

ImmTAV, a new Immunotherapy targeting the source of HBV infection.

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Several therapeutic options have been developed to improve our ability to cure chronic HBV infection(1). In this issue of Hepatology, Fergusson et al describe the development of HBV-specific “Immune mobilizing monoclonal T cell receptors Against Virus” (ImmTAV), a novel and elegant immune therapeutic strategy designed to substitute and potentially restore HBV-specific CD8T cells in chronic Hepatitis B (CHB) patients.

Before explaining their structure, specificity and mode of action, it is important to summarize the role of CD8T cells in HBV infection and why a considerable number of immune-based therapies are targeting them.

CD8T cells represent the component of our immune system that has evolved to selectively recognize and eliminate virus infected cells, the source of viral replication within the infected host. The approach of CD8T cells to recognize the virus differs completely from the one of antibodies. Antibodies (particularly the so-called neutralizing antibodies) have the primary scope to prevent viral infection by blocking the interaction of the virus with their specific receptor on the cell surface. Therefore, antibodies recognize the antigenic conformation of viral proteins expressed on the virions. Instead, CD8T cells recognize short fragments of viral antigens presented on the surface of the infected cells by HLA-class I molecules. The recognition of each HLA-class I/viral-peptide complex is mediated by a specific T cell receptor (TCR). The presented viral peptide (usually 9-10 AA long) results from the processing of viral proteins actively synthesized within the infected cells. This feature is extremely important as it ensures that CD8T cells can distinguish between virus infected or non-infected cells and thus eliminate the primary source of the infection from the host (2). Thus, the presence of functionally active HBV-specific CD8T cells is important for HBV control.

Functional HBV-specific CD8T cells have been demonstrated in chimpanzees able to control HBV and in patients who resolved acute hepatitis B (reviewed in (1)). In contrast, quantitative and functional defects of HBV-specific CD8T cells are the hallmark of CHB and many different therapeutic approaches are currently trying to restore their defects(3).

Such therapeutic strategies can be divided into two broad categories: the first includes those designed to augment the function and quantity of the few remaining HBV-specific T

cells that have escaped deletion induced by persistent HBV antigen presentation. The typical representative of this group is anti-PD1/PD-L1 antibody therapy aiming to restore exhausted HBV-specific T cells, which has shown some therapeutic effect in selective patients(4). In addition, therapeutic vaccines are also designed to restore an endogenous HBV-specific T cell response. The second category is exemplified by therapies like CAR/TCR-engineered T cells, that have been developed to re-create "tout court" a new, functional source of HBV-specific T cells. Such therapies have proven their potential in animal models(5) and are waiting to be tested in patients.

The "Immune mobilizing monoclonal T cell receptors" described by Fergusson et al., are a hybrid between these two categories. They are not engineering cells ex vivo, but instead are using a soluble HBV-TCR to compensate for defective HBV-specific CD8T cells and, through the engagement with CD3, employ the global T cell population (non HBV-specific) present in the patients that is not affected by functional defects(6).

Indeed, the Immune mobilizing monoclonal T Cell Receptors Against Virus (called ImmTAV molecules) are, as described by the authors, "soluble bispecific T cell engaging fusion proteins comprised of an affinity enhanced TCR... fused to a humanized anti-CD3 single chain antibody variable fragment." In other words, the variable region of the TCR, that specifically recognize a viral peptide-HLA complex, is fused with an antibody variable region that binds CD3 molecules on the surface of T cells. Such products have already been developed for the recognition of HIV-latently infected cells(7). Here, Fergusson et al., first produced three ImmTAV molecules specific for HLA-A*02:01 restricted HBV epitopes derived from the viral core, polymerase and envelope proteins,. These distinct TCRs are derived from TCR phage display libraries and from T cells isolated from healthy donors. Their affinity for their target (HLA-class I viral peptide complex) was enhanced by a phage display method developed by the authors. After a functional test, consisting of an in vitro analysis of the ability of the ImmTAV to recognize target cells pulsed with different HBV peptide concentrations, the authors selected the ImmTAV specific for an envelope epitope, as it showed the ability to stably bind (more than 12 hours) target cells pulsed with picomolar concentration of the envelope peptides, and given the appeal of targeting HBsAg/envelope epitopes to eliminate the source of ongoing antigen production.

A series of experiments was then performed to demonstrate that ImmTAV-Env can recognize target cells that were not peptide-pulsed but that endogenously synthesized the envelope proteins, both from integrated HBV-DNA or directly from HBV infection. Such data obtained using Hepatoma-like cell lines are a necessary step, since they suggest that in vivo, ImmTAV-Env will be able to recognize hepatocytes with integrated HBV-DNA coding for the envelope protein or HBV infected hepatocytes; cells that notoriously express very low levels of HLA-class I. The stable binding of the ImmTAV-Env to the HBV antigen expressing hepatoma cells (PLC/PRF/5 A2B2M) allowed the anti-CD3 portion of the ImmTAV molecule (see Figure 1) to activate different families of T cells. Interestingly, the ImmTAV molecule activated CD8, CD4, MAIT and gamma delta T cells and their activation was demonstrated to specifically eliminate HBV-infected cells.

Thus, these data suggest that a new method to overcome the defect of HBV-specific T cell immunity present in CHB has potentially been developed, which now needs to be tested in patients to really understand its efficacy. This last step is still missing, and the authors did not analyze the ability of ImmTAV-Env to redirect T cells towards HBV infected target cells in animal models in vivo. This is, however, not an easy task, since it requires the use of a chimeric mouse model reconstituted not only with human HLA-A*02:01 HBV-infected hepatocytes but also with HLA-matched T cells, to avoid alloreactivity between human hepatocytes and T cells. I am not convinced that such efforts would be informative since such animal models could not, in any case, recapitulate the complexity and heterogeneity of chronic HBV infection, nor the complexity of the human immune system. Moreover, the number of infected hepatocytes and level of fibrosis, features that can affect the accessibility of T cells to infected hepatocytes(8), will greatly differ among patients and could affect the efficacy of this novel therapy.

Like all therapies aiming to boost the immune system in CHB patients, the difficulty of implementing ImmTAV-Env, will be to find the level of lysis of infected hepatocytes that is therapeutically necessary to achieve functional cure without compromising liver function. In this respect, new findings have provided some hints that the danger of triggering severe liver damage might be manageable: the number of infected hepatocytes within a chronically HBV infected liver appears to be robustly reduced by NA therapy(9) and the presentation of HBV-epitope peptides on infected hepatocytes in vivo is not

homogeneous(10). Even though such data are derived from a small cohort of CHB patients, they start to provide evidence that therapies targeting HBV-infected hepatocytes are unlikely to affect all liver parenchyma. As such, it might be safe to evaluate different posologies of the product in patients.

The ultimate goal of immune therapy is to eliminate not only infected hepatocytes but also hepatocytes with HBV-DNA integration, which favors regions coding for HBV envelope. However, it will be crucial to proceed with utmost caution, to minimize the risk of decompensation. While in my opinion the safest strategy might be to start by targeting only infected hepatocytes through core or polymerase epitopes, targeting both infected cells and those with HBV DNA integration via Env epitopes could be achieved safely by implementing a conservative clinical strategy and may have the advantage of a significant response. Regardless, a step by step approach is still needed to demonstrate that such antigen-specific T cell-based therapies are safe to be tested in CHB patients.

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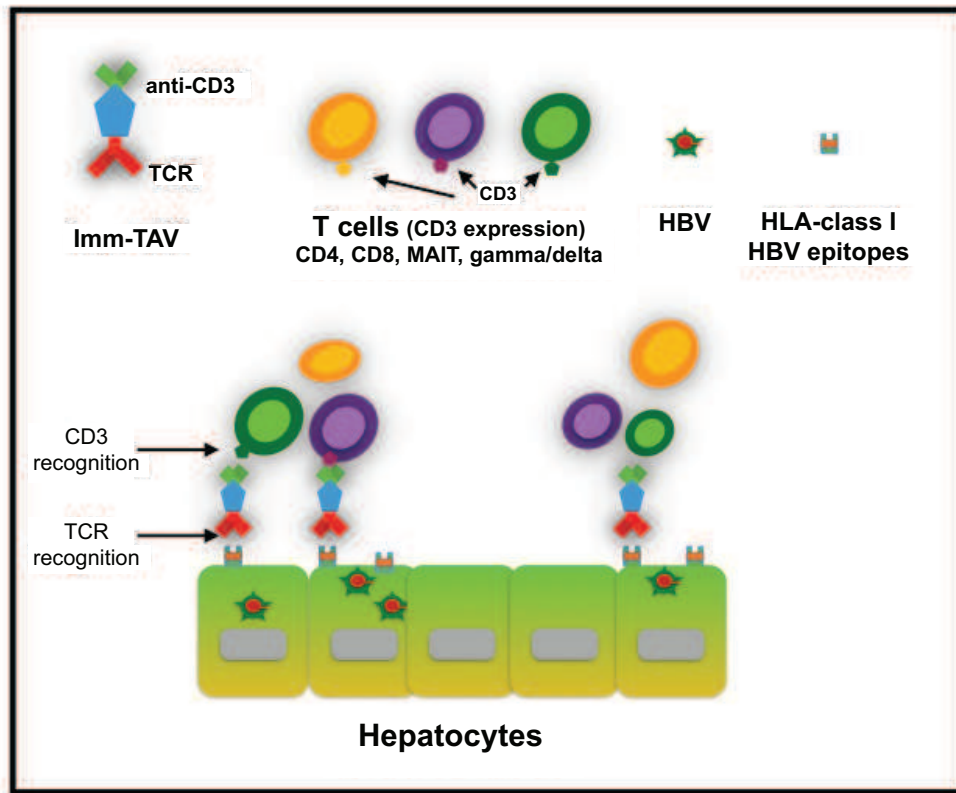


Figure 1. Schematic representation of Imm-TAV. The Immune mobilizing monoclonal T Cell Receptors Against Virus (Imm-TAV) is a soluble bispecific T cell engaging fusion proteins comprised of an affinity enhanced TCR fused to a humanized anti-CD3 single chain antibody variable fragment. The Imm-TAV specific for HBV envelope epitope recognises the complex HBV-envelope epitope/HLA-class I molecule display on the surface of HBV infected hepatocytes. It redirects and activated T cells expressing CD3 (CD4, CD8, MAIT, Gamma/delta) towards them.

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