Cell Research (2012) 22:1637-1639.

www.nature.com/cr

RESEARCH HIGHLIGHT

Mechanisms involved in the Abacavir-mediated hypersensitivity syndrome

Anuska Llano[1], Christian Brander[1, 2, 3]

[1]Irsicaixa AIDS Research Institute – HIVACAT, Hospital Universitari Germans Trias i Pujol, Badalona, Spain; [2]Universitat Autònoma de Barcelona, Hospital Universitari Germans Trias i Pujol, Badalona, Spain; [3]Institució Catalana de Reserca i Estudis Avançats (ICREA) Barcelona, Spain

Cell Research (2012) **22**:1637-1639. doi:10.1038/cr.2012.105; published online 10 July 2012

The potentially life-threatening adverse reactions to Abavacir (ABC), a nucleoside analog reverse transcriptase inhibitor for the treatment of HIV infection, have been known for several years to be limited to individuals expressing the HLA-B*57:01 gene. Why the ABC hypersensitivity syndrome is only seen in HLA-B*57:01-expressing subjects and what the precise mechanisms underlying this intolerance are remain however controversial. A series of recent studies, particularly a study by Illing *et al*. recently published in *Nature*, now answer some of these questions and offer new opportunities to better understand autoimmune disorders and prevent adverse reactions to other drugs.

Delayed hypersensitivity reactions (DHS) are a form of adverse drug reactions that are caused by drug-specific T cell responses. To date, two main mechanisms have been used to explain such adverse reactions, including the hapten/pro-hapten concept and the "pharmacological interaction with immune receptor" concept (also known as "p-i concept") [1, 2]. In the hapten concept, the drug reacts with a self-protein or peptide, generating a haptenated determinant with antigenic potential. Such antigenic determinants can be created on the surface of antigen-presenting cells (APC) where the drug directly interacts with T cell epitopes in the context of presenting HLA structures or, in case of haptenized proteins, by intracellular antigen processing and representation of drug-modified epitopes by HLA molecules on the cell surface. A classical example of drug hypersensitivity reactions that is generally explained by the hapten concept, is the case of penicillin and its derivatives that bind covalently to lysine residues of serum proteins generating new antigenic determinants [3]. In the p-i concept, the drug may interact in a non-covalent manner with the T cell receptor (TCR) or with the HLA molecules without the antigen processing pathway being involved in this process. In contrast to the hapten model, such drug hypersensitivity reactions are often associated with specific HLA class I or class II alleles, as it is for instance the case with carbamazepine (CBZ)-induced Stevens-Johnson syndrome (SJS), where individuals with the HLA-B*1502 allele have a 1 000-fold higher risk to experience adverse reactions than subjects not carrying this gene [4]. Although the p-i concept has been suggested to explain many cases of drug hypersensitivity syndromes strongly associated with specific HLA alleles, this model cannot satisfactorily explain the HLA-B*5701-associated ABC hypersensitivity syndrome (AHS). Particularly, extensive earlier studies by the McCluskey lab have shown that AHS is TAP and Tapasin dependent and requires conventional antigen-presentation pathways [5], which are not required for drugs acting via the p-i concept mechanisms. In addition, the p-i concept is not compatible with the absence of AHS in individuals carrying HLA class I allele closely related to HLA-B*57:01, such as HLA-B*58:01 and B*58:02, which would offer conserved sequence segments for ABC to bind and to be recognized by drug-reactive T cells. Thus, alternative models, including scenarios in which the peptide cargo of HLA-B*57:01 molecules could be subject to ABC-mediated alterations remain to be tested.

In their elegant and in-depth analyses, Illing *et al*. [6] used HLA-B*57:01-expressing cells cultured in the absence or presence of ABC to probe the repertoire of self-protein-derived epitopes presented in the context of HLA-B*57:01. They show that in the presence of ABC, up to 25% of the total peptides that can be eluted from HLA-B*57:01 were novel self-peptides not seen in the absence of ABC. Most re-

Correspondence: Christian Brander
Tel: +34 93 465 63 74; Fax: +34 93 46 53 968
E-mail: cbrander@irsicaixa.es

markably, when aligning the sequences of these new self-epitopes, they noted marked changes in amino acids that were favored at the C-terminal end, which under normal condition serves as the anchor position for the F-pocket of the presenting class I molecule: While conventional HLA-B*57:01 epitopes possess most frequently a Tryptophan in this position, peptides eluted from HLA-B*57:01 molecules derived from cells cultured with ABC showed a preference for Isoleucine and Leucine instead. This effect was not observed in the closely related HLA-B*57:03 and HLA-B*58:01 alleles and no changes were seen for the B-pocket anchor residue (position 2) for HLA-B*57:01-eluted epitopes, suggesting that ABC in some way specifically interacts with the F-pocket in the HLA-B*57:01 peptide-binding groove. Subsequent crystallographic structural analyses indeed showed that ABC binds in the cleft of the class I molecule and interacts with residues (particularly residue 116) that form the F-pocket of HLA-B*57:01. These residues differ between the HLA-B*57:01 sequence and the HLA-B*57:03 and HLA-B*58:01 alleles as well as other closely related alleles HLA-B*57:02 and HLA-B*57:11. Furthermore, ABC binding also appears to impact the C, D and E but not the B pocket of HLA-B*57:01, in line with unchanged anchor preferences at position 2 of peptides eluted from HLA-B*57:01 in the presence of ABC. These data are consistent with two other recent reports in which changes from conventional anchors to Isoleucine, Leucine (and Valine) containing peptides were observed in HLA-B*57:01 molecules derived from cells cultured in the presence of ABC [7, 8].

Together, these data help to explain the highly allele-specific adverse effects of ABC: given its requirements for binding across several pockets of HLA-B*57:01, there is likely hardly any other sufficiently closely related allele that could bind ABC in the same manner. Although not tested in the present study and likely not captured in epidemiological studies due to their low frequencies, this may also be the case for alleles of the HLA-B63 serotype (HLA-B*15:16 and -B*15:17), which share epitope-binding motifs with HLA-B57 and likely have emerged through a recombination event that transferred the HLA-B17-binding pockets to the HLA-B15 background [9, 10]. These alleles share many of the residues considered crucial for ABC binding to HLA-B*57:01 (including 116 in B*15:17) but differ at some others (e.g., 97), which may render them insensitive to ABC effects. At the same time, the strict allele specificity of the ABC effect also speaks against a dominant hapten effect by which ABC would bind to proteins or epitopes presented by HLA-B*57:01. In such a case, the broad epitope sharing between HLA-B*57:01 and other alleles of the B58 supertype would lead one to expect ABC adverse events in the context of these other alleles as well. However, a recent report did find that for a minority of ABC-reactive T cells, ABC could directly stimulate the cells without the need for antigen-presenting cells, presumably by binding to presented epitopes or presenting HLA alleles [11]. As ABC could bind into the peptide groove of transiently empty HLA class I molecules on the cell surface, this could change their structure sufficiently to make them appear non-self and elicit a reaction, which would be equally allele specific as the process described by Illing *et al*. [6].

Through the analysis of TCR repertoires of ABC-reactive T cells in HLA-B*57:01-positive individuals, the study by Illing *et al*. [6] also suggest that ABC induces a broad spectrum of polyclonal T cell responses. This is in line with the fact that ABC-loaded molecules is still able to present a wide array of different epitopes (since there are only few contact sites between ABC and the presented epitopes), all with the potential to induce T cell responses utilizing various TCR repertoires. The elution studies by Illing *et al*. [6] support this view but it is interesting to note that there was no increase in shorter peptides being presented by HLA-B*57:01 with embedded ABC in its cleft. While Illing and colleagues emphasize the possibility that bulged epitopes could be presented in the presence of ABC, this does not need to be necessarily so as a partly occupied binding groove could accept shorter epitopes [12]. The data by Norcross *et al*. [7] and Ostrov *et al*. [8] point towards peptides with shortened side chains on the F-pocket and also show some examples of shorter epitopes eluted from ABC-loaded HLA-B*57:01 molecules, and among them epitopes derived from a self-protein that is expressed in the skin were suggested to be the target of autoimmune reactions.

Illing *et al*. [6] also show additional studies where they translate the findings of the ABC effect on HLA-B*57:01 to a similar, but mechanistically possibly different hypersensitivity reaction to CBZ. This antiepileptic drug causes severe adverse effects in individuals expressing HLA B*15:02. Although the odds ratio is above 1 000 in some Asian populations, its positive and negative predictive values are lower [13] and the data are not as impressive as those for ABC. Particularly, CBZ seems to cause fewer changes in the peptide cargo of HLA B*15:02 molecules (15% compared to 25% for ABC) and induce less dramatic changes in preferred primary and secondary anchor residues. The authors link these more limited changes in the peptide cargo with the more oligoclonal TCR repertoire reported for CBZ-reactive clones compared to the broad TCR usage for ABC. It remains however unclear why such limited TCRs have been observed, as even a single neo-epitope could induce a T cell response comprising many different Vb families, left alone the likely several thousand new epitopes that CBZ-modified HLA B*15:02 could present *in vivo*. In fact, the effect that

CBZ has on the evolving T cell response resembles more what has been seen for drugs such as the classical haptenizing Penicillin and its derivatives, where limited TCR repertoires have been described [14-16] .

All together, the study by Illing *et al*. [6] and some of the other recent papers on this topic, greatly further our understanding of the possible mechanisms of drug hypersensitivity reaction in general and AHS in particular and pave the road for approaches that could be taken to prevent such adverse effects during drug design [6-8, 11]. An already important question that still awaits an answer is why only 50% of HLA-B*57:01-expressing, ABC-treated individuals will develop AHS. Evidently, some crucial co-factor(s) may be needed for AHS, which may include limitations in T cell receptor repertoires or differences in the pre-existing immunity that would partly cross-react with ABC-modified HLA and self-epitopes. In addition, a danger signal, simultaneously present when the drug(s) are being delivered, may be required and would likely be present in many cases, such as in HIV infection (as a form of generalized immune activation and inflammation at the time of treatment), in bacterial infections, or (minor) tissue damage in other conditions that require medications. Mechanistically, it also would be interesting to see whether for instance children born to HIV-infected, HLA-B*57:01-negative mothers who were ABC treated during pregnancy would remain tolerant to ABC's effects, at least *in vitro*. Such analyses could be extended to many other drugs that pass the placenta and may tolerize children for later exposure, if they express the correct HLA risk alleles and if the drug indeed acts via an alteration of peptide cargo of the HLA class I molecules in question.

References

1 Bharadwaj M, Illing P, Theodossis A, Purcell AW, Rossjohn J, McCluskey J. Drug hypersensitivity and human leukocyte antigens of the major histocompatibility complex. *Annu Rev Pharmacol Toxicol* 2012; **52**:401-431.

2 Pichler WJ. Pharmacological interaction of drugs with antigen-specific immune receptors: the p-i concept. *Curr Opin Allergy Clin Immunol* 2002; **2**:301-305.

3 Pichler WJ. T cells in drug allergy. *Curr Allergy Asthma Rep* 2002; **2**:9-15.

4 Wu Y, Sanderson JP, Farrell J, *et al*. Activation of T cells by carbamazepine and carbamazepine metabolites. *J Allergy Clin Immunol* 2006; **118**:233-241.

5 Chessman D, Kostenko L, Lethborg T, *et al*. Human leukocyte antigen class I-restricted activation of CD8+ T cells provides the immunogenetic basis of a systemic drug hypersensitivity. *Immunity* 2008; **28**:822-832.

6 Illing PT, Vivian JP, Dudek NL, *et al*. Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. *Nature* 2012; **486**:554-558.

7 Norcross MA, Luo S, Lu L, *et al*. Abacavir induces loading of novel self-peptides into HLA-B*57: 01: an autoimmune model for HLA-associated drug hypersensitivity. *AIDS* 2012; **26**:F21-F29.

8 Ostrov DA, Grant BJ, Pompeu YA, *et al*. Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. *Proc Natl Acad Sci USA* 2012; **109**:9959-9964.

9 Barber LD, Percival L, Arnett KL, Gumperz JE, Chen L, Parham P. Polymorphism in the alpha 1 helix of the HLA-B heavy chain can have an overriding influence on peptide-binding specificity. *J Immunol* 1997; **158**:1660-1669.

10 Frahm N, Adams S, Kiepiela P, *et al*. HLA-B63 presents HLA-B57/B58-restricted cytotoxic T-lymphocyte epitopes and is associated with low human immunodeficiency virus load. *J Virol* 2005; **79**:10218-10225.

11 Adam J, Eriksson KK, Schnyder B, Fontana S, Pichler WJ, Yerly D. Avidity determines T-cell reactivity in abacavir hypersensitivity. *Eur J Immunol* 2012; **42**:1706-1716.

12 Burrows SR, Rossjohn J, McCluskey J. Have we cut ourselves too short in mapping CTL epitopes? *Trends Immunol* 2006; **27**:11-16.

13 Phillips EJ, Chung WH, Mockenhaupt M, Roujeau JC, Mallal SA. Drug hypersensitivity: pharmacogenetics and clinical syndromes. *J Allergy Clin Immunol* 2011; **127(3 Suppl)**:S60-S66.

14 Brander C, Mauri-Hellweg D, Bettens F, Rolli H, Goldman M, Pichler WJ. Heterogeneous T cell responses to beta-lactam-modified self-structures are observed in penicillin-allergic individuals. *J Immunol* 1995; **155**:2670-2678.

15 Mauri-Hellweg D, Zanni M, Frei E, *et al*. Cross-reactivity of T cell lines and clones to beta-lactam antibiotics. *J Immunol* 1996; **157**:1071-1079.

16 Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ. Activation of drug-specific CD4+ and CD8+ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepine. *J Immunol* 1995; **155**:462-472.