Research project objectives/Research hypothesis

Infections with hepatitis C virus, an etiologic factor of chronic hepatitis C concern approximately 170 million of individuals. They represent an important epidemiological problem, since chronic infection may lead to severe clinical consequences, including liver cirrhosis and hepatocellular carcinoma. Subclinical course of infection, limited availability of antiviral treatment, especially in highly endemic areas as well as absence of effective vaccines cause further problems with the eradication of the virus. Increasingly observed HIV-1/HCV coinfection worsens the prognosis for long-term survival and outlook for anti-HCV treatment success.

Adaptive immune responses related to activity of cytotoxic T cells play a critical role in the clinical course of infection with hepatitis C virus (HCV). Approximately 20-50-% of infected persons spontaneously eliminate the virus, mainly due to multispecific response of these cells. In contrast, HCV-specific cytotoxic T cell responses in chronic hepatitis C are functionally exhausted which manifests as decline in pro-inflammatory molecules release (IFN-γ, TNF-α and IL-2), impaired elimination of infected cells, and decreased proliferative potential after virus recognition. These disturbances, mediated by continuous HCV stimulation of the immune system, progress along with time of infection and are accompanied by characteristic changes, i.e. expression of PD-1 and Tim-3 receptors, on total and HCV-specific T cells, which have been shown to inhibit ability to proliferate, eliminate infected cells and secrete pro-inflammatory molecules. Moreover, T cell exhaustion in HCV infection is related to the increase in anti-inflammatory molecule secretion, i.e. IL-10.

To date, studies of T cell exhaustion in HCV infection were focused on the mechanisms, reversibility of this phenomenon and its impact on the development of chronic infection, whereas other consequences are very scarcely investigated. Most of all, the relationship between T cell exhaustion and heterogeneity of immunogenic fragments of HCV proteins (epitopes) recognized by T cells, is unraveled. Furthermore, it is uncertain how anti-HCV treatment modifies T cell exhaustion.

The aims of the present study are to assess:
1) the relationship between the extent of T cell exhaustion and the extent of heterogeneity of HCV epitopes recognized by T cells, in patients infected with HCV and HIV-1/HCV;
2) antiviral therapy effect on T cell exhaustion in patients infected with HCV and HIV-1/HCV.

Research project methodology

The study will involve 100 patients with chronic HCV infection, and 50 patients with HIV-1/HCV coinfection qualified for hepatitis C therapy. Exhaustion markers will be assessed before treatment and six months after treatment (evaluation of response to treatment).
T cells exhaustion be assessed using:
1) analysis of gene expression of inhibitory receptors (PD-1, Tim-3) and IL-10 in blood leukocytes;
2) analysis of expression of inhibitory receptors PD-1 and Tim-3 on total and HCV-specific T cells from peripheral blood;
3) analysis of IL-10 levels in plasma.

Heterogeneity of HCV T cell epitopes (within NS3/4a protein) will be evaluated by next-generation sequencing (NGS), a relatively new technique, which allows for parallel sequencing of millions of genes. Next, heterogeneity parameters of reconstructed epitope variants will be assessed by bioinformatics programs. Differences in expression of exhaustion markers will be compared with differences in heterogeneity of epitopes and hepatitis C treatment outcome.

Reasons for undertaking the topic of the study

Recent developments in molecular biology create new possibilities for studying immunological aspects of infections as well as molecular features of viruses. Pathogenic and clinical consequences of HCV-induced T cell exhaustion and their relationship with viral heterogeneity are poorly understood, but may be a relevant factor determining a course of chronic HCV infection. Better understanding of these consequences may be of significant importance and in a larger perspective, could be translated into individualized treatment programs and vaccine design. This would decrease the burden of HCV infections related to drug side effects, treatment costs, hospitalizations and premature deaths.