ImmunoTools *special* Award 2014

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BCG immunotherapy of HPV-associated diseases

Recurrent respiratory papillomatosis (RRP) is a rare disease characterized by recurrent proliferation of benign squamous papillomas within the respiratory tract causing severe airway obstruction. The papillomas occur more frequently in the larynx but also in various extralaryngeal sites (oropharynx, trachea, bronchi, lung parenchyma and esophagus). Despite its benign nature, in 3 - 5% of patients, respiratory papillomas may undergo malignant transformation to squamous cell carcinoma with quite poor prognosis. More than 90% of RRP cases are caused by human papillomavirus (HPV) type 6 and 11.

Efficient control of viral infections requires NK cell activation, differentiation of Th1 and cytotoxic T-cell (Tc1) effectors in combination with regulatory $CD4^{+}FoxP3^{+}$ T cells with inhibitory function (Treg) to prevent terminal effector cell differentiation and restrict tissue damage caused by non-specific immune activation. Recent studies suggest that the effective Th1-antiviral immune response to HPV-6 and HPV-11 is disturbed as a result of polarization of the immune response to the Th2/Treg phenotype. It was shown that tumor-infiltrating lymphocytes (TILs) in papillomas and peripheral blood mononuclear cells (PBMC) dominantly produced Th2 cytokines (IL-4, IL-5, IL-10, IL-13) that inhibit IFN-g and IL-2 secretion by Th1-cells. The impaired cytokine balance correlates with disease severity.

Currently, surgery is the mainly therapeutic approach in RRP. The adjuvant therapy as IFN-a, some antiviral drugs (Ribavirin, Cidofovir) and inhibitors of cyclooxygenase-3 or EGFR do not completely prevent relapses in RRP. Thus, understanding the mechanism(s) by which HPV-6 and HPV-11 polarize the immune response towards tolerance in RRP, as opposed to the development of cell-mediated immune

clearance of these viruses, is critical in developing novel therapies that would prevent disease recurrence and/or reduce disease severity.

Bacillus Calmette–Guérin (BCG) is a potent stimulator of Th1 response and has been successfully applied for treatment of superficial bladder tumors and malignant melanoma. However, data about the effects of combined surgical/ BCG immunotherapy in RRP patients are scarce.

The aim of our project is to investigate the effects of BCG on the antiviral immune response in RRP patients subjected to combined CO_2 microsurgery /BCG therapy. For this purpose we will collect blood samples and autologous papilloma tissues from RRP patients subjected to combined CO_2 laser microsurgery / BCG-immunotherapy on approved scheme. The control group will include age- and sex-matched healthy individuals corresponding to the CLSI standards.

The percentages of peripheral blood lymphocyte subsets (T ($CD3^+CD56^-$), B ($CD19^+CD3$-), Th ($CD4^+CD3^+$), Tc ($CD8^+CD3^+$) and NK ($CD56^+CD16^+CD3^-$) cells) will be study by multicolor flow cytometry using ImmunoTools fluorophore-conjugated monoclonal antibodies (CD3, CD4, CD8, CD19, CD45, CD56). Since plasmocytoid dendritic cells (pDCs) contribute to Th1 differentiation by a massive secretion of type I interferons (IFN-a and IFN-b) and provide an essential link between innate and adaptive immune response to chronic viral infections, we will determine the percentages of circulating pDCs (Lin^{neg}HLA-$DR^+CD123^+CD11c^{lo}$) and mature (Lin^{neg}HLA-DR^+ $CD86^+$) DCs using ImmunoTools general and subset-specific monoclonal antibodies (CD3, CD14, CD16, CD19,CD20, CD56, CD86, CD11c, HLA-DR). In addition, ImmunoTools ELISA sets will facilitate us to evaluate the basic Th1 (IFNγ, TNFa, IL-2) and Th2 (IL-4, IL-6) cytokines secreted in response to non-specific stimulation and in this way to determine changes in the cytokine background that play a pivotal role in the effective anti-HPV response.

ImmunoTools *special* AWARD for **Evelina Vetskova** includes 25 reagents

FITC - conjugated anti-human CD3, CD86, HLA-DR,

PE - conjugated anti-human CD3, CD8, CD14, CD19, CD20, CD56,

PerCP - conjugated anti-human CD45,

APC -conjugated anti-human CD3, CD4, CD11c, CD16, CD19, CD25,

human IL-4 ELISA-set, human IL-6 ELISA-set, human TNFa-ELISA-set

(each 3 reagents) DETAILS more AWARDS