



**Karolinska
Institutet**

Department of Laboratory Medicine

Immune response during hepatitis B virus infection and reconstitution of HBV-specific immunity using T cells redirected against HBV

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Föreläsningssalen 4Z Alfred Nobels allé 8, Karolinska Universitetssjukhuset, Huddinge

Fredagen den 20 september, 2013, kl 09.00

av

Sarene Koh

Huvudhandledare:

Professor Matti Sällberg
Karolinska Institutet
Institutionen för Laboratoriemedicin

Fakultetsopponent:

Professor Robert Thimme
Universitätsklinikum Freiburg
Abteilung Innere Medizin II

Bihandledare:

Professor Antonio Bertoletti
A*STAR
Singapore Institute for Clinical Sciences

Betygsnämnd:

Professor Johan K. Sandberg
Karolinska Institutet
Centrum för infektionsmedicin (CIM)

Professor Edvard Smith
Karolinska Institutet
Institutionen för Laboratoriemedicin

Professor Jorma Hinkula
Linköping Universitet
Institutionen för klinisk och experimentell
medicin, IKE

Stockholm 2013

ABSTRACT

Hepatitis B is one of the most prevalent viral diseases worldwide and is a major public health concern, particularly in Asia. Of the 350 million people suffering from chronic hepatitis B virus (HBV) infection worldwide, approximately 75% are found in Asia. HBV is a noncytopathic, hepatotropic virus that causes acute and chronic hepatitis and hepatocellular carcinoma (HCC). Virus-specific T cells have been found to be associated with the control of HBV infection, but have also been implicated as the principal effectors of liver damage. Although the mechanisms responsible for liver inflammation have been nicely demonstrated in animal models, we still lack evidence for these events in humans. We therefore studied the role of HBV-specific CD8 T cells in different phases of HBV infection. Analysis of the kinetics of innate and adaptive immune activation during hepatic flares in chronic hepatitis B revealed that the rebound of HBV replication following therapy withdrawal occurred without triggering any innate immune activation, with the exception of increased serum CXCL-8. Hepatic flares were temporally associated with high serum levels of the IFN- γ inducible chemokines CXCL-9 and CXCL-10. Both were differentially produced in liver injury present in acute or chronic patients and displayed different *in vitro* requirements for activation. The inflammatory potential of virus-specific T cells was characterized by analysing their ability to produce IL-17 and CXCL-8 during different phases of HBV infection. We also determined whether cytokines present in the liver during inflammation (IL-7 and IL-15) could license virus-specific T cells with additional cytokine profiles that contribute to tissue inflammation. Our results showed that HBV-specific T cells produced CXCL-8, but not IL-17, during periods of liver inflammation in acute or chronic patients. HBV-specific T cells producing CXCL-8 could be expanded from acute/resolved patients in the presence of IL-7 and IL-15, suggesting that virus-specific T cells can acquire through exposure to environmental factors, a cytokine/chemokine profile capable of contributing to parenchymal inflammation.

Despite that today's antiviral drugs efficiently decrease HBV viral load to undetectable levels, they fail to eradicate infection due to the persistence of HBV covalently closed circular DNA in hepatocytes. Long-term treatment is also expensive, and may result in problems of toxicity and emergence of resistant viruses. Attempts to restore HBV-specific immunity in chronic patients with therapeutic vaccines have had little success. We therefore developed a new strategy based on T cell receptor (TCR) gene transfer to reconstitute the defective antiviral immunity of chronic patients. DNA encoding the HBV-specific TCR alpha/beta chain was cloned from immunodominant HBV-specific CD8 T cells from acute/resolved patients. The TCR genes were transferred to primary human T cells using retroviral transduction or mRNA electroporation. Transgenic TCRs were efficiently expressed on T cells of chronic HBV/HCC patients and reconstituted fully functional HBV-specific T cells. Furthermore, these TCR-redirected T cells recognized and lysed natural HCC lines with HBV DNA integration. Despite a transient functionality, the TCR mRNA electroporated T cells efficiently prevented tumor seeding and suppressed the growth of established tumors in a xenograft model of HCC. Overall, we developed a method that represents a practical approach to cell therapy of HCC and its inherently self-limiting toxicity suggests potential for application in other HBV-related pathologies.