INFLUENCE OF THE MICROENVIRONMENT OF HEPATITIS B VIRUS-INFECTED HEPATOCYTES ON INNATE IMMUNITY'S MECHANISMS

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HBV virus is an infectious agent that replicates in the hepatocytes and causes an acute form of hepatitis, but it can also lead to a chronical inflammation, and consequently, to a hepatocellular carcinoma. HBV virus is also described as "stealth virus" due to its ability to avoid recognition by the innate immunity system and the immune activation, as it does not cause the immune response[1].

The goal of our project is to discover the immune inhibitory mechanism, as this knowledge can be useful in clinical research.

Currently, our attention is focused on the main interferon α (IFNα) producing cells – plasmacytoid dendritic cells (pDC). After the activation of the pattern recognizing receptors (PRR), pDCs are starting the massive production of pro-inflammatory cytokines, as IFNα, IL-6, and TNFα[2]. As any other inflammatory process, pDC's activation is strictly regulated. It was suggested that miRNA 146 is the element of negative feedback loop to control the cytokine production due to its ability to silence key proteins of the TLR signaling[3].

miRNAs are small non-coding RNAs approximately 22 nucleotide long. miRNA, together with Ago (1 - 4) proteins and Ago-bound proteins GW182, forms the RISK complex, which binds to the target mRNA and leads to its silencing[4]. It was also described that the mature miRNAs in the complex with Ago2 and GW182 can be sorted through the Multivesicular Body (MVB) into extracellular vesicles and can be transported to different cells[5,6]. Among different miRNAs, miRNA-146a plays a special role, as a major miRNA expressed in hepatocytes, having in addition immunosuppressive properties.

EVs are small single membrane vesicles of 30 - 200 nm of diameter. They contain cargo molecules that can be cellular/viral proteins, RNA, and lipids. Nowadays, EVs are considered to be part of the active cell-to-cell communication[7]. Our hypothesis is that HBV-infected hepatocytes produce the inhibitory molecules packaged in EVs, and that, in turn, are actively up-taken by pDC, leading to the inhibition of the immune response.

Our results, as the results of other laboratories[8], show that the intracellular level of miRNA 146 is higher in infected hepatocytes.

At the same time, the activation of the immune pDC, cell line, Gen2.2., after exposure to the supernatant from HBV-producing cell line HepG2.2.15, and infected hepatocytes, HepG2 NTCP cell line, is losing its effectivity. The level of IFNα production is lower after incubation with the infectious supernatant.

Our results show that HBV infected hepatocytes produce EVs that are actively up taken by the pDC cells and contains inhibitory molecules that are able to down regulate the signaling, that in turn leads to the decrease of the IFNα production.

Our future investigations will be focused more closely on the fractionation and characterization of EVs. We want to explore the content of different EV fractions by their ability to inhibit the immune response of the pDC, and by the presence of cellular/viral proteins and levels of miRNAs, or other RNA.

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