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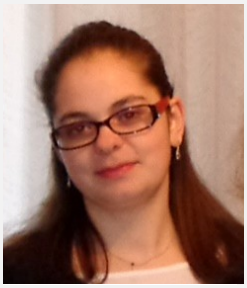

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Study of the effects induced by the HIV-1 Nef protein in plasmacytoid dendritic cells

Several viruses manipulate host innate immune responses to avoid recognition and improve viral replication and spreading. The viral protein Nef of Human Immunodeficiency Virus type 1 (HIV-1) is a well-established virulence factor and it is mainly involved in this “hijacking” activity.

In the last few years, there have been remarkable advances in outlining a defined framework of its functions. In particular, Nef appears to be a shuttling molecular adaptor able to exert its effects both on infected and non-infected bystander cells. In acute HIV-1 infection, the increase in plasma viremia was found to be associated with elevations in plasma levels of multiple cytokines and chemokines (a phenomenon named “early cytokine storm”). Afterwards, the wearing down of immune system during HIV disease progression is accompanied with chronic inflammation, T cell exhaustion and viral immune evasion *[1]*. It is emerging fact that Nef has an important impact on the chemo-cytokine network possibly contributing to chronic inflammation. Studies carried out on primary monocyte derived macrophages (MDMs) treated with Nef showed that the protein is rapidly internalized and triggers NF-κB, MAPKs and IRF-3 activation inducing a set of cytokines and chemokines including IFN-β *[2, 3, 4]*.

Not only monocytes/macrophages but also plasmacytoid dendritic cells (pDCs) play a pivotal role in the immunologic network. pDCs are innate immune cells that are specialized to produce large amounts of type I IFN and for this reason they are also called Interferon-Producing Cells (IPC). In the context of HIV infection, although pDCs do not represent a reservoir of infection, they are the majors producers of type I IFN in response to the virus, but their relevance in the pathogenesis of AIDS as well as the specific effects induced by Nef are not yet well known *[5,6]*.

Therefore, the aim of this research is to better understand Nef-pDC interactions analysing in details the effects induced by the viral protein with respect the

production of pro-inflammatory cytokines. To this purpose, human pDCs will be treated with a recombinant myristoylated HIV-1 Nef_{SF2} and later analysed for the production of inflammatory cytokines, such as IL-6, IL-1β and TNF-α by means of specific ELISA kits. At the same time, cells will be treated with Nef mutants in order to identify the motives of the protein possibly involved in this response. In addition, signal transduction pathways involved in pro-inflammatory response will be analysed.

Since primary human pDCs are present in very low amount in blood (0.2-0.5% of the circulating peripheral blood mononuclear cells, PBMCs), the experiments will carry out using both primary human pDCs isolated from buffy coats of healthy donors and a plasmacytoid dendritic cell line obtained by a French laboratory. This pDC cell line grows in presence of murine cells which are used as feeder layer, therefore the purity of the pDC population and the possibly variations in its phenotype will be assayed by cytofluorimetric analysis. To this purpose, specific fluorochrome coniugated antibodies would be required.

This study will provide important information regarding the effects induced by Nef protein in plasmacytoid dendritic cells that can be useful for the development of Nef inhibitors aimed at reducing the inflammatory response, which persists in HAART-treated patients (Highly Active Anti-Retroviral Therapy).

The ImmunoTools selected products would be of great benefit to this project as they would be used to characterize the purity of the pDC cell line and to analyse the cytokine pattern induced by Nef.

References:

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PE - conjugated anti-human TNFa

APC - conjugated anti-human CD63

PE - conjugated anti-mouse CD29, CD44, CD90

APC - conjugated anti-mouse CD45

recombinant human cytokines: rh IL-3, rh GM-CSF

recombinant mouse cytokines: rm IFNgamma

human ELISA-set (for one 96 plate): human IL-1beta, human IL-6, human IP-10, human TNF-a

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