Predicting Drug Hypersensitivity by *In Vitro* Tests

Werner J. Pichler
Department for Rheumatology and Clinical Immunology/Allergology, Inselspital, University of Bern, Switzerland

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

Background Information

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, W.J 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.

Predicition 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 pro-haptens, which rapidly become haptens. Nevertheless, these tests lack a reliable prediction of generalised forms of drug hypersensitivity (Bala et al., 2005). Furthermore, immune-mediated 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 prohaptens 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.

www.forschung3r.ch/en/projects/pr_80_01.html

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.

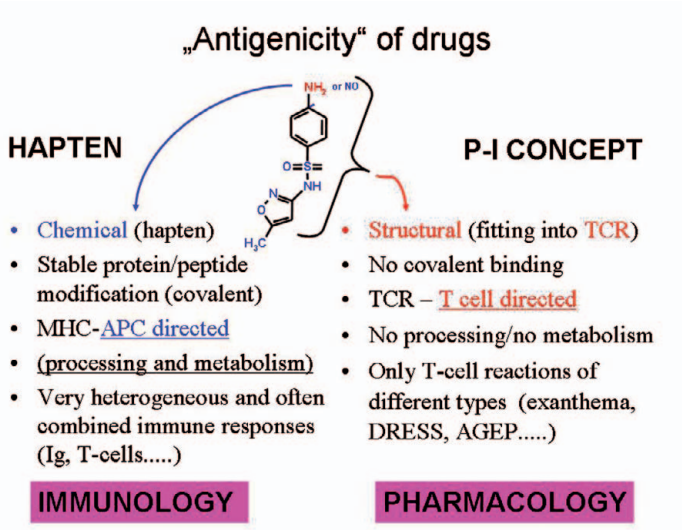

Fig. 1: Antigenicity of drugs: Drugs gain antigenicity by two pathways.
A) they can bind covalently as haptens to peptides and proteins, modify them and make them immunogenic. This pathway is APC directed and determined by the chemical reactivity of a drug. B) the p-i concept relies on the fitting of the drug into a certain T cell receptor for an antigen which happens to have a stimulating effect and leads to T cell expansion (for details see Pichler, 2003).

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.

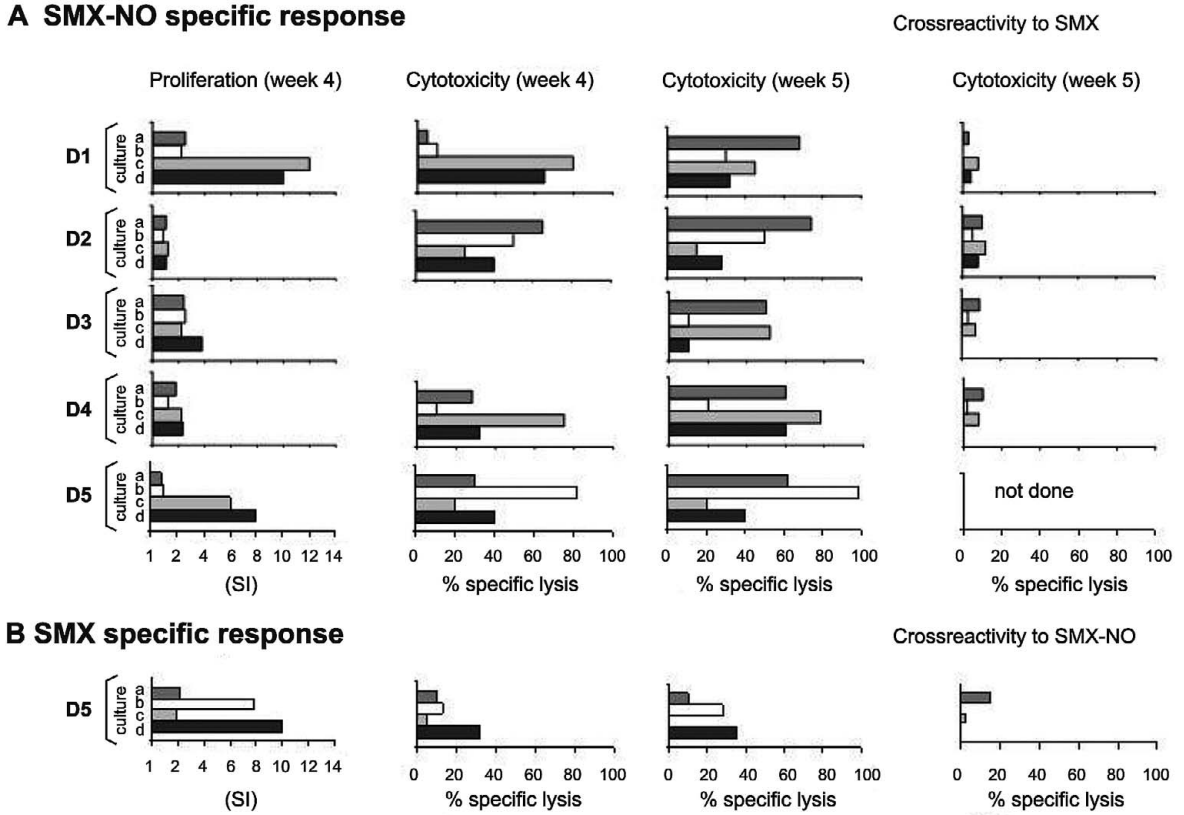

Fig. 2: Induction of a primary immune response or expansion of SMX-reactive T cells from non-sensitised individuals.
The induction method consisted of the weekly stimulation of naive PBMCs with irradiated autologous PBMCs, IL-2 and the drug. The proliferative and cytotoxic response of 5 donors (D1-D5) 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**)**

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

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.

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Correspondence to

Prof. Dr. Werner Pichler
Department for Rheumatology and Clinical
Immunology/Allergology
Inselspital
University of Bern
3010 Bern
Switzerland
e-mail: werner.pichler@insel.ch