytokine production of drug-reactive T cells was determined after 36 hours of cell culture with the drug or tetanus. Both analysis gave quite similar values: a high frequency of drug-specific T cells were found in individuals with an allergy to the corresponding drug whereby the analysis was always negative to other drugs to which the patients had been exposed but not sensitised. The frequency was actually higher than the simultaneously measured tetanus response, as 1:250 to 1:10,000 of CD4+ T cells reacted with the different drugs. This detailed analysis of drug precursor frequencies is a good basis to establish tests to detect such cells for the *in vitro* diagnosis of delayed drug hypersensitivity reactions (Beeler et al., 2006).

**From an individual (clinical) to a predictive test**

The present data shows the ability to stimulate T cells “pharmacologically” via the T cell antigen receptor (p-i concept). This stimulation of T cells of non-sensitised individuals required repetitive stimulations and was strong enough to cause the T cells to divide and to expand. This drug-mediated stimulation via T cell receptors is per se a quite astonishing finding as it underlines the high potency of certain drugs to stimulate the immune system.

Clinical data indicate that the p-i pathway is probably more relevant than the hapten concept in eliciting generalised drug

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**A SMX-NO specific response**

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**Fig. 2**: Induction of a primary immune response or expansion of SMX-reactive T cells from non-sensitised individuals.

The induction method consisted of the weekly stimulation of naive PBMCs with irradiated autologous PBMCs, IL-2 and the drug. The proliferative and cytotoxic response of 5 donors (D1-D5) to the reactive drug metabolite SMX-NO and its inert parent compound SMX is shown. During week 4 and 5, 4 individual induction cultures from each donor (a-d) were tested for specific proliferation, cytotoxicity and during week 5, also for cross-reactive cytotoxic responses. The stimulation index (SI) represents the factor of specific proliferation – measured by thymidine incorporation after 2 days of stimulation – in presence and absence of the drug. Cytotoxicity was analysed by standard chromium release assay using EBV lines as targets. Mean values of duplicates are indicated for an effector-to-target ratio of 40:1 (for details see Engler et al., 2004).
hypersensitivity reactions, while contact dermatitis is more due to the hapten mechanism.

In order to develop a predictive test based on the p-i concept and suitable for preclinical testing, several technical problems remain to be solved: The test must become far simpler, more robust and needs to be standardised. The human cells used for the in vitro tests must be carefully characterised. But the most important aspect is a better understanding of systemic drug hypersensitivity reactions in general. These questions are: What is the relationship of T cell stimulation to the clinical picture and why do only some individuals react - both in vivo as well as in vitro: What is the role of co-stimulation of T cells to enhance reactivity to the drug? Is the ability to react to drugs via the p-i mechanism due to the T cell receptor repertoire, immune regulation, genetic background, or is it, as some data would indicate, a combination of all? With this knowledge, one could create sophisticated in vitro models with human cells which might even replace a substantial number of animal tests for the detection of hypersensitivity inducing drugs.

References


Engler, O. B., Strasser, I., Naisbitt, D.J. et al. (2004). A chemically inert drug can stimulate T cells in vitro by their T cell receptor in non-sensitised individuals. Toxicology 197, 47-56.


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