ImmunoTools *special* Award 2023

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Autophagy mechanisms regulating HIV-induced MDSC survival and functions

Autophagy is the main catabolic process by which intracellular components can be degraded through lysosome delivery. This mechanism plays an important role in the host defence by removing and degrading invading pathogens. On the other hand, pathogens have evolved strategies to inhibit the immunity-supporting roles of autophagy and to hijack autophagy protein activities for their benefit. Several studies report an interplay between HIV and autophagy suggesting that early steps of HIV infection allow the virus to modulate autophagy pathways preparing cells to be permissive for viral infection. However, autophagy dysregulation in the late steps of the HIV replication cycle may promote autophagic cell death of $CD4^+$ T cells, contributing to disease progression and infection persistence. Although the roles of autophagy during HIV infection are multiple, several pieces of evidence pointed out a potential beneficial effect of inducing autophagy, potentiating the immune response to HIV and counteracting viral pathogenesis in non-progressor-HIV-infected patients.

Several substances are documented to be involved in autophagy modulation, including vitamin D. Vitamin D activates autophagy to influence a broad spectrum of physiological functions and protective mechanisms inhibiting oxidative stress and apoptosis. This process effectively regulates cell proliferation, differentiation, and immune modulation, ultimately controlling inflammation and enhancing host immunity by activating antimicrobial defence mechanisms.

In particular, autophagy has been demonstrated to have a role in the regulation of myeloid-derived suppressor cells (MDSC), an immune suppressive cell population that expands during different pathologies, including HIV infection, and is associated with disease progression. Indeed, MDSC can inhibit HIV-specific T cell response and could play a role in hindering CD4 T cell recovery.

Since the detrimental role of MDSC expansion during HIV infection, detailed studies on the mechanisms regulating their survival and suppressive function are needed to evaluate new therapeutic approaches targeting this cell population

The aim of this project is to evaluate the role of autophagy in the regulation of MDSC suppressive activity during HIV infection.

We will explore the autophagic flux in MDSC from HIV-infected patients at different stages of the disease and with different plasmatic vitamin D levels, evaluating the correlation with suppressive capacity. To this aim, the determination of autophagy flux will be performed in purified MDSC from HIV^+ individuals infected patients at different stages of the disease. MDSC subsets will be purified from peripheral blood mononuclear cells by cell sorting. MDSC identification will be performed on PBMC by flow-cytometry using specific cell markers (CD11b, HLA-DR, CD14, CD33, CD80, DRAQ7, CD56, CD19, CD3, CD15, CD45). Immunocytochemistry will evaluate the autophagy flux by analysing BECN1, AMBRA 1, ATG5, LC3.

Also, purified MDSC will be treated with autophagy inducers (Vitamin D and rapamycin) or inhibitors (chloroquine and bafilomycin) and the apoptotic level and suppressive function will be tested. Flow cytometry will be employed to assess annexin V and propidium iodide (PI) expression on MDSCs. The capacity of MDSC to suppress T lymphocyte proliferation and cytokine production will be analysed. T cell proliferation will be evaluated by stimulating with anti-CD3/CD28 in the presence of MDSC treated with autophagy inducers or inhibitors. The proliferation rate will be analysed by flow cytometry. HIV-specific T cell response will be evaluated by analysing the production of IFN-gamma, TNF-a, IL17A, and IL-10 by T cells stimulated with virus-specific peptides.

ImmunoTools' generous support will aid this project in deepening the understanding of the HIV/MDSC/autophagy interplay, potentially leading to the development of innovative therapeutic strategies aimed to overcome the HIV-induced immune suppression.

Key References:

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Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. Nat Rev Cancer. 2013 Oct;13(10):739-52. doi: 10.1038/nrc3581. PMID: 24060865; PMCID: PMC4358792.

ImmunoTools *special* AWARD for **Flavia Giannessi** includes 10 reagents

FITC - conjugated anti-human CD11b

PE - conjugated anti-human CD8

PerCP - conjugated anti-human CD8, HLA-DR

APC - conjugated anti-human CD3, CD66b, CD80, CD86, Annexin V

anti-human CD63 purified

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