

## Specific elimination of Hepatitis B virus infected hepatocytes by modified human T cells which express chimeric T-cell receptors and establish a cytotoxic T-cell response

For a proportion of patients, HBV infection results in chronic disease with severe consequences like liver cirrhosis or hepatocellular carcinoma. This results from a weak and oligoclonal T-cell response targeting only limited HBV epitopes. A major goal in the therapy of chronic HBV infection is the elimination of the episomal replication template, the so called covalently closed circular DNA (cccDNA). The therapeutic approaches are inhibitors of viral replication (interferon alpha) or inhibitors of viral reverse transcription (Lamivudin). By these means replication is controlled partially but cccDNA persists, generating a relapse of infection after therapy has ceased. A possible immunotherapeutical approach for a successful treatment could be achieved by receptor modified T-cells inducing an artificial T-cell response. Therefore, T-cells were equipped with recombinant T-cell receptors, using single chain antibody fragments (scFv) for antigen recognition and hence being independent of peptide antigen presentation via MHC-molecules. The chimeric T-cell receptors, generated in this work, are directed against two HBV surface proteins, which are present on the surface of infected cells, as a result of virus production and secretion. Modified T-cells were activated upon antigen recognition resulting in the generation of a T-cell receptor signal and a costimulatory signal provided by a modular signalling domain. HBV-infected primary human hepatocytes were specifically eliminated and the episomal replication template was removed. Furtheron activation led to the secretion of proinflammatory interferon gamma and of interleukin-2. This generated a proliferative signal inducing an antigen specific expansion of receptor modified T-cells. Apoptosis of target cells was mediated through degranulation of perforin and granzyme containing vesicles and activation of NFkappaB and proapoptotic effector caspases 3 and 7 was observed. These results demonstrate that a genetherapeutic modification of T-cells results in specific elimination of HBV infected hepatocytes. Since the therapeutic goal, the elimination of cccDNA was achieved, this system could be a promising alternative to the actual standard therapeutics against HBV.