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Analiza zależności pomiędzy wyczerpaniem komórek T, zmiennością genetyczną epitopów wirusa zapalenia wątroby typu C (HCV) rozpoznawanych przez komórki T, a leczeniem przeciwwirusowym względem HCV

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

STRESZCZENIA

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Summary

Analysis of relationships between T-cell exhaustion, genetic heterogeneity of hepatitis C virus (HCV) epitopes recognized by T-cells and anti-HCV treatment

During chronic hepatitis C (CHC), caused by hepatitis C virus (HCV) infection, T-cell functions become exhausted, which is manifested in decline in effector cytokines release, impaired elimination of infected cells, and decreased proliferative potential. This phenomenon is mediated by continuous antigenic stimulation, progresses along with time of infection and is accompanied by the expression of "inhibitory" receptors, including: PD-1 (programmed cell death protein 1) and Tim-3 (T-cell immunoglobulin and mucin domain containing protein 3) on total and HCV-specific T-cells. These receptors inhibit T-cell activation of upon antigen recognition. In addition, there is an increase in the secretion of anti-inflammatory cytokines, e.g., IL-10.

To date, studies on T-cell exhaustion in chronic hepatitis C were focused on characterization of this phenomenon, whereas it is still largely unknown whether T-cell exhaustion may be determined by HCV immune epitope variant. It is also uncertain how anti-HCV treatment modifies T-cell exhaustion.

The aims of the PhD thesis were:

1) to analyze of the relationship between T-cell exhaustion and variability of HCV epitopes recognized by $CD8^+$ T-cells;

2) to assess the effect of successful anti-HCV therapy on T-cell exhaustion.

The study material included whole blood of 97 patients with chronic hepatitis C, qualified for therapy with direct antiviral drugs. Samples were collected before the start of therapy and six months after its completion. Controls comprised blood from 18 healthy controls (anti-HCV^-). Exhaustion markers (PD-1 and Tim-3 expression on total and HCV-specific T-cells) were determined by multiparametric flow cytometry, while plasma IL-10 levels were assessed by ELISA. The genetic variability of HCV T-cell epitopes was assessed by next-generation sequencing (Illumina).

Aim 1

The results showed a relationship between the sequences of the analyzed epitopes and the phenotype of $CD8^+$ T lymphocyte exhaustion. Infection with an epitope $NS3_{1406}$ sequence not representing the HCV 1b prototype (KLSGLGLNAV) or a cross-reactive variant (KLSSLGLNAV, KLSGLGINAV or KLSALGLNAV) was associated with a higher percentage of HCV-specific $CD8^+PD\text{-}1^+Tim\text{-}3^+$ T-cells. Variability (at least two variants) of the $NS3_{1406}$ epitope sequence was associated with an increased percentage of $CD8^+PD\text{-}1^+Tim\text{-}3^+$ peripheral T-cells and a lower percentage of $CD8^+PD\text{-}1^-Tim\text{-}3^-$ T-cells. Infection with a dominant variant of the $NS3_{1073}$ epitope other than the prototype for HCV 1b (CVNGVCWTV) was associated with a lower percentage of peripheral $CD8^+PD\text{-}1^+Tim\text{-}3^+$ T-cells. These results indicate that there is a relationship between the percentage of T-cells expressing PD-1/Tim-3 receptors and the HCV epitopes sequence and their variability. They also suggest the importance of evaluating autologous viral epitope sequence in the investigation of $CD8^+$ T-cell exhaustion in HCV infection.

Aim 2

Before treatment the percentages of peripheral $CD4^+PD\text{-}1^+$, $CD4^+PD\text{-}1^+Tim\text{-}3^+$ and $CD8^+PD\text{-}1^+Tim\text{-}3^+$ T-cells and plasma IL-10 levels were statistically significantly higher, and the percentages of $CD4^+PD\text{-}1^-Tim\text{-}3^-$ and $CD8^+PD\text{-}1\text{-}Tim\text{-}3^-$ lower in patients than in the control group. Treatment resulted in significant decrease in the percentages of $CD4^+Tim\text{-}3^+$, $CD8^+Tim\text{-}3^+$, $CD4^+PD\text{-}1^+Tim\text{-}3^+$ and $CD8^+PD\text{-}1^+Tim\text{-}3^+$ T-cells and plasma IL-10 levels, and concomitant increase in the percentages of the $CD4^+PD\text{-}1^-Tim\text{-}3^-$ and $CD8^+PD\text{-}1^-Tim\text{-}3^-$ T-cells. There was no significant change in the percentage of $CD4^+PD\text{-}1^+$ T cells, while the percentage of $CD8^+PD\text{-}1^+$ T cells significantly increased.

An important additional finding was that patients with advanced liver fibrosis had higher PD-1 as well as lower Tim-3 expression levels on $CD4^+$ T-cells, and treatment had little or no effect on the expression of exhaustion markers in these patients.

The frequency of peripheral HCV-specific $CD8^+$ T-cells has significantly declined after treatment, but the expression level of PD-1 and Tim-3 on these cells remained unchanged.

Based on the above results, it can be concluded that successful treatment of chronic hepatitis C is associated with a reduction in plasma IL-10 levels and a reduction in the immune exhaustion markers expression on T-cells, but this effect was not present in patients with

advanced liver fibrosis. This suggests that long-term chronic HCV infection is related to irreversible changes in the phenotype of these cells.