Isolation of NY-ESO-1-specific T cell receptors restricted by non-HLA-A*02:01 allotypes for TCR gene therapy

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Background: To date, application of T cell receptor (TCR) gene therapy has been mostly limited to patients with the HLA-A*02:01 allotype since suitable TCRs with this restriction specificity have been more readily available. To extend TCR gene therapy to patients with other HLA allotypes requires isolation of suitable TCR recognizing tumor-associated antigens (TAA) presented by other MHC alleles. We have shown in previous studies that non-selected T cell repertoires still having high-avidity T cells for self-antigens can be tapped by presenting TAA-derived peptides to responding T cells of healthy donors by allogeneic MHC molecules. Our approach to obtain allo-restricted, peptide-specific T cell clones concentrates on dendritic cell (DC) priming using RNA-loaded DC as antigen-presenting cells. After successfully obtaining HLA-A*02:01-restricted TCRs specific for tyrosinase, MART-1, survivin, and HMMR, among others, we have begun the search for TCRs restricted by non-A2 alleles, with our first interest directed to the HLA-Cw*06:02 and HLA-Cw*07:02 allotypes. The cancer/testis antigen NY-ESO-1 was chosen as our first candidate TAA for two reasons: NY-ESO-1 has a restricted expression in normal tissues but is overexpressed in numerous cancers and it has been shown to be a suitable antigen for TCR gene therapy using an HLA-A*02:01-restricted TCR.

Methods: Naïve CD8-enriched T cells were primed using autologous DC electroporated with RNA encoding the allogeneic MHC-molecules HLA-Cw*06:02 or HLA-Cw*07:02 and the TAA NY-ESO-1. After seven days, T cells were stimulated again with RNA-loaded DC and 2 weeks later specific T cells were enriched via CD137, a marker that is upregulated upon T cell response to antigen, since specific epitope knowledge was not available. After limiting dilution cloning, specificities of emerging T cell clones and lines were tested in co-cultures with autologous RNA-loaded DC and allogeneic LCL cell lines sharing one or more MHC-molecules, using IFN-γ ELISA as read-out.

Results: T cell priming using DC loaded with RNA encoding HLA-Cw*06:02 and NY-ESO-1 resulted in isolation of unspecific or allo-reactive T cell clones that recognized HLA-Cw*06:02 molecules as foreign alloantigens, independent of specific NY-ESO-1 peptide, but allo-HLA-Cw*06:02-restricted NY-ESO-1 peptide-specific T cell clones were not found. In our DC priming approach, NY-ESO-1 RNA is processed and epitopes are also presented by autologous MHC-molecules of the DC. Indeed,we isolated one HLA-A33-self-restricted T cell clone that recognizes a NY-ESO-1 epitope that lies in the first 44 amino acids. In contrast to HLA-Cw*06:02, the preliminary screening for HLA-Cw*07:02-allo-restricted clones has shown one candidate to be of potential interest, which is under further characterization.

Conclusions: The combination of DC priming and CD137 enrichment of responding T cells offers an unbiased method to build a library of TCRs for adoptive T cell therapy that goes beyond the common HLA-A*02:01 allotype. While HLA-C alleles represent interesting restriction specificities because of their high prevalence in various populations, the frequency of responding T cells seems to be very low compared to our experience with HLA-A2-restricted T cells. Nevertheless, the rare T cell clones that can be found may open the door for future inclusion of more patients in studies of TCR gene therapy.