# Abstract

GT-specific sequence differences are certainly important in the antiviral immune response against HCV. The present study for the first time systematically analyzed the degree of cross-GT reactivity of HCV-specific T cells at the epitope level. To determine the impact that these GT-specific mutations have on the CD8+ T cell responses we analyzed a group of IVDUs including subjects with undetectable viremia and individuals with HCV GT1 and HCV GT3 infection. Overlapping peptide sets covering local consensus sequences from HCV GT1b and 3a were used to analyze the immune responses of each individual. The total strength and number of HCV-specific T cell responses detected were higher in the HCV-RNA negative group when compared to the GT1 or GT3 infected groups. Interestingly in the chronically infected subjects CD8+ T cells preferentially targeted the heterologous GT. These responses more possibly are memory CD8+ T cells of a previously resolved heterologous infection.

Cross-reactive CD8+ T cells are a high precious repertoire for HCV protection against heterologous infections. In this work we wanted to identify cross-reactive peptides that could be useful for the development of a broad-protective vaccine. 22% of the 28 epitopes identified was cross-reactive. This includes epitopes where the targeted sequence was identical in GT1 and GT3 and epitopes that targeted both GT-specific variants. Although the majority of these cells showed only limited cross-reactivity, we were able to identify a number of subjects with T cells active against both genotypes. Interestingly, T cells active against both genotypes were preferentially detected in HCV-RNA negative subjects. This demonstrates that CD8 responses targeting different HCV genotypes can be primed in the same individual and that these responses are potentially linked to protection from chronic infection. In the face of a heterogeneous genotype distribution in many areas of the world, this has important implications for vaccine design.

To define the determinants of a successful versus an unsuccessful CD8+ T-cell response in HCV infection we decided to perform phenotypic studies on memory HCV-specific CD8+ T cells. We evaluated the expression level of the memory marker CD127 and of the pro-apoptotic protein Bim in individuals with different clinical outcomes to highlight the role that peripheral deletion of peptide-specific CD8+ T cells could have on the paucity of CD8+ T cell responses in chronically infected subjects. During our work it became evident that the autologous sequence of the circulating virus had a strong influence on the phenotype of specific CD8+ T cells. Upon spontaneous clearance of an infection, virus-specific cells typically acquire a memory phenotype with upregulation of the memory marker CD127. In line with this specific CD8+ T cells from HCV-RNA negative IVDUs showed high CD127 expression. Interestingly, patients with an autologous

sequence - that differed from the prototype epitope sequence and that was not recognized by the CD8+ T cells - also showed upregulation of CD127. We expected that the latter group of CD8+ T cells would be continuously activated in the liver and potentially also have upregulation of the pro-apoptotic molecule Bim. This would also explain the difference not only in the quality of HCV-specific CD8+ T cells but also in the quantity of CD8+ T cells between patients with chronic HCV infection and those who spontaneously resolved the infection. Unfortunately we were unable to identify any differences in the expression levels of Bim between the different patients groups here analyzed even when we controlled for the presence of the correct antigen. The number of CD8 responses that have been analyzed is still rather low. It will therefore be necessary to increase the number of patients for this analysis before coming to any final conclusions.

The last part of this study was focused on the establishment of an assay for the priming of naïve CD8+ T cells with HCV immunodominant epitopes. For this purpose monocytes from healthy individuals were matured into DCs and co-cultured with autologous PBMCs in presence of the immunodominant HCV-A2-peptide 1073. The successful detection of HCV-specific T cells in polyclonal cultures was analyzed through IFN-γ staining and MHC class I-Pentamer staining. CD8+ T cells are thought to be one of the major responsible for clearance in HCV infection, therefore the establishment of a peptide-specific functional CD8+ T cell line is an interesting tool that could be applied as adoptive immune transfer for example after liver transplantation to prevent reinfection of the allograft.