ImmunoTools *special* Award 2013

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Characterization of the contribution of HIV regulatory proteins to immune dysfunctions during HIV infection.

HIV infection is characterized by several immune dysfunctions like chronic immune activation and immune senescence (*Appay, Almeida et al. 2007*). Immune activation is directly linked with disease progression and lack of control of co-infections (*Giorgi, Hultin et al. 1999*) while immune senescence, which is present also during antiretroviral therapy (ART), leads to premature ageing and thus to a number of pathologies usually seen in elderly people as non-AIDS defining cancer or cardiovascular diseases (*Bini, Green et al. 2009; Desquilbet, Jacobson et al. 2011; Deeks, Verdin et al. 2012*). The causes of both immune activation and immune senescence are poorly understood, although the two phenomena share some features like T cells activation and increased inflammation (*Deeks 2011; Haas, Zimmermann et al. 2011*). It has been proposed that some regulatory proteins of HIV, known to be released by infected cells and directly affect CD4+ and CD8+ T cells functionality, may contribute to immune dysfunctions (*Haas, Zimmermann et al. 2011*; *Chang, Samaniego et al. 1997; Ott, Lovett et al. 1998; Bouzar, Villet et al. 2004; Neri, Giolo et al. 2011*). However, studies characterizing the effects of HIV regulatory proteins on activation and senescence in T cells are missing. The aim of this proposal is to dissect the contribution of HIV regulatory proteins to immune activation or immune senescence.

Activities:

Purified CD4+ e CD8+ T cells (total or after sorting of naïve, effector and memory cells) from healthy donors will be cultured in resting conditions or will undergo multiple rounds of stimulation. Stimulation will be provided by TCR engagement (anti-CD3/CD28 or CD8 peptide epitopes) or by cytokines whose level/secretion is known to be dysregulated during HIV infection.

Short-term or long-term cell cultures, treated or not with HIV regulatory proteins, will be assayed for:

- Expression of surface molecules (Flow cytometry) markers of exhaustion/apoptosis, activation, senescence, memory;
- Release of cytokines (ELISA) known to be involved in immune activation and/or senescence processes;
- Proliferation and resistance to apoptosis (flow cytometry), respectively hallmarks of immune activation and immune senescence in HIV infected subjects;
- Responsiveness (Elispot, cytotoxic assay) of memory CD8+ T cells from EBV-positive donors to EBV-derived CTL epitopes after being cultured with Tat protein;

- Expression (qPCR, Western Blot) of transcription factor important for T cell programming and functionality and involved in immune activation and immune senescence (Hasley, Hong et al. 2013);
- Expression (qPCR) of genes markers of immune senescence;
- Expression (qPCR) of genes modulated in HIV-infected individuals during chronic immune activation: γ-chain cytokines and type I interferon pathways (Catalfamo, Wilhelm et al. 2011).

Thus, ImmunoTools reagents will result fundamental to:

- Provide co-stimulation to T cells (cytokines)
- Assess the phenotype (conjugated antibodies) and apopostosis (Annexin V) of T cells by flow cytometry
- Determine cytokine release in treated cells (ELISA kits)

Expected results will be important to understand the contribution of HIV regulatory proteins to immune senescence and accelerate ageing and, thus, to HIV pathogenesis. ART-treated individuals still maintain residual excess disease risks, and further strategies are needed to prevent immune dysfunction and thus increase their life expectancy and quality of life (*Deeks, Lewin et al. 2013*). This study will help to understand whether the induction of immunity against specific HIV regulatory proteins may be one of these strategies.

ImmunoTools *special* AWARD for **Francesco Nicoli** includes 25 reagents

FITC - conjugated anti-human CD27, CD38, CD69, HLA-DR,

PE - conjugated anti-human CD8, CD25, IL-6, Annexin-V,

PerCP - conjugated anti-human CD4, CD8,

recombinant human cytokines rh IFNgamma, rh IL-4, rh IL-6, rh IL-7, rh IL-8, rh IL-10, rh IL-12, IL-17a, rh TGF-beta3, rh TNFα,

human IL-4 ELISA-set, human IL-6 ELISA-set, human IL-8 ELISA-set, human IL-12p40 ELISA-set, human TNF alpha ELISA-set,

DETAILS