Stojanović, M.; Živković, I.; Inić-Kanada, A.; Petrušić, V.; Marinković, E.; Stojićević, I.; Dimitrijević, I. Phenotypic and Functional Characteristics of Splenocytes in Tetanus Toxoid-Hyperimmunized Balb/c Mice Is Influenced by the Context of Tetanus Toxoid Application. *Immunology (Abstracts of the European Congress of Immunology, 5-8 September 2012, Glasgow, Scotland)* **2012**, *137* (s1), 423–423.

response or impacts on the immunological activation phase favouring virus replication. On the other side, our results establish a new basis for the reliable characterization of the role of FOXP3+ T cells in HIV-1 individuals.

P0717

Phagocytosis of infected apoptotic cells promote PGE2 production by dendritc Cells

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Purpose/Objective: Phagocytosis of apoptotic cells promotes the synthesis of anti-inflammatory mediators such as PGE_2, TGF-β and IL-10 that may result in the suppression of host immune defense. However, a study using infected apoptotic cells showed that phagocytosis of these by dendritic cells (DC) promotes the production of anti-inflammatory cytokines such as TGF-b but also pro-inflammatory cytokines as IL-6 and IL-23, resulting in an immunostimulatory effect, the differentiation of Th17 cells. The role of PGE_2 in adaptive immunity has been investigated regarding lymphocyte differentiation and activation. Our aim was to evaluate the PGE_2 production from DC when co-cultured with different ratios of infected apoptotic cells.
Materials and methods: Infected apoptotic neutrophils were prepared as follows: C57BL/6J mice were injected i.p. with 3 ml thioglicollate with 3×10^6 live *Escherichia coli*, after 13 h, the cells were collected from peritoneal cavity lavage. Apoptosis of neutrophils were confirmed by flw cytometry using Annexin-V/PI staining. Bone marrow derived dendritic cells were co-cultured at different ratios (1:1, 1:3, 1:5) with infected apoptotic neutrophils during 18 h. Supernatant from co-culture was collected and PGE_2 production was determined by ELISA. We also evaluated the maturation level of DC after co-culture.
Results: Our results show that phagocytosis of infected apoptotic cells induces the production of high levels of PGE_2 at 1:3 (1500 pg/ml) and 1:5 (3400 pg/ml) ratio. Interestingly, the DC co-cultured with infected apoptotic cells deviated for immature state, with low levels of CD11c, MHC-II and CD86, contrasting with the activating stimulus LPS. Furthermore, the proportion of phenotypically immature cells increased in higher infected apoptotic cells rates.
Conclusions: DC does not acquire a predominant activated state when co-cultured with infected apoptotic cells and further characterization are needed to understanding this phenotypical state and its role in Th17 development. Also, the higher levels of PGE_2 suggest a role for this lipid mediator in Th17 differentiation in this context. However, the involvement of PGE_2 and the mechanism by which PGE_2 can work synergistically with TGF-b, IL-6 and IL-23 in the process of Th17 cell differentiation needs further characterization.

P0718

Phenotypic and functional characteristics of splenocytes in tetanus toxoid-hyperimmunized Balb/c mice is influenced by the context of tetanus toxoid application

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Purpose/Objective: The hyperimmunization with tetanus toxoid (TTd) induces protective TTd-specific as well as autoreactive β2-glycoprotein I (β2GPI)-specific immune responses in BALB/c mice. The overall immune response characteristics, especially its pathogenic potential, depended on adjuvants applied prior and in combination with TTd. Beside structural homology between TTd and β2GPI, tolerance toward β2GPI could be impaired by adjuvants acting as polyclonal stimulators. In order to clarify the impact of adjuvants, phenotypic and functional analyses of immune system cells within spleen were done upon immunization completion.
Materials and methods: Non- or CFA-pretreated BALB/c mice were immunized with TTd (3×100 μg/dose; 2-week intervals) mixed with alum or 2.5M glycerol. *Ex vivo* analyses of CD3, CD4, CD8, CD19, CD 25, CD27 and mIgM expression on age-matched control and immunized mice's splenocytes were done by flow cytometry. Changes in TLR2, TLR4 and TLR9 expression were assessed indirectly, by measuring cytokine production, following *in vitro* stimulation of splenocytes with appropriate agonist.
Results: TTd-immunization diminished CD27 expression on T cells implying on their differentiation into potent effector cells. T cell activation (increase in CD25 expression and the raise of percentage of $CD4^+$ $CD8^+$ $CD3^+$) and B cell activation (rise in percentage of $CD19^+$ $CD25^+$ cells and the increase of mIgM density) occurred in all immunized mice, being more intensive in CFA-pretreated groups. Irrespective to the applied immunization protocol, statistically significant rise in abundance of CD4- CD8- cells (often cited as cells having suppressive potential) within T cell pool was registered too. Differences in cytokines production (IL4, IL10, IFNγ) registered upon *in vitro* stimulation with peptidoglycan, LPS and CpG ODN implied on context-dependant modulation of TLR2, TLR4 and TLR9 expression on splenocytes.
Conclusions: TTd-hyperimmunization promoted concomitant rise in abundance of activated cells and the cells that have suppressive potential. This could be regarded as an attempt of the system to retain control. Imbalance in percentages and activities between activated cells and those having suppressive potential, highly influenced by the context of TTd application, is most likely the cause for the observed pathology appearance after TTd hyperimmunization.

P0719

Processing of particle bound antigens by peripheral phagocytes induces immunological tolerance

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Purpose/Objective: Phagocytes are key players in the upkeep of body homeostasis, e.g. by removing apoptotic material as well as forming a primary line of defense against invading microbiota. In this study we investigated the effect of systemically distributed particle bound antigens under sterile conditions to address the immunoregulatory capacities of phagocytes throughout the body in homeostasis.
Materials and methods: 0.5 μm fluorescent latex particles were covalently linked to ovalbumin (OVA) and injected i.v. into recipient mice. Latex particle distribution was followed up to 14 days in wildtype, CX3CR1.eGFP and CCR2.eGFP mice using flow cytometry and multiphoton microscopy to investigate uptake by distinct phagocyte subsets. Bone marrow transplants using CFP^+ donors were performed to assess latex particle distribution between resident macrophages and monocyte-derived phagocytes. Tolerance induction was measured by activation of regulatory T cells (Treg) and inhibition of OVA-specific CTL mediated target cell lysis. Interactions between latex-positive phagocytes and T cells were followed using intravital time lapse multiphoton microscopy in liver tissue as well as secondary lymphatic organs.