ImmunoTools *special* Award 2018

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Characterization of the MHC class I cross-presentation pathway for pathogenic antigens by human dendritic cells

Cross-presentation describes the mechanism of exogenous antigen presentation by antigen presenting cells (APCs) through major histocompatibility complex (MHC) class I molecules to induce cytotoxic $CD8^+$ T lymphocytes (CTLs). Cross-presentation represents a central pathway to initiate protective immune responses against tumour and pathogenic antigens. Dendritic cells (DCs) are the major cross-presenting APCs. So far, pathways used by human DCs for MHC class I cross-presentation as well as the role of cross-presentation on the magnitude of specific T-cell induction are not well understood.

This project aimed to characterize cross-presentation of exogenous antigens by human DCs using *Aspergillus fumigates* lysate (AspL) as target of interest. Components involved at different stages of cross-presentation will be in examined with regard to induce highly protective CTL responses and to increase frequencies of antigen-specific CTLs. Healthy donors will be pre-tested in response to AspL-specific memory T cells by interferon-gamma (IFN-γ) EliSpot assay, in which only donors with AspL-specific T-cell responses will be included. Monocyte-derived DCs (moDCs) as professional APCs will be generated *in vitro* by using an established seven day protocol. Isolated monocytes from healthy blood donors will be cultured in the presence of human interleukin-4 (IL-4) and granulocyte macrophage colony-stimulating factor (GM-CSF) for five days to obtain immature DCs. For differentiation into mature DCs, immature DCs will be additionally stimulated with a maturation cocktail including IL-1β, tumor necrosis factor α (TNF-α), IL-6, and prostaglandin E_2 (PGE2). The morphological characteristics and specific phenotype of moDCs (CD83, CD86, CD14, HLA-DR) will be analysed at different stages of differentiation and maturation by light microscopy and flow cytometry. In the presence of the maturation cocktail, generated immature DCs will be loaded with the *Aspergillus fumigates* lysate consisting of disrupted *Aspergillus fumigatus* mycel from the ATCC strain 46645. Isolated autologous human $CD8^+$ T cells will be stimulated with AspL-loaded DCs to induce and assess AspL-specific CTL responses. The efficiency of cross-presentation

of AspL-derived peptides via MHC class I to induce AspL-specific CTLs will be analysed by IFN-γ EliSpot assay.

Furthermore, to verify cross-presentation of AspL-derived peptides and to investigate the role of antigen up-take, processing and MHC class I presentation by human DCs, non-toxic inhibitors for the specific stages of the antigen cross-presentation pathway will be used. Among inhibitors of interest, brefeldin A will be used to block the export of MHC class I complexes out of the ER to the Golgi and the cell surface. Generated moDCs treated with or without specific inhibitors will be examined by flow cytometry and western blot assays with regard to molecules involved in the cross-presentation blocking process. $CD8^+$ T cells will be stimulated with AspL-loaded moDCs treated with or without inhibitors. To evaluate the impact of cross-presentation on the induction capacity of AspL-specific CTLs, phenotype (CD8, CD45RA, CD62L, CD27), activation levels (CD25, CD69, IL-2), and function (IFN-γ, TNF-α, granzyme B) of stimulated AspL-specific CTLs will be assessed by flow cytometry, intracellular staining, ELISA, and EliSpot assay.

The results of the present study will contribute a better understanding on the cross-presentation pathway of exogenous antigens. Moreover, new findings will provide more inside into the role of cross-presentation of exogenous pathogens by human DCs on the induction capacity of protective CTL responses.

For the project human cytokines and growth factors from ImmunoTools will be used for human moDC generation. Additionally ELISA kits and anti-human antibodies for flow cytometry allow analysing moDCs phenotype and evaluating AspL-specific CLT phenotype, activation levels, and function.

ImmunoTools *special* AWARD for **Sabine Tischer** includes 25 reagents

FITC - conjugated anti-human CD14, CD62L, CD69

PE - conjugated anti-human CD25, CD27, IFN-gamma, TNFa

APC - conjugated anti-human CD8, CD86

PerCP - conjugated anti-human CD45RA, HLA-DR

recombinant human cytokines: rh GM-CSF, rh IL-1beta /IL-1F2, rh IL-2, rh IL-4,

rh IL-6, rh TNFα

human ELISA-set (for one 96 plate): human IFN-gamma, human TNF-a