cible protein — 10, CXCL10) may be an alternative biomarker of tuberculosis infection, as it provides a greater range of measured concentrations compared with IFNγ and a higher threshold that is its unquestionable advantage. But these results can not be approximated for HIV patients because it is known that immune deficiency caused by HIV due primarily to a decrease in the number and the functions loosening of CD4 T-lymphocytes, which leads to a reduction of production and biological action of other cytokines, especially IFNγ.

In view of this, in the our last study we enrolled patients with HIV infection and pulmonary tuberculosis (n=40) and patients with HIV infection without evidence of active tuberculosis (n=47). All persons included in the study were carried out QuantiFERON®-TB Gold In-Tube and we determined the content of the antigen-induced (AG) and the spontaneous production (NIL) of IP-10. For IP-10 we reached the sensitivity of detection of tuberculosis infection 67% and specificity of 92%, whereas QuantiFERON-TB Gold was comparable sensitivity of 70%, but significantly lower specificity 73% (p=0,021, chi-square). And our results showed the possibility of using IP-10 as an alternative biomarker to IFN for the detection of tuberculosis in patients with HIV infection. Prospects for further development of the theme.

Actual is further study of the features of immunopathogenesis of TB in patients living with HIV, search the informative biomarkers of HIV, the development of approaches to assess the effectiveness of specific anti-tuberculosis therapy. At present we obtained blood from 29 patients of TB+HIV+ patients and 24 HIV−TB+. Also we are forming the following groups of patients: TB−HIV− and TB−HIV+.

In addition to the QuantiFERON®-TB Gold In-Tube, by flow cytometry, we assess the degree of maturity of T lymphocytes and NK-cells from the peripheral blood of patients with severe immunosuppression (HIV+) in combination with pulmonary tuberculosis, in the evaluation of effector cell populations.

Peripheral blood samples would be stained with antibodies to CD3, CD4, CD8, CD27, CD28, CD45, CD45RA, CD62L, as well as CD56 and CD57. Samples will be acquired using Navios flow cytometer, equipped with 405, 488 and 635 lasers (Beckman Coulter, USA). T-helpers will be identified as CD3+CD4+ and cytotoxic T-cells as CD3+CD8+. Based on initial expression of CD27 and CD28 and on the following analysis of CD45RA and CD62L expression on CD27+CD28+ subset CD3+CD4+ and CD3+CD8+ lymphocytes will be divided into the following subpopulations: «naïve» CD27+CD28+ CD45RA+CD62L+, central memory CD27+CD28+ CD45RA−CD62L+, transitional memory CD27+ CD28+CD45RA−CD62L−, as well as effector memory cells and effector cells (CD27+CD28− and CD27−CD28−, respectively). For T-cell subsets identification we also plan to use the «alternative» gating strategy based on initial analysis of CD45RA and CD62L expression. So CD3+CD4+ and CD3+ CD8+ cells will be divided into «naïve»» (CD45RA+ CD62L+), central memory (CD45RA−CD62L+), effector memory (CD45RA−CD62L−,) and «terminally differentiated» effector memory (CD45RA+ CD62L−) cells. Based on the expression of CD27 and CD28 in EM and TEMRA further populations will be distinguished, which within EM cells will be CD27+CD28+, CD27+CD28− CD27−CD28− and CD27−CD28+. While within TEMRA CD27+ CD28+ (pE1), CD27+CD28− (pE2) and CD27− CD28− (E) subpopulations will be isolated. The frequencies of all these T-cell subsets will be expressed in percentages from the total lymphocytes and in absolute counts. Furthermore, the presence of CD56 and CD57 — the key molecules, that characterize the effective capacity of cytotoxic T-cells and, in some measure, of T-helpers — was studied on all the above mentioned T-cells subsets. NK-cells will be purified from peripheral blood as CD3-CD56+. In order to identify the main subpopulations of NK-cells the expression of CD8, CD27, CD62L and CD57 will be measured.

The results allow to conclude that, if the level of immunosuppression effect on the functional activity of T-lymphocytes, which are the most useful cell populations in HIV detection and in diagnosis of tuberculosis in patients with HIV infection.

The evaluation of immune dysregulation during opioid withdrawal treatment and opioid abstinence among HIV infected injection drug users

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Introduction: Neurocognitive impairment (NCI) is an important complication of chronic HIV infections. Previous research found that Injection drug use (IDU) of opioids increase risk of NCI. We hypothesize that dysregulation of the neuroendocrine (hypothalamic pituitary axis, HPA and sympathoadrenalmedullary, SAM) and immune systems during opioid withdrawal and opioid addiction treatment may contribute to heightened neurological risk in HIV infected IDU. Our overall concept is that this dysregulation represents one of the mechanisms that sets the stage for HIV associated brain injury as evidenced by NCI.

Methods: This study will recruit 80 HIV infected IDU who are HAART naive during last month and entering opioid addiction treatment (detoxification). Participants

will complete four assessments: 1 — the first day of detoxification; 2 — the peak of opioid withdrawal; 3 — the last day of detoxification; 4— the last day of stabilization. The assessment protocol consists of gathering structured information on patient demographic characteristics, drug abuse history, and medical history, followed by physical examination, neurocognitive assessment, mood and withdrawal severity assessment, comorbidities (viral hepatitis, syphylis) and blood sampling for biomarkers of interest. In addition, we will collect the data from the medical cards.

The specific measures of HPA dysregulation will include:

1) plasma ACTH;

2) cortisol;

3) DHEA, DHEA-sulfate.

The specific measures of SAM dysregulation will include:

1) plasma neuropeptide Y;

2) plasma epinephrine;

3) norepinephrine.

The specific measures of impaired viral regulation and potential underlying mechanisms will include:

1) HIV RNA;

2) T-cell subsets: activated CD4+ cells (HLA-DR+) and activated CD8+ cells (HLA-DR+, CD38+);

3) SCD14 (evidence of microbial translocation);

4) MCP-1 (CCL2; evidence of monocyte activation);

5) IL-6 (acute phase response & chronic inflammation indicator).

Results: Preliminary results demonstrate an HPA/SAM dysregulation during the peak of opioid withdrawal. In addition, the extent of this dysregulation correlated with level of immune impairment. HPA/SAM systems and neurocognitive functions were normalized at 4 weeks of opioid abstinence in comparison with the last day of detoxification. The primary hypothesis is dealing with immediate effect of HPA and SAM dysregulation during the 1-day period of detoxification on progress HIV viral load and worse neurocognitive performance. We will estimate the changes in measures of HPA dysregulation and SAM axis perturbation during the 1-day period and use them as predictors. This approach will help to answer on question on immediate effect of neuroendocrine dysregulation on viral regulation, immune activation and neurocognitive processes. Further analyses will investigate potential underlying mechanisms of impaired viral regulation (SCD14; MCP-1; IL-6) by linking HPA and SAM axis perturbation to immune activation measures.

Conclusion: This study will provide data about clinical and immunological dynamics during detoxification at the medical settings and after in-patient treatment. The data could inform future policy on pharmacotherapy which focused on immune therapy during detoxification.

Modified HIV-1 Env proteins in VLPs enhance induction of neutralizing antibodies

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Introduction: Broadly neutralizing antibodies can prevent HIV-1 infection via binding to envelope (Env) glycoprotein complexes on the virion surface. The HIV-1 envelope glycoprotein exhibits a number of features that help the virus to evade humoral immunity including variable loops, extensive N-linked glycosylation, and conformational flexibility. Purified Env proteins as well as virus-like particle (VLP) vaccines presenting membrane-anchored HIV-1 spikes have been explored, but none have yet demonstrated a high potential to induce neutralizing antibodies even against moderately sensitive (tier 2) strains of HIV-1

Purpose of research: To investigate the effects of specific modifications of the transmembrane spanning (TMS) and cytoplasmic tail (CT) domains on assembly and antigenic properties of HIV-1 clade C transmitted/founder (T/F) ZM53 Env glycoprotein.

Materials and Methods: To generate modified Env proteins we used plasmid pBlueSF162 containing the SF162 *env* gene (accession number EU 123924) and plasmid pcr3.1−53M21-env containing the ZM53 *env* gene (accession number AY 423984). HIV-1 *env* coding sequences were modified by replacing the TMS-CT domains with domains from the mouse mammary tumor virus (MMTV) Env glycoprotein with or without a GCN4 trimerization sequence in the cytoplasmic (CT) domain. Standard HIV VLPs (Env/Gag VLPs) were produced by coinfection of insect cells (Spodoptera frugiperda Sf9 cells) with rBVs expressing Gag and modified Env proteins for immunization studies. Female guinea pigs were obtained from Charles River Laboratory (Wilmington, MA) and immunized (four animals in each group) two times with SF162 or ZM53 pCAGGS DNA vaccines (50 μg per guinea pig) separately followed by two boosting immunizations by the intramuscular (I.M.) route with VLPs using doses containing 10 μg Env. Env-specific antibody (Ab) titers in immune sera were determined by enzyme-linked immunosorbent assay (ELISA) using recombinant ZM53 or SF162 Env gp120 proteins (5μg/ml) as coating antigens. Neutralization activity was determined using the JC53-BL indicator cell assay. The avidity index values of serum antibodies for the native viral envelope were determined by measuring the resistance of antibody-envelope glycoprotein complexes in the ConA ELISA to treatment with 1,5 M sodium thiocyanate (NaSCN).

Results: We investigated the effects on assembly and antigenic properties of specific modifications of the transmembrane spanning (TMS) and cytoplasmic tail (CT)