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Multicolor flow cytometry analysis of innate responses following in vitro interaction of PBMC with Hepatitis C virus

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Background

Alterations in innate immunity responses might be implicated in the establishment of a chronical infection with hepatitis C virus (HCV) in more than 80% of infected patients. This hypothesis is supported by the relative success of IFN-alpha-based therapy. Our aim has been to evaluate the consequences of HCV interaction with PBMC on global innate immune functions and to compare it to interaction with other RNA viruses, influenza and HIV-1.

Methods

The complex setting and diversity of interactions among cellular sub-populations involved in the innate response was approached by a short-time virus stimulation of total PBMC population. Multicolor cytometry is a unique tool for these multi-parametric investigations. By the mean of a 17-color LSRII, we have identified simultaneously plasmacytoid dendritic cells (pDCs), myeloid dendritic cells (mDCs), NK, monocytes and CD8+ T lymphocytes and analyzed their functional response to virus stimulation by measurment of expression levels of activation markers (CD69, CD83, CD86) and intra-cellular cytokines (IFN-alpha, IFN-gamma, TNF-alpha, IL-12).

Results

The global overview of the functional markers expression in each cell sub-population shows differences between the RNA viruses tested. Influenza induces pDC and NK activations but not the activation of mDC and monocytes. Interistingly, responses to HCV and HIV clusterize together and are characterized by a sustained IL-12 production in mDC and monocytes associated to a low pDC and NK activation. HCV-infected patients cells show a lower response to TLR7/8 agonist or HCV re-stimulation as compared to uninfected donors.

Discussion

In conclusion, with the help of multicolor cytometry technology, we were able to take a time-dependant picture of innate immune responses to RNA viruses stimulations from a complex cell system. Our results show importance of mDCs for a deeper understanding of HCV interactions with the innate immunity. (ANRS grant 2007/306)

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