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POSTER PRESENTATIONS

P-0235

Activation and functional reponsesivness of macrophages cells to aluminum adjuvanted tetanus-diphtheria vaccine

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The purpose of this study is to evaluate the impact of aluminum-adjuvanted vaccines on macrophage activation and functional responsiveness. Murine monocyte/macrophage J774. A1 cells were exposed to two different dilutions of aluminum-adjuvanted tetanus-diphtheria vaccine (Td) in the presence or absence of LPS. The change in macrophage maturation (morphology and marker expression), activation (ROS, NO and phagocytosis) and viability were determined by flow cytometry. Exposure to aluminum-adjuvanted Td vaccine supported F4/80 and CD11b and MHC-II maturation markers either in LPS-stimulated or control macrophages. CD80 expression was increased dramatically by LPS treatment however negatively correlated in combinations with Td. A positive trend was observed in cellular granularity and Td concentration with or without LPS combination. The phagocytosis of latex beads was decreased significantly by increasing Td amount in all conditions. ROS production capacity was increased in both Td and LPS administrations whereas NO production was decreased with combination of LPS and Td vaccine. Our preliminary results may be useful for understanding the impact of aluminum adjuvants on macrophage activation.

Keywords: Adjuvants and vaccines, infectious disease, macrophage, phagocytosis

P-0236

CD28 individual signaling up-regulates IL-22 expression and IL-22–mediated effector functions in human T lymphocytes

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IL-22 is a member of the IL-10 cytokine family involved in host protection against extracellular pathogens, by promoting epithelial cell regeneration and barrier functions. Dysregulation of IL-22 production has also frequently been observed in acute respiratory distress syndrome (ARDS) and several chronic inflammatory and autoimmune diseases. We have previously described that human CD28, a crucial co-stimulatory receptor necessary for full T cell activation, is also able to act as a TCR independent signalling receptor and to induce the expression of IL-17A and inflammatory cytokines related to Th17 cells, which together with Th22 cells represent the main cellular source of IL-22. Here we characterized the role of CD28 autonomous signalling in regulating IL-22 expression in human CD4+ T cells. We show that CD28 stimulation in the absence of TCR strongly up-regulates IL-22 gene expression and secretion. As recently observed for IL-17A, we also found that CD28-mediated regulation of IL-22 transcription requires the cooperative activities of both IL-6-activated STAT3 and RelA/NF-κ transcription factors. CD28-mediated IL-22 production also promotes the barrier functions of epithelial cells by inducing mucin and metalloproteases expression. Finally, by using specific inhibitory drugs, we also identified CD28-associated class 1A phosphatidylinositol 3-kinase (PI3K) as a pivotal mediator of CD28-mediated IL-22 expression and IL-22-dependent epithelial cell barrier functions.

Keywords: Cell signalling, cytokines and mediators, molecular immunology

P-0237

Phenotypical and functional characterization of dapsone treated-neutrophils *in vitro*

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Clinical experiences confirm that dapsone is effective in treating inflammatory diseases, characterized by neutrophil-rich infiltrations. We examined different aspects of reshaping neutrophil's function by dapsone. Human neutrophils were isolated from the venous blood of healthy donors. Neutrophils were treated with dapsone (10μg/mL; 5μg/mL or 2,5μg/mL), and stimulated by phorbol-12 myristate-13-acetate (PMA), N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) or calcium ionophore (CaI). In some experiments, dapsone-primed neutrophils were treated with interferon-gamma (IFNγ) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Phenotypical characterization and apoptosis were measured by flow cytometry. Oxidative burst and NETosis were determined by a multimode microplate reader using luminol and Sytox Green, respectively. Production of IL-8 was measured by ELISA. We found that dapsone did not change the rate of spontaneous apoptosis after 6h and 24h. Dapsone at all tested concentrations significantly decreased oxidative burst of neutrophils activated by all stimuli compared to control. The highest concentration of dapsone inhibited the CaI-triggered NETosis. Also, dapsone increased the expression of CD16, CD15, CD62L on resting neutrophils and suppressed down-regulation of these markers in the presence of fMLP. The opposite effect of dapsone was seen with CD11b which was up-regulated by both resting and fMLP-treated neutrophils. The production of IL-8 was decreased in both, stimulated (fMLP) and unstimulated conditions. INFγ and GM-CSF increased oxidative burst of neutrophils in stimulated and unstimulated conditions, while priming with dapsone reduced the oxidative burst of these cells. Our results indicate the complexity of dapsone action on neutrophils with the predominance of anti-oxidative mechanisms.

Keywords: Drugs for immune modulation, granulocytes, neutrophils

P-0238

Myelodysplastic syndromes and acute myeloid leukemia display distinctive patterns of bone marrow NK cell maturation and KIR expression

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Myelodysplasic syndromes (MDS) are pre-malignant hematological disorders characterized by ineffective hematopoiesis that frequently progress towards acute myeloid leukemias (AML). Natural Killer (NK cells) are key antitumor elements of the innate immune system and understanding the differences in the NK functioning between MDS and AML is crucial in predicting the progression of MDS to AML and defining the corresponding therapy. The bone marrow aspirates of newly diagnosed patients with MDS (n=25), AML (n=8) and controls (normal bone marrow – NBM, n=30) at the Lucien Neuwirth Institute of Cancerology (Saint-Priest-en-Jarez, France) were collected between March-July 2020, and the resident NK cells were investigated by flow-cytometry. Based on the expression of CD56, CD94, CD16 and CD57 molecules, the three distinct maturation NK subsets were isolated (immature NK as CD56brightCD94hiCD16−CD57− cells, mature as CD56dimCD94medCD16+CD57- cells, and hypermature as CD56dimCD94lowCD16+CD57+ cells) and analyzed for the expression of inhibitory KIR receptors: NKG2A, CD158a, CD158b, and CD158e1. The NK/T cell distribution was impaired and accompanied by an increase in the immature NK subset in the bone marrow of MDS and AML cases compared to the NBM settings. Furthermore, the KIR expression was differentially expressed on the three distinct NK subsets in AML compared to MDS and NBM. This study provides important evidence for the undermined NK cell antitumor response in AML compared to MDS and NBM and explains the failure of this arm of the immune response during the pathogenesis of myeloid malignancies.

Keywords: Biology of the immune system, cancer immunology, innate lymphoid cells, NK cells