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

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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 tricomplex 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 complex 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 mechanism by which TCR contacts MHC/drug/peptide remains unknown and is the subject of this study.

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).

Results
CBZ specific T-cell lines and clones were produced from an HLA-B*57:01 SJ5/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 HSR. Shown in Figure 3 are representative flow plots showing T-cell reactivity to CBZ.

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 TCRs will be used to define the structure of the tricomplex of the drug/MHC/TCR.

References

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