Single cell analysis of drug responsive T cells; identification of candidate drug reactive T-cell receptors in abacavir and carbamazepine hypersensitivity

Alec Redwood[1], Kaija Strautins[1], Katie White[2], Abha Chopra[1], Katherine Konvinse[2], Shay Leary[1], Rebecca Pavlos[1], Simon Mallal[1,2] and Elizabeth Phillips[1,2]

[1]IIID, Murdoch University, WESTERN AUSTRALIA; [2]Vanderbilt University Medical Center, TN, USA.

Background

T-cell mediated drug hypersensitivity requires three essential components, a target drug, a scaffold for the drug (MHC class I or class II) and a pathogenic T-cell receptor (TCR). For several drugs including abacavir (ABC) and carbamazepine (CBZ), two components of this trimolecular complex have been defined. For ABC hypersensitivity reaction (HSR) the associated MHC is HLA-B*57:01 and for CBZ induced Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), the associated MHC is HLA-B*15:02. In the former instance the association of drug with MHC has been further defined with the successful resolution of the crystal structure of the ABC complexed to HLA-B*57:01 (1, 2).

Objective

Several models have been proposed to explain the induction of T-cell responses to small drug molecules (3-4). These are the hapten/pro-hapten theory, the p-i model and the altered peptide model (Figure 2). The structure of ABC bound to MHC class I is consistent with the altered peptide model. For other drugs the p-i or the hapten model may be more relevant. For all models the mechanisms by which TCR contacts MHC/drug/peptide remains unknown and is the subject of this study.

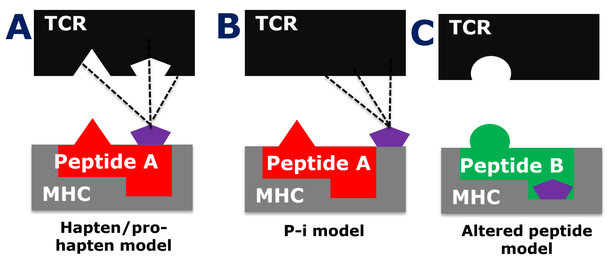
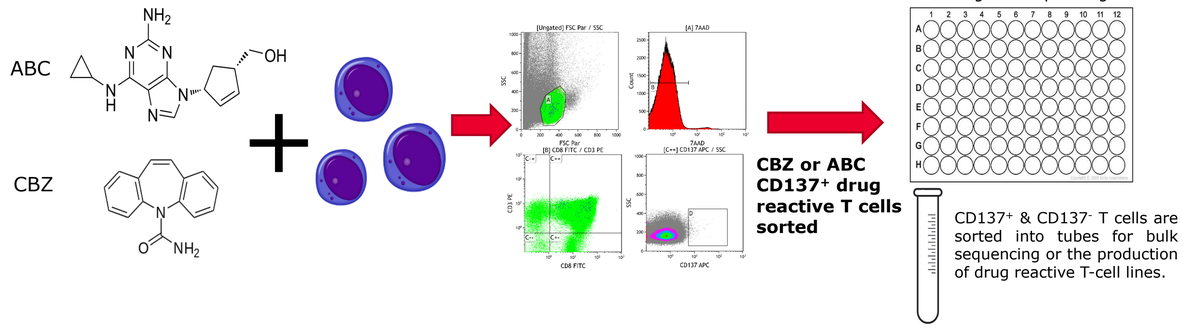


Figure 2. Models of T-cell drug specificity. (A) The *hapten/pro-hapten model* posits that drug binds to a carrier protein which is processed and presented on MHC molecules as a neo-antigen. **(B)** the *p-i model* postulates that drugs directly bind the TCR. **(C)** The *altered peptide model* posits that drug binding - within the peptide binding cleft - alters peptide binding allowing presentation of novel self-antigens to T cells.

Methods

Working from a cohort of cryopreserved PBMCs on HLA-typed patients with confirmed histories of T-cell mediated drug hypersensitivities (ABC HSR and CBZ induced SJS/TEN and DRESS) we performed flow cytometry, single cell cloning and single cell TCR sequencing and used these to develop and interrogate T-cell responses (Figure 2).



CD137+ & CD137- T cells are sorted into 96 well plates for direct sequencing or cloning and sequencing.

Figure 2. T cell activation and sorting strategy. PBMC samples with confirmed T-cell drug reactivity (ELISpot) were treated with drug (CBZ – 12.5 or 25 ug/ml; ABC – 10 ug/ml) for 18-20 hours. Upregulation of the activation marker CD137 was used to sort drug reactive CD8+ or CD4+ T cells into 96 well plates to produce T-cell clones or into tubes to produce T-cell lines. T cells were subsequently non-specifically expanded using protocols developed to expand anti-viral T cells (5) and then sequenced. Alternatively, T cells were sequenced directly ex vivo following activation and sorting.

Results

CBZ specific T-cell lines and clones were produced from an HLA-B*57:01 SJS/TEN patient and a drug reaction with eosinophilia and systemic symptoms (DRESS) patient. ABC reactive T-cell lines and clones were produced from two patients with ABC HSS. Shown in Figure 3 are representative flow plots showing T-cell reactivity to CBZ.

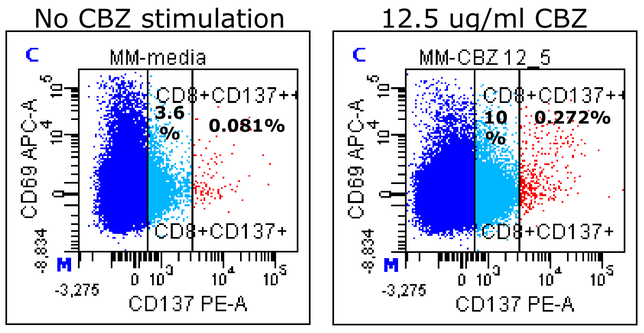


Figure 3. Representative sorting. PBMCs from a patient with SJS/TEN were incubated o/n with CBZ and then sorted. Left panel - spontaneous CD137 and CD69 expression on CD8 T cells. Right panel up-regulation of CD137 and CD69 in response to CBZ. Note sorts were performed on the basis of CD137 only. Index sorting allows for all data to be linked to T-cell phenotype

The specificity of T-cell lines and clones was determined by assessment of CD137 up-regulation following addition of the relevant drug (not shown) and by IFNγ ELISpot (Figure 4).

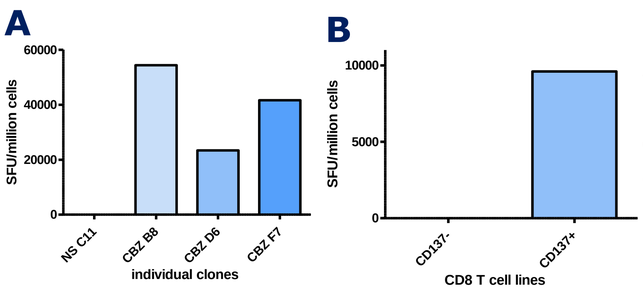


Figure 4. CBZ and ABC clones and lines retain specificity for drug. T-cell lines or clones were incubated over night with relevant drug and then tested by IFNγ ELISpot. (**A**) CBZ reactive T cell clones B8, D6 and F7 from a CBZ SJS/TEN patient, NSC11 is a non CBZ reactive clone from the same patient. (**B**) IFNγ production of ABC reactive line (CD137+) compared to a control line.

TCR usage of drug specific T cells was assessed directly ex vivo or following stimulation and T-cell cloning from patients with CBZ induced DRESS or SJS/TEN or in patients with ABC HSR.

Figure 5. TCR repertoire of CBZ reactive T cells - DRESS. Comparison of CDR3 (β chain) sequences from T-cells lines derived from samples taken 7.5 months apart; (**A**) CD4 T-cell lines**, (B)** CD8 T-cell lines. Coloured text represent multiple distinct nucleotide sequences encoding a common CDR3.

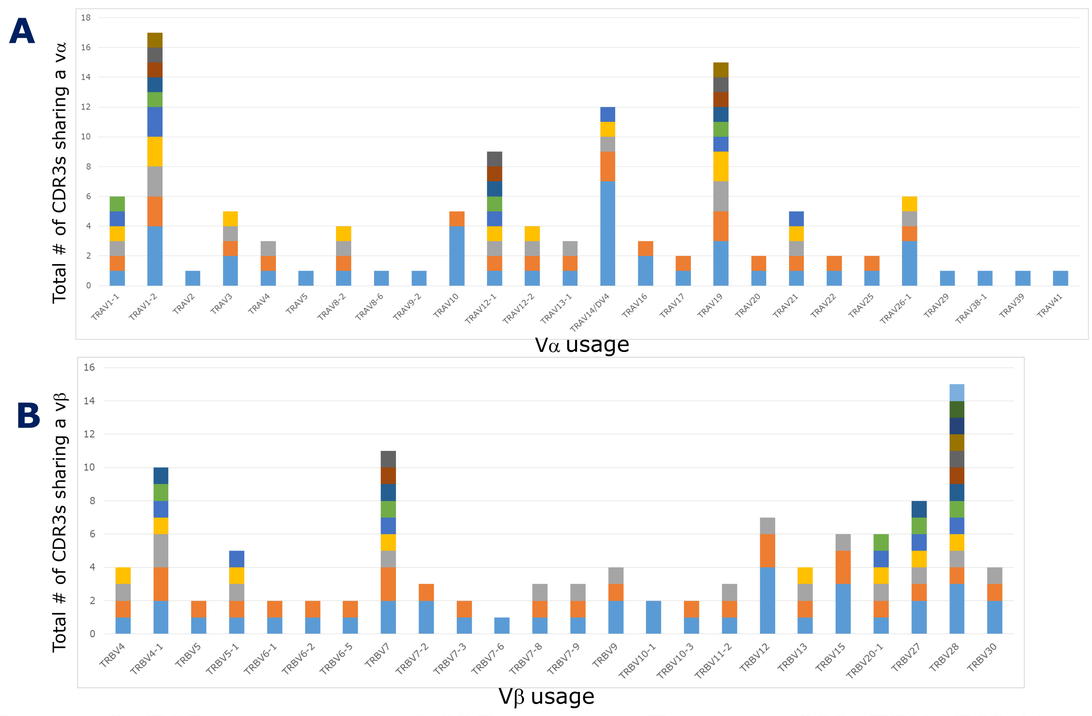


Figure 6. TCR repertoire of CBZ reactive T cells – SJS/TEN. TCR Vα (**A**) and Vβ (**B**) chain usage from CBZ reactive T cells from a patient with SJS/TEN. Column infill shows number of CDR3 sequences using an individual Vα or Vβ chain. Most common α/β pairs CAMRGSGGFKTIF (TRAV14)/CASYHGTGVAGELFF (TRBV28) and CASYHGTGVAGELFF (TRAV1-2)/CASSYSHIQYF (TRBV12).

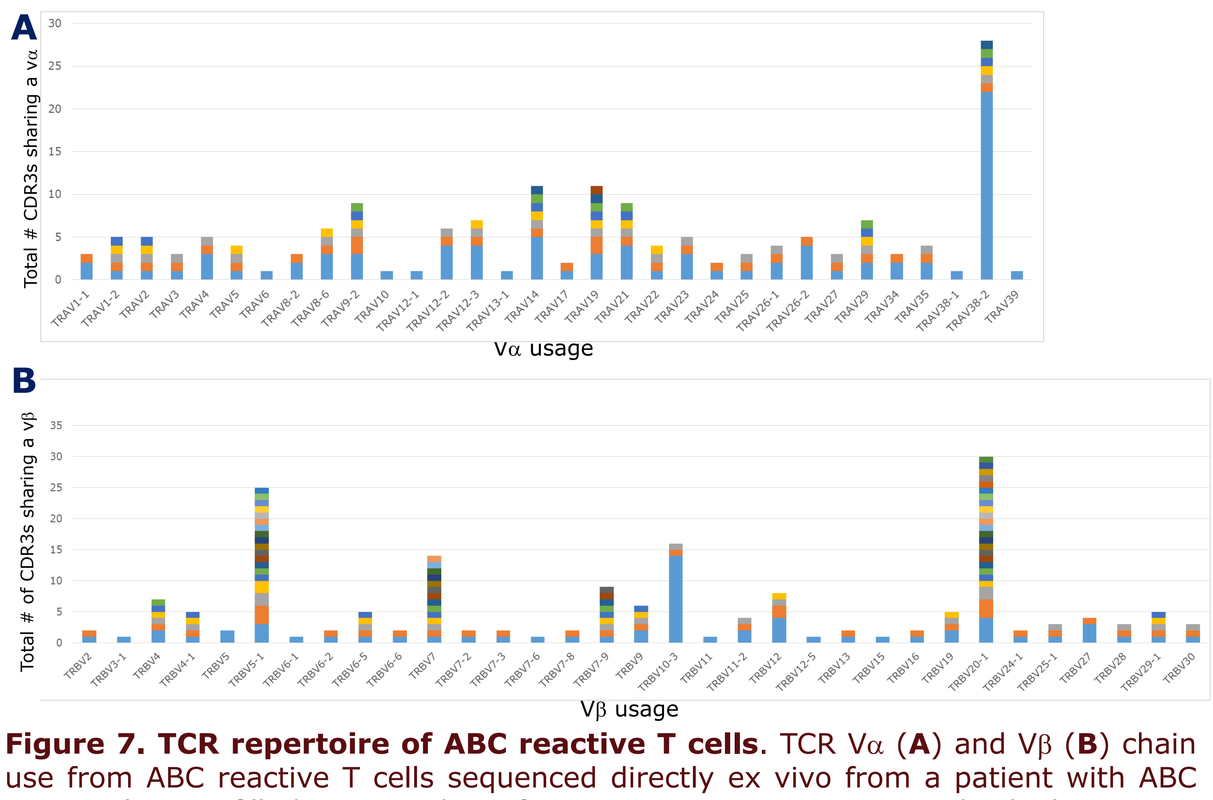


Figure 7. TCR repertoire of ABC reactive T cells. TCR Vα (**A**) and Vβ (**B**) chain use from ABC reactive T cells sequenced directly ex vivo from a patient with ABC HSR. Column infill shows number of CDR3 sequences using an individual Vα or Vβ chain. Most common α/β pair CAYRSARYNFNKFYF (TRAV38-2)/CAISERDTHQPQHF (TRBV10-3)

Conclusions

Several candidate pathogenic TCRs have been identified in patients with hypersensitivity to abacavir or carbamazepine. These putative TCRs will be cloned into Jurkat T-cell lines to determine if multiple T-cell clones contribute to hypersensitivity or if only a single clone in each patient is pathogenic. Ultimately these cloned TCR will be used to define the crystal structure of the trimolecular complex of drug/MHC/TCR.

References

1. Illing, et al. (2012). Nature.486:554-8.
2. Ostrov, et al. (2012). Proc Natl Acad Sci USA.109:9959-64.
3. White KD, et al. (2015). J Allergy Clin Immunol. 136: 219-234
4. Pavlos, et al. (2015). Annu Rev Md.66:439-54.
5. Jing et al. (2016). J Immunol. 122:654-673

IIID Public Presentations

Correspondence:
a.redwood@iiid.Murdoch.edu.au, elizabeth.j.phillips@vanderbilt.edu