ImmunoTools special Award 2013

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Metabolism of dendritic cells: Setting the stage for adaptive immune responses

BACKGROUND:
Dendritic cells (DCs) are potent antigen presenting cells that can initiate and orchestrate immune responses against viruses, intracellular bacteria or tumors by promoting inflammatory T helper (TH) 1 or 17-type responses. On the other hand, these cells can also suppress immune responses that might be essential to maintain self-tolerance. Tumor cells or viruses, like HIV, often demonstrate a strong ability to subvert antigen-specific immune responses by reprogramming DCs. There is an increasing demand for controlling T cell responses via DC-based therapies, which initiated a quest for reliable and feasible DC modulatory strategies that would facilitate inflammation and cytotoxicity against tumors, viruses or bacteria or tolerance in autoimmunity or allergy.

PRELIMINARY RESULTS:
We have identified a powerful autocrine pathway in developing monocyte-derived DCs (MoDCs) that strongly interfered with inflammatory DC differentiation (Nasi A. et al. J Immunol 2013). MoDCs differentiating in sparse cultures had superior ability to produce inflammatory cytokines, to induce TH1 polarization and to migrate towards the lymphoid tissue chemokines. On the contrary, MoDCs that originated from dense cultures produced IL-10 but no inflammatory cytokines upon activation. We showed that the molecule responsible for the density-dependent DC regulation was lactic acid, a side product of glycolytic ATP production, that accumulated in dense cultures and induced a potent anti-inflammatory program in developing MoDCs. These results suggest that metabolic pathways may be efficiently manipulated in developing MoDCs to modulate the immunogenicity or tolerogenicity of DC vaccines.

RESEARCH PLAN:
Project 1. Metabolic regulation of inflammatory and non-inflammatory DC differentiation: We aim at understanding how metabolic intermediates can trigger inflammatory, suppressed or suppressor DC differentiation and whether we can induce these differentiation programs by facilitating a more glycolytic or oxidative metabolism.
Project 2. DC vaccine programming via metabolism regulation: Influencing metabolism in developing DCs, as our preliminary results suggest, may lead to the increased immunogenicity of DC vaccines. To test the potential therapeutic applications of our data in vivo we investigate whether we could improve DC chemotaxis to lymphoid tissues as well as

the magnitude of DC-induced TH1 responses by down-modulating the anti-inflammatory effects of lactic acid during DC development. We generate functionally polarized DC vaccines by influencing metabolic pathways and compare their ability to prime protective T cell responses against M. tuberculosis, an infection where immunity depends on an efficient TH1 response, and against melanoma in mice.
Project 3. Reprogramming of inflammatory and suppressed DCs by HIV: DCs are extensively manipulated by HIV and therefore we hypothesized a viral recoding of the inflammatory or non-inflammatory differentiation programs in DCs. We plan to clarify the molecular details of the HIV-induced DC modulatory pathways and we aim to understand how the viral rewiring of DC functions can contribute to HIV pathogenesis.

In summary, we aim at achieving significantly improved DC vaccines and at providing novel knowledge on functional DC reprogramming in infection models, potentially contributing to the development of future preventive or therapeutic approaches.

We will use rh GM-CSF and rh IL-4 for the MoDC generation. Inflammatory and suppressed MoDCs differ in their CD14 and CD1a expression and therefore the anti-CD1a and anti-CD14 antibodies will be used to monitor their differentiation (*Nasi A. et a. J Immunol 2013*). To analyze the effects of metabolism regulation or the presence of HIV on DC activation antibodies against CD40, CD80, CD86 and HLA-DR will be used as well as the IL-6 ELISA. T cell activation and survival in the presence of inflammatory or suppressed DCs will be studied using antibodies against the activation markers CD25, CD38, CD69 and Annexin V staining of the apoptotic cells. T cells and DCs in co-cultures will be distinguished by flow cytometry using antibodies against CD11c and CD3. Isotype control antibodies will be used for confirming staining specificity by flow cytometry. We propagate HIV virus for our experiments using PHA-activated PBMC in the presence of IL-2.

ImmunoTools *special* AWARD for **Aikaterini Nasi** includes 25 reagents

FITC - conjugated anti-human CD1a, CD14, CD25, CD38, CD86, HLA-DR, Control-IgG1, Control-IgG2a, Annexin V,

PE - CD11c, CD80, Control-IgG1, Annexin V,

PerCP - conjugated anti-human CD3,

APC -conjugated anti-human CD3, CD11c, CD14, CD40, CD69, Control-IgG1, Annexin V,

recombinant human cytokines rh GM-CSF, rh IL-4, rh IL-2,

human IL-6 ELISA-set,

DETAILS