ABSTRACT

Immune consequences of *in vitro* infection of human peripheral blood leukocytes with vesicular stomatitis virus

Vesicular stomatitis virus (VSV) is a pathogen of hoofed mammals, including livestock. So far, few cases of human infection have been documented, which were characterized by mild, flu-like symptoms. However, VSV has been shown to cause viral encephalitis in animal models. Increasing interest in the use of VSV as a vaccine and oncolytic virus requires careful evaluation of its clinical safety following intravenous administration. Despite the previous achievements of the Laboratory of Virology in determining the level of non-specific antiviral response using VSV, the leukocyte population responsible for the production of VSV during infection and the effects of viral replication in these cells in the *in vitro* model has not yet been evaluated.

The aim of the study was to identify the population of peripheral blood leukocytes susceptible to vesicular stomatitis virus (VSV) and to characterize the effects of this infection in the *in vitro* model on: the interferon-stimulated gene expression, the proportions of major leukocyte populations, the surface markers expression, and the production of cytokines and chemokines. So far, the results of many research teams, including the Laboratory of Virology, confirmed the possibility of VSV multiplication in human leukocytes, but they have not focused on explaining the details of this process, which is becoming increasingly important.

In the first stage of the study, the method of fluorescent identification of VSV-infected cells was optimized using flow cytometry with the use of antibodies specifically recognizing virions during replication. Based on the curve of the percentage of infected cells and the results of a study published by the Laboratory of Virology, the incubation time of infected cells was set for 18 hours. The utility of UV inactivated virus as a negative infection control has also been tested. The differential susceptibility of human leukocytes to VSV infection was confirmed, which allowed for the isolation of the two groups: less susceptible and highly susceptible to VSV infection. The levels of cytokines TNF-α, IFN-γ and IL-10 were determined in mock- and VSV-infected

samples, divided into aforementioned groups. It was shown that the cells of the individuals less and highly susceptible to VSV infection differed in the level of spontaneously produced TNF-α.

We have also examined immune responses to VSV via determining the expression profile of cytosolic RNA sensors (RLRs) and IFN–stimulated genes. VSV infection led to the marked induction of RLRs and ISGs expression respectively, however the greatest effect was observed with the gene encoding the MxA protein.

In the next stage of the study, populations of leukocytes susceptible to VSV infection were identified as monocytes and dendritic cells. It was also shown that replication of the virus in monocytes resulted in decreased CD14 expression on their surface. In order to exclude the possible effect of soluble factors secreted by activated leukocytes, cells were stimulated with supernatants obtained from VSV infected cells. Both this type of stimulation as well as lymphocyte incubation with TLR3 and TLR4 ligands or inactivated virus (VSV-UV-I) did not cause such significant changes in the $CD14^+$ cell count, suggesting that the highest decline of monocyte population required virulent live VSV.

In order to clarify this phenomenon, the possible causes, i.e. differentiation of monocytes into dendritic cells and apoptosis of $CD14^+$ cells, were analyzed. We have determined that the infection of leukocytes with VSV lead to a significant differentiation of monocytes into $CD11c^+CD123^-$ dendritic cells that exhibited immature phenotype due to very low expression of CD40, CD83 and CD86 markers. In turn, the analysis of apoptosis with flow cytometry revealed a significant increase in apoptotic cells mainly in $CD14^+$ cells after VSV infection. The luminometric assay showed some evidence of an increase in caspase-3/7 activity in VSV-infected PBLs compared to mock-infected samples. Taken together, these results indicate that the decrease in percentage of $CD14^+$ monocytes caused by VSV infection was the result of two different processes - the differentiation of these cells into dendritic cells and their apoptosis, with the results suggesting that the former process plays a more significant role.

In light of the above observations it was important to show the impact of VSV infection in leukocytes on cytokine and chemokine production, also in samples divided into groups of individuals who were susceptible and less susceptible to the virus. Viral

replication increased production of IL-2R and IFN-α, as well as decreased production of RANTES. VSV-infected cells from individuals less susceptible to infection produced less IL-10, IL-12, IL-15 and IL-1β in comparison to non-infected cells. However, increased production of IL-2R was observed in a group of cells from susceptible individuals. Both groups differed in spontaneous cytokine production of eotaxin, IL-2, IFN-γ, IL-15, and IL-1β.

The results obtained in the presented thesis were discussed in light of available literature and the possible impact on the safety of VSV-based oncolytic therapies.