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REVIEW ARTICLE

# Consequences of drug binding to immune receptors: Immune stimulation following pharmacological interaction with immune receptors (T-cell receptor for antigen or human leukocyte antigen) with altered peptide-human leukocyte antigen or peptide

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ABSTRACT

Drugs may stimulate the immune system by forming hapten–carrier complexes or via their pharmacological features, namely by noncovalent binding to proteins such as immune receptors. The latter type of immune stimulation is called the p-i concept, meaning pharmacological interaction with immune receptors, which implies stimulation of the immune system by noncovalent binding of a drug to T-cell receptors for antigens (p-i TCR) or human leukocyte antigens (p-i HLA). The functional consequences of these interactions are heterogeneous: clinically, it can lead to T-cell mediated reactions such as Stevens–Johnson syndrome/toxic epidermal necrolysis, drug rash with eosinophilia and systemic symptoms, acute generalized exanthematous pustulosis, and maculopapular eruptions. If the drug binds to the TCR, it can become stimulatory, and an additional interaction with HLA/peptide complexes is necessary for full stimulation. The T-cell reaction can be oligoclonal or polyclonal. Binding of drugs to an HLA molecule can have two consequences: if the drug can modify the HLA molecule, a distinct repertoire of peptides might be presented: this is the altered peptide model. However, peptide exchange is not necessary to make the peptide-HLA complex immunogenic: if the drug binds to HLA, already the complex of altered HLA and *normal* peptide is immunogenic and able to stimulate T-cells (altered peptide-HLA model). The immunological and clinical consequences of different forms of the p-i concept are described with typical p-i binding drugs such as abacavir, carbamazepine, flucloxacillin, allopurinol, and sulfamethoxazole. Thereby the role of drug binding to HLA or TCR, the affinity of drug binding, additional TCR binding, and potential oligoclonality are described and compared.



## Introduction

Drug hypersensitivity (DH) reactions are a modern and largely man made (iatrogenic) heterogeneous group of diseases, linked to the wide use of chemicals that are orally, parentally, or locally/topically applied. DH represents only a part of drug-related side effects. It is usually described as unpredictable and the clinical picture is not explained by the drug action or underlying disease: these unexpected clinical manifestations and the sometimes fulminant course make it an enigmatic area for clinicians and researchers. In addition, in DH, two highly variable systems meet: on one hand the endless number of novel small molecules, the majority chemically synthesized; and on the other, the highly variable immune system with $> 10^{11}$ different T-cell receptors (TCRs) and antibodies per individual, and a large number of human leukocyte antigens (HLA) molecules (> 9300) in the population (http://www.ebi.ac.uk/imgt/hla/stats.html).

The predominant antigens for both T-cells/TCRs, as well as B-cells/immunoglobulins, are proteins; in particular structural or sequential epitopes [mostly 8~20-amino acid (AA) long peptide stretches] within or derived from larger proteins. The highly variable immune receptors are supposed to *not* interact with small molecules (< 1000 Da), as the high specificity of the immune receptors requires a certain size of its antigens to be recognized as antigen and be differentiated from other structures. If the antigen was very small, e.g. methanol ($CH_3OH$), it could bind to many different regions within the protein receptor. However, even if it would fit into the receptor binding site, it would probably not

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provide enough surface interactions to cause signal transduction. A further checkpoint to exclude small molecules from activating the immune system is the need for cross-linking immune receptors to elicit an effector mechanism. Small molecules are too small to cross-link two adjacent immunoglobulins and their Fc-IgE-receptors; thus no cell signaling occurs.

In spite of these limitations, there are enough examples of drugs eliciting DH, drug-induced autoimmunity, and drug-induced immunodeficiency. Until recently, all these immune interactions were explained by the hapten features of the drug or drug metabolite (see below). However, during the last 15–20 years it has become clear that drugs have more possibilities to interact with the immune system than just by inducing an immune reaction by forming hapten–carrier complexes[1–10]: drugs are able to stimulate the innate immune system by binding to TLR (imiquimod),[1] drugs can bind to major histocompatibitlity complex (MHC) molecules and interfere with peptide loading onto MHCs (MHC loading enhancer, MLE),[2] which is similar to the altered peptide hypothesis, where drug binding to certain HLA alleles may affect the presentation of peptides presented by a certain allele.[3–5] In addition, drugs may also bind directly to a TCR and stimulate, in the presence of HLA/peptide interactions, specific T-cells via TCR.[6,7] The latter concepts, drug binding to the TCR or HLA with functional consequences, were originally described as *pharmacological interactions of drugs with immune receptors* (p-i concept).[8–10] In this review I will analyze briefly the hapten and prohapten concept, the initial findings leading to the p-i concept, the refinement of p-i (TCR) and p-i (HLA) concepts and extensions as elaborated during the last years. I compare immune stimulations by drug binding to TCR or HLA and their functional consequences and illustrate different possibilities of p-i using different drugs. Importantly, I indicate the possibility that peptide exchange is not a prerequisite of p-i (HLA) stimulations elicited by drugs, as drug binding to HLA *per se* already forms a highly immunogenic structure, which leads to strong T-cell activation in the presence of *normal* peptides (altered pHLA). Comparison of the hapten and p-i concepts is summarized in Table 1.

## The hapten concept

The limited interactions of small molecules with the immune system have been recognized by studying the (humoral) immune system—and soon it became clear that there are ways to overcome it. The hapten concept goes back to the 1930s; a small molecule can gain antigenicity if it is bound to larger proteins[11,12]: stable, covalent binding is required to modify the larger protein structure. Thereby the modified protein could be a foreign or an endogenously produced protein, to which tolerance has been developed. In both instances, the modified protein becomes a new antigen, as the stable hapten-binding modifies the protein. The modified protein (hapten–carrier complex) can under certain circumstances elicit B-cell reactions, antibody production and secretion and—after processing to small peptides presented by MHC—T-cell reactions. The antibody specificity is often predominantly directed to the small hapten itself as even a small modification of the hapten can already abrogate the recognition of the whole hapten–carrier complex (Table 1).[12]

**Table 1** Comparison of the hapten and p-i concepts.

| Hapten concept | p-i concept |
|---|---|
| Chemical binding to proteins or peptides | Structural binding to HLA or TCR |
| Covalent interactions | Noncovalent interactions |
| Often dependent on processing and metabolism | Processing and metabolism not required |
| Activation of the innate immune system | Bypass of the innate immune system |

Haptens are chemicals that are chemically reactive and have a tendency to build covalent bonds to some AAs within a protein. For example, at least 13 lysine groups within the albumin molecule have been shown to bind piperacillin and were processed to different modified epitopes within the same protein.[13] For the immune system, accessible modifications may elicit antibody responses to these hapten-modified epitopes. If the antibodies react with the hapten bound to different sites on the same protein, cross-linking of the bound antibodies can occur. This requires a certain sterical distance between these hapten-modified epitopes, otherwise the rather large antibody molecule (their Fab part) would interfere with binding. If the antibody response is predominantly directed to the hapten and distant enough to allow two antibody bindings, cross-linking of the hapten-specific antibodies (including antibodies with the *same* specificity!) can occur by a single protein. This may enhance and explain why hapten-specific IgE reactions are often fulminant and occasionally even fatal.

Later, the work of Landsteiner et al showed that the delayed reaction to haptens (later shown to be T-cell mediated) is also very specific.[14] Proteins are processed and presented as small 8–20-AA long peptides by MHC-encoded molecules, which as proteins (HLA) appear on the cell surface. Thereby the 14–18 HLA molecules expressed per individual present different peptides (mostly 8–10 AA for HLA class I, ~14–16 AA for HLA class II), which fit into the peptide binding groove of HLA-molecules. Further work by Weltzien et al demonstrated that the location of the hapten modification (in the middle or at the end of the 9-mer peptide) may influence the functional consequence of the evolving immune response, in particular cross-reactivity and autoimmunity.[15]

It is important to realize that hapten-specific immune responses are complete immune responses, involving stimulation of antibodies and T-cells. Actually, if a drug is able to elicit both B- and T-cell immune responses, it is most likely to have hapten-like characteristics. Haptens are immunogenic and antigenic: their immunogenicity is linked to the ability to activate the innate immune system, mostly by binding to molecules that cause cell activation or damage. For quite a number of molecules it has been shown that haptenization leads to the activation of dendritic cells (DCs) *in vitro*,[16,17] and this capacity of haptens is used to identify contact allergens by *in vitro* tools. The immunogenicity is supplemented by antigenicity, which is the provision of antigenic determinants for the specific immune receptors (B- and T-cell receptors).

Not every hapten modification may result in an efficient immune response: if, for example, the hapten modifies a peptide sequence, which is not presented by the available HLA alleles, the hapten modification remains unnoticed by the immune system. If the hapten induced modification does not simultaneously activate the innate immune system it may remain ignored, as no efficient immunity will be developed.

An unexplained issue of the hapten (or prohapten) theory is the fact that hapten-formation is common for given drugs such as penicillin and happens in the majority of treated patients. IgG antibody formation to penicilloyl-determinants seems to be frequent. Why only a minority of patients develops an allergic, clinically symptomatic immune reaction is unclear.

## The prohapten concept

Many drugs are not chemically reactive but are still able to elicit immune-mediated side effects. The prohapten hypothesis reconciles this phenomenon with the hapten hypothesis by stating that a chemically inert drug may become reactive upon metabolism.[18,19] Sulfamethoxazole (SMX) is a prototype of such a prohapten. It is