Editorial

Priming persistence of HCV

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Acute infection with hepatitis C virus (HCV) is cleared in about 30% of cases with $CD8^+$ T-cells being the main effector cells in viral elimination. Yet, about 70% of patients progress to chronic HCV infection that can result in chronic immunopathology. This immune-mediated liver diseases can progress to liver cirrhosis and ultimately to hepatocellular carcinoma.

Chronic HCV infection is characterized by an impaired virus-specific $CD8^+$ T-cell response caused by several viral and host factors. However, the relative contribution of those and the exact mechanisms of $CD8^+$ T-cell failure are still not fully illuminated. Viral escape and $CD8^+$ T-cell exhaustion due to prolonged antigen exposure, for example, cause impaired virus-specific $CD8^+$ T-cell responses and therefore contribute to HCV persistence. Exhausted HCV-specific $CD8^+$ T-cells are characterized by impaired effector functions and the co-expression of inhibitory receptors such as PD-1, 2B4 and KLRG1 (Figure.1). In contrast, HCV-specific $CD8^+$ T-cells that target epitopes that underwent viral escape mutations express fewer inhibitory receptors and are marked by CD127 expression [1] (Figure 1). Importantly, the identification of these distinct $CD8^+$ T-cell phenotypes as markers for different mechanisms of $CD8^+$ T-cell failure (viral escape versus $CD8^+$ T-cell exhaustion) was based on analyses performed in patients with HCV-specific $CD8^+$ T-cell populations that were detectable directly *ex vivo* by conventional peptide/MHC multimer staining. However, HCV-specific $CD8^+$ T-cells are not detectable in most chronically HCV-infected patients by this method. The lack of *ex vivo* detectable HCV-specific $CD8^+$ T-cells in the majority of chronically HCV-infected patients has raised the question whether this is due to technical constraints or to the absence of HCV-specific $CD8^+$ T-cells in these patients.

Recently, a protocol adopted from mouse studies [2] elegantly combined peptide/MHC tetramer staining and magnetic bead enrichment to detect and analyze rare epitope-specific $CD8^+$ T-cells *ex vivo* not only in infected individuals, but also in unexposed donors [3, 4]. Using this approach Nitschke *et al.* [5] were able to enrich HLA-A*02:01-restricted HCV-specific $CD8^+$ T-cells specific for two different viral epitopes in seventeen analyzed patients as well as in twelve uninfected individuals. Hence, HCV-specific $CD8^+$ T-cells were present in all chronically HCV-infected patients. These results indicate that the absence of virus-specific $CD8^+$ T-cells does not contribute to HCV persistence in this cohort. As expected, chronically HCV-infected patients with virus-specific $CD8^+$ T-cell responses that have been detectable directly *ex vivo* showed significantly higher frequencies of enriched HCV-specific $CD8^+$ T-cells compared to patients without virus-specific $CD8^+$ T-cell responses detectable by conventional peptide/MHC multimer staining methods (enriched detectable). Yet, enriched detectable HCV-specific $CD8^+$ T-cell populations were more frequent in chronically HCV-infected patients than HCV-specific $CD8^+$ T-cell precursor populations in healthy donors. A detailed phenotypic characterization of enriched HCV-specific $CD8^+$ T-cells revealed that HCV-specific $CD8^+$ precursor T-cells obtained from healthy donors predominantly displayed a naïve phenotype ($CD45RA^+CCR7^+CD27^+$). In contrast, directly detectable virus-specific $CD8^+$ T-cell populations in chronically HCV-infected patients had an antigen-experienced phenotype ($CD45RA^-CCR7^-CD27^+$). Interestingly, enriched detectable HCV-specific $CD8^+$ T-cell populations had a highly heterogeneous differentiation profile with an antigen-experienced phenotype in two third and a predominantly naïve-like phenotype in one third of chronically HCV-infected patients. In addition, enriched HCV-specific $CD8^+$ T-cells with an effector memory phenotype also expressed PD-1 according to previous data obtained with directly detectable HCV-specific $CD8^+$ T-cells that have been defined as exhausted. Notably, the presence of both

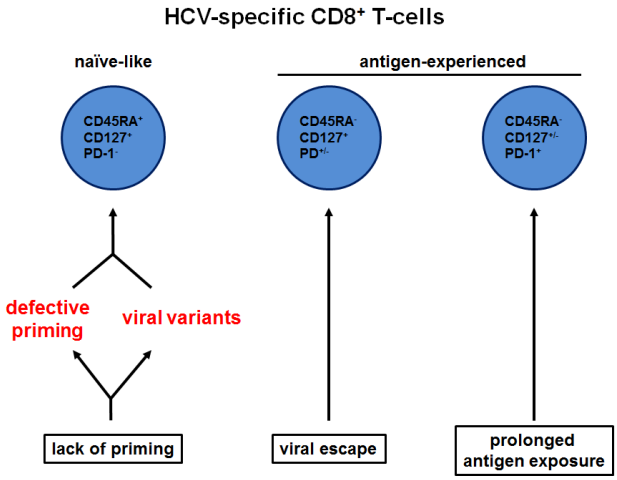

Figure 1: Mechanisms underlying HCV persistence reflected by $CD8^+$ T-cell differentiation. Beside viral escape and prolonged antigen exposure, lack of priming can cause impaired $CD8^+$ T-cell responses and viral persistence in chronic HCV infection. These different mechanisms are reflected by diverse differentiation states of HCV-specific $CD8^+$ T-cells.

antigen-experienced and naïve-like phenotypes in different HCV-specific CD8$^+$ T-cell populations within the same patients argues against a role of host genetic factors.

In a next series of experiments, the authors analyzed the functionality of enriched detectable HCV-specific CD8$^+$ T-cells after co-culture with peptide-pulsed autologous monocyte-derived DC. As expected, enriched detectable HCV-specific CD8$^+$ T-cells with an antigen-experienced phenotype accompanied by high PD-1 expression did not expand after co-culture, indicating CD8$^+$ T-cell exhaustion. Of note, bona fide virus-specific CD8$^+$ T-cell memory populations should expand upon antigen-specific stimulation. Surprisingly, only two of five naïve-like enriched detectable HCV-specific CD8$^+$ T-cell populations expanded and produced effector cytokines such as IFN-γ and TNF and mobilized CD107a, a surrogate marker for degranulation. Those two patients harbored viral escape variants carrying mutations in the corresponding HCV epitope. Consequently, stimulation with the autologous variant peptide sequence did not lead to recognition and thus to successful priming of the naïve-like HCV-specific CD8$^+$ T-cells in these two patients. This indicates an initial infection with a variant HCV strain, since viral escape occurring at later time points during infection would engender HCV-specific CD8$^+$ T-cells with a non-naïve phenotype (Figure 1). In contrast, the three enriched detectable naïve-like HCV-specific CD8$^+$ T-cell populations that did not expand upon *in vitro* priming indicate a functional impairment in priming of naïve-like HCV-specific CD8$^+$ T-cells. In sum, these results indicate that in addition to viral escape and CD8$^+$ T-cell exhaustion lack of priming may also represent a mechanism for HCV-specific CD8$^+$ T-cell failure in chronically infected patients promoting viral persistence. However, it remains to be elucidated whether intrinsic CD8$^+$ T-cell defects account for this phenomenon or whether impaired activation by DC, lack of CD4$^+$ T-cell help or the presence of immunomodulatory cytokines such as IL-10 or TGF-β may play a role. It will also be important to analyze whether similar observations can be made in other chronic infections, such as chronic HBV infection, to gain further insights into the contribution of priming defects to CD8$^+$ T-cell impairment and viral persistence in general.

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