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Poster presentation

P16-56 LB. Novel tetramer technology for the detection of high affinity CD8 T cells

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Background

Defining the quality of HIV T cell responses is a major hurdle in the development of T cell based vaccines. A key determinant of viral control is the affinity of the T Cell Receptor (TCR) for the HLA/epitope complex. We report for the first time in HIV the development of Class I HLA tetramers, which allow detection of CD8 with high TCR affinity that may prove to be invaluable in assessing the quality of T cell immunity.

Methods

HLA Class I molecules A*0201 and B*0702 were mutated at positions D227K and T228A to nullify CD8 binding and refolded with HIV epitopes: SLYNTVATL (A*0201), ILKEPVHGV (A*0201) and GPGHKARVL (B*0702). BIACORE confirmed abolition of CD8 binding and HLA molecule conformation. Mutated HLA monomers were termed CD8null. Peripheral blood mononuclear cells from HLA-matched acute patients in the SPARTAC trial (n = 30) were stained with A*0201 or B*0702 CD8 wild-type and CD8null tetramers. Real-time Image Flow Cytometry, directly examined the CD8null and CD8 wild-type tetramer/TCR interaction on an individual cell level.

Results

HLA Class I A*0201 and B*0702 CD8 null monomers had undetectable CD8 binding. Wild-type monomers had comparable CD8 binding capacity for A*0201 and B*0702 (KD = 5.9 × 10-4 and 6.0 × 10-4 M, respectively). Both CD8wild-type and CD8null monomers are bound by the anti-HLA w6/32 antibody with equal affinity (KD = 4.2 × 10-10 M). Ex vivo imaging showed slower internalisation of CD8null, compared to CD8wild-typetetramers, indicating prolonged HLA/TCR interaction. HIV patients stained with CD8null and CD8wild-type had distinct high affinity CD8 populations for A*0201 SLYNTVATL and ILKEPVHGV ($p < 0.05$) but not for B*0702 GPGHKARVL.

Conclusion

CD8null tetramers represent a novel technology that allow the direct ex vivo detection and characterisation of high affinity CD8 T responses. This represents a crucial new tool for assessing the quality of T cell responses to vaccination.