Review of: "Risk Assessment in Drug Hypersensitivity: Detecting Small Molecules Which Outsmart the Immune System"

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Drug hypersensitivities (DH) are inherently difficult to predict. Here, Pichler, Watkins and Yerly outline the difficulties of, and propose a new approach for, identifying the possibility of a drug to cause hypersensitivities (DH) early in drug development. The proposed approach involves a combination of 1) *in vitro* cell culture assays that asses off-target effects of drugs involving the interaction with immune receptors, including particularly those that are highly polymorphic and/or polygenetic (HLA molecule, TCR, antibody), 2) structural analysis (computational modelling and crystal structures) of the interaction between the drug and the relevant protein and 3) upon identification of a drug that modulates an immune receptor, clinical monitoring to assess the incidence of DH. The authors also appeal to pharmaceutical companies to enhance their investment into understanding adverse drug reactions (ADRs), for carefully documented data of ADRs being openly shared and that a database of clinical DH and associated drug-protein interactions gets established.

1. There is great logic in an approach for risk assessment, as proposed here, that identifies if and how a drug in development induces DH: it prevents individuals from getting DH and allows the redesign of a new drug to achieve pharmacological effect whilst avoiding DH, thus increasing the chance of its clinical approval, and thereby providing multiple clinical and economic benefits. An increased mechanistic understanding of individual adverse drug reactions would also feed into an overall better understanding of the plethora of possible mechanisms of drug modulations which could then be taken into account at the earliest stages of novel drug design. There is also great logic in specifically including human *in vitro* and structural biology studies during the preclinical phase of drug development in order to predict DH, as animal models are not suitable to address the interactions between drugs and human receptors, although relevant humanized or transgenic animal models can sometimes provide useful information, as stated by the authors.

   Whilst there might be no better alternatives, we think it is worthwhile considering the pitfalls of the proposed approach: (i) Notwithstanding the value of determining the mechanisms of how drugs induce drug hypersensitivities, to date there are very few drugs for which the mechanisms have been identified, indicative of the complexity of the mechanisms and associated challenges in identifying them.

Indeed, the examples of know mechanisms involve strong associations with particular HLA allotypes and strong functional phenotypes, suggesting that the first two aspects of the proposed approach are difficult to address. (ii) What exactly does the *in vitro* cellular assay look like, especially when the drugs in question require metabolic alteration to create the active agent causing hypersensitivity, perhaps in the liver or other organs? Without any pre-existing knowledge of relevant HLA, TCR or antibody involved, the possibility in cells/blood donors that would need to be tested is vast and even the proposed use of blood from individuals with multiple drug hypersensitivity syndromes (MDH) would not provide sufficient coverage. (iii) Structural analysis is only possible when the protein-drug interaction has been sufficiently understood at a functional level, with the interaction partners clearly defined. Whilst there have been advances in computational modelling, crystallography is preferred over computational modelling, especially to correctly predict entirely new mechanisms. (iv) As touched on in the conclusion of the article, in light of the existence of DH suppressing and enhancing factors *in vivo,* a stepwise approach that only considers close clinical monitoring of drugs that light up in *in vitro* cellular assays is expected to follow up on both false-positives and false-negative DH causing drugs, somewhat similar to results gained in preclinical animal models. (v) Extending on the latter, DH suppressing and enhancing factors differ between individuals beyond genetic differences in immune receptors, such as HLA and TCR, leading to some DH being rare in the population (low penetrance), and thus difficult to identify in first place. Following on it would then also be difficult to understand the mechanisms (less clear association) and hence it would also be difficult to predict the relevant DH using the outlined approach, whilst not less important to do so at the individual patient level.

2. As part of the conclusion, the authors raise that 'by challenging the hapten as the sole explanation for DH, a first step to improve the situation (to address the risk assessment in DH) may have been made. We may now be closer to understanding these interactions, but the explanations are still complicated'. We agree generally speaking that in the absence of understanding DH in most cases, models of DH can be helpful in better understanding DH. However, the authors construct a largely theoretical set of parameters that might underpin immune mediated hypersensitivity unsupported by very much experimental evidence. The theories are not particularly novel despite renaming old concepts with new names (pi-concept).

   The very best understood T cell mediated hypersensitivity remains abacavir hypersensitivity in HIV-infected patients with HLAB*57:01 (Illing *et al*, Nature 2012, DOI: 10.1038/nature11147). The structural biology and T cell assays are well described, and the mechanism is clear and could not be further from the pi-concept coveted by the authors. The biochemistry and immunology unequivocally demonstrate altered self-peptide presentation due to abacavir-binding to the cleft of HLA-B*57:01 leading to T cell immunity towards the novel peptide repertoire.

   Both the original and 'new models' do not account accurately for above-described altered self-peptide presentation and remain highly speculative. Also, those models do not provide a useful logical framework for possible drug modulations and do not lend themselves to experimental confirmation, suggesting to us that the models are outdated. New models, that accurately reflect the existing mechanistic insights and extend to include variations of those on the background of the current immunological, biochemical and pharmacological knowledge would be desirable. For example, the authors appear to be convinced

that drug-protein interactions are unusual in that the underlying immune stimulations are (i) not antigen driven and (ii) they are non-covalent and thus labile, reversible, and transient. These two statements do not make sense and are neither here nor there in relation to the larger question of mechanism. In terms of the first statement: Are the authors trying to convey the difference between direct antigen recognition (by TCRs and antibodies) versus indirect antigen recognition resulting from conformational or allosteric alterations to self-antigens? Importantly, the latter are not examples of hapten recognition which is direct recognition of an antigen generally too small to elicit antibody immunity in its own right but immunogenic when linked to a carrier protein. In terms of the 2nd statement: Most protein-protein interactions are in fact non-covalent, and indeed such interactions are the hallmark of how TCRs recognise antigen-loaded HLA molecules, with the antigen also non-covalently bound to the HLA molecule, and how antibodies recognise their antigens.

3. We would like to add, that more recently, we and others have shown that drugs and drug-like molecules can also modulate non-polymorphic MHC-I like molecules, reviewed in de Lima Moreira, Souter *et al* Allergy 2020 (DOI: 10.1111/all.14279):

   (i) The MHC-I related protein 1 (MR1), which otherwise presents derivatives of an intermediate in microbial riboflavin biosynthesis, such as 5-OP-RU, to Mucosal-Associated Invariant T cells (MAIT cells) (Kjer-Nielsen *et al* Nature 2014, DOI: 10.1038/nature11605; Corbett, Eckle, Birkinshaw, Liu *et al* Nature 2014, DOI: 10.1038/nature13160), can instead present drugs or drug-like molecules, some of which were cross-recognised by MAIT cells *in vitro,* including metabolites of diclofenac (Keller, Eckle, Xu *et al* Nature Immunology 2017, DOI: 10.1038/ni.3679), although these observations have not been linked to DH yet.

   (ii) Cluster of differentiation 1 (CD1) molecules, including CD1a, present lipid antigens to αβ TCR^+ T cells. CD1a can also present the poison ivy allergen-derived antigen urushiol (Kim, Hu *et al* Nature Immunology 2018, DOI: 10.1038/ni.3523) and farnesol present in cosmetics and perfumes (Nicolai*et al* Science Immunology 2020, DOI: 10.1126/sciimmunol.aax5430). (iii) CD1d, which is known to present lipids to type I and type II natural killer T cells (NKT cells), can co-present sulfa-drug molecules and self-lipids, otherwise not recognised by themselves, to a subset of type II NKT cells, expressing αβ or γδ TCRs (Almeida, Smith, Cheng *et al* PNAS 2021, DOI: 10.1073/pnas.2104420118).

   Accordingly, in the case of MHC-I like molecules, drugs themselves could act as antigens, as per the above examples (i, ii). Alternatively, drugs could be co-presented with their regular type of MHC-I like ligand, which by itself is not recognised by T cells, as per the above example (iii), thus sharing the aspect of co-presentation with the mechanism underlying abacavir hypersensitivity syndrome, referred to as the altered repertoire concept (Illing *et al*, Nature 2012, DOI: 10.1038/nature11147). Other mechanisms of DH involving MHC-I like molecules are expected to be identified, with the biology of MHC-I like molecules representing emerging fields in immunology.

4. We would like to re-emphasise the need for careful clinical, functional and structural understanding of DH and the creation of associated databases towards a better understanding and thus risk assessment of DH, as appealed to by Pichler *et al*. We also would like to add that a more collaborative approach between clinicians, clinical researchers, immunologists and biochemists will enhance the chances of delineating the mechanisms underlying DH.