In search of a virotope: Identification of herpesvirus specific CD8 T cells

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Introduction. Immunologically-mediated adverse drug reactions (IM ADRs) cause significant economic burden, and patient morbidity and mortality. Many severe T-cell mediated reactions are restricted by class I and/or II HLA alleles. The rapid onset and tissue specificity of some IM-ADRs is indicative of resident effector memory T cell involvement, perhaps specific for persistent pathogens such as herpes simplex virus (HSV). In collaboration with others we have shown that hypersensitivity to the drug abacavir is mediated by alterations to the repertoire of self-peptide bound to HLA risk alleles and tissue specificity of reactions such as carbamazepine (CBZ) induced Stevens-Johnson-syndrome/toxic epidermal necrolysis (SJS/TEN) associated with HLA-B*15:02, we propose a "heterologous immunity/altered peptide model" that requires the identification of the HLA-B*15:02 restricted virotope (foreign peptide) as the first step to identifying cross-reactivity with self-peptide in the presence of CBZ.

Methods.

Global ORF screening

Global CD8 T cell response to HSV-2 were assessed by ORFeome screening, as shown in Figure 1 below, to identify ORF targets of patient derived CD8 T cells.

ORFeome screening

1. HSV-2 genes cloned into expression vectors
2. HSV-2 specific CD8+ T cells activated via cross-presentation
3. Activated (CD137+) CD8+ T cells sorted and expanded to generate HSV-2 specific CD8+ T cell lines
4. T-cell lines screened for reactivity to HSV2 ORF
   a) Subject specific HLA
   b) An individual HSV-2 ORF

Epitope identification. Epitopes within candidate ORF were identified by a combination of overlapping peptide screening or epitope prediction. Candidate epitopes were screened using the expanded CD8 T cell lines above, plus peptide. Readout for T cell responses was ELISA for IFN-γ.

Results.

Global ORF screening. A CD8 T cell line from a B*15:02 patient with a history of SJS/TEN was produced following T cell stimulation with UV inactivated HSV-2 and GM-CSF induced PBMC derived DC. Activated (CD137+) CD8 T cells were sorted and expanded by repeated rounds of non-specific stimulation (PMA followed by anti-CD3). This line, maintaining at least 15% HSV-2 specificity was used to screen the full complement of HSV-2 ORF. Figure 2 shows strong T cell reactivity to epitopes within UL20, UL23 and UL27.

Herpesvirus core genes are highly conserved. Therefore the capacity of HSV-2 specific T cells to cross react against HSV-1 and VZV epitopes was assessed (note this patient was HSV-2 and VZV seropositive but HSV-1/seronegative).

Future: Tetramer study of this? TCR NGS of bulk line see if we can find Taiwan