A3.13 INVESTIGATING T-CELL SUBSETS IN LYMPH NODE BIOPSIES OF AUTOANTIBODY POSITIVE INDIVIDUALS AND EARLY ARTHRITIS PATIENTS

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Background Rheumatoid arthritis (RA) is an immune-mediated inflammatory disease of unknown aetiology. Recent work has shown that systemic autoimmunity precedes inflammation of the synovium in RA patients. We developed a method to study the cellular composition of lymph nodes in the earliest phases of RA.

Objective Cross-sectional analysis of the phenotype and functional characteristics of T cells from lymph nodes of individuals in different phases of rheumatoid arthritis.

Materials and Methods Seven individuals with arthralgia but without any evidence of arthritis upon physical examination who were/but positive for IgM rheumatoid factor (IgM-RF) and/or anti-citrullinated protein antibodies (ACPA; RA risk group) and 7 DMARD and biological naïve RA patients were included in the study. All study subjects underwent ultrasound-guided inguinal lymph node biopsy. T cell subsets T-helper (Th)1, cytotoxic T cell (Tc)1, Th2, Tc2, Th17, Tc17, regulatory T cells (Treg) and follicular T cells (Tfh) were analysed by multi-colour flow cytometry. Cytokine profiles were determined after stimulation with Phorbol Myristate Acetate (PMA) and Ionomycin in the presence of Brefeldin A and Golgi Stop. We used directly labelled antibodies for CD45, CD3, CD4, CD8, IFN- γ (Th1/Tc1), IL-4 (Th2/Tc2), IL-17A (Th17/Tc17) Foxp3, and IL-10 (Treg).

Results Different T-helper cell subsets could be distinguished in the RA risk and arthritis group. An increase of CD4+IL-17A T cells (Th17; $p = 0.04$) and of CD4+IL-10 producing T cells ($p = 0.014$) could be observed in the early arthritis group compared to the RA risk group. Interestingly, a significant correlation between CD4+IL-10 producing T cells and ACPA titers ($r = 0.78$; $p = 0.0016$) and between CD4+IL-17A producing T cells and IgM-RF levels ($r = 0.57$; $p = 0.04$) was found when combining both study groups. In addition, within the RA risk group the levels of CD4+ IL-10 producing T cells correlated significantly with the TOTTJC28 ($r = 0.8097$; $p = 0.03$). Within the early arthritis group no significant correlations between T cell subsets and disease activity were found.

Conclusions Flow cytometry analysis of cells collected using ultrasound-guided inguinal lymph node biopsy suggests an increase in activated T cells producing IL-17A and IL-10 in patients with early RA. Moreover, the number of these T cells correlates with autoantibody levels suggesting an important role of these T cells in the humoral autoimmune response. These data indicate that inflammatory changes in lymph nodes are present during the earliest phases of RA which may provide new insights in early immunological changes associated with RA pathogenesis.

A3.14 PD-1 SIGNALLING AND LOW RORC MRNA CONTENT INFLUENCE THE TREG/TH17 BALANCE IN HUMAN CORD BLOOD

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Background and Objectives In neonates, the immune system is less responsive than in adults. Explanations for this involve differences in T helper (Th) 1 versus Th2 cell numbers and altered cytokine profiles after stimulation. We hypothesised that neonatal immune deviation may also involve FOXP3⁺ regulatory T cells (Treg) and Th17 cells.

Materials and Methods In order to investigate this, we compared CD4⁺CD25⁻CD45RO⁻ naïve T cells from human cord blood (CB) with cells from adult peripheral blood (APB). Cells were activated in vitro by anti-CD3 in the presence of viable antigen presenting cells (APB). The induction of regulatory T cells and Th17 cells was analysed by flow cytometry. Cytokine production was measured by multiplex immunoassays and RORC mRNA content by PCR. Specific interactions between APC and T cells were blocked with monoclonal antibodies during cell culture.

Results CB cells show lower numbers of Treg ex vivo than APB. However, upon activation, high percentages of functionally suppressive FOXP3⁺ Treg are induced in CB. Replacement of viable APC by irradiated APC or anti-CD28 abrogated this effect, suggesting that live APC from CB in particular may imprint a Treg phenotype. Indeed, CB APC were able to induce high numbers of FOXP3⁺ cells in alloreactive immune reactions with APB T cells as well. Addition of blocking antibodies against CD80, CD86 or CTLA-4 to CB cultures did not affect the number of Tregs induced. Blockade of the PD-1/PD-Ligand (PD-L)1 interaction however showed a marked decrease in FOXP3⁺ cell numbers. CB cells showed a consistently reduced protein kinase B (PKB)/c-Akt phosphorylation upon activation. On top of that, in contrast to APB, low concentrations of APC derived pro-inflammatory cytokines were detected in CB cultures and no Th17 cells were induced. Addition of Th17 inducing cytokines reduced FOXP3⁺ cell numbers in CB, but did not induce IL-17 production. PCR of the Th17 defining transcription factor RORC showed a significantly reduced concentration of this protein in CB.

Conclusions Human cord blood cells have an increased propensity to become FOXP3⁺ Treg as compared to APB. This effect is due to low production of inflammatory cytokines and PD-1/PD-L1 interaction. Inability to induce Th17 cells in CB could not be abrogated by Th17 inducing cytokines, but was due to reduced RORC mRNA content. This study shows mechanisms involving the human Th17/Treg induction switch that can also be important in inflammation and auto-immune disorders.

A3.15 RECONSTITUTED HIGH DENSITY LIPOPROTEIN (rHDL) MODULATES T EFFECTOR CELL FUNCTION IN A MURINE MODEL OF RHEUMATOID ARTHRITIS

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Background and Objectives High density lipoprotein (HDL) has a variety of functions which confer protection from cardiovascular and other human diseases. Increased cardiovascular risk is observed in humans with autoimmune disorders such as rheumatoid arthritis (RA), which is associated with either low levels or dysfunctional HDL. Recent evidence suggests a role of HDL in modulating both innate and adaptive immune responses. We sought to investigate the role of rHDL during the development of an autoimmune response in the antigen-induced arthritis (AIA) mouse model.

Materials and Methods C57BL/6 mice were subcutaneously immunised with ovalbumin (OVA) in complete Freund's adjuvant (CFA) and the inguinal lymph nodes (LN) were excised 9 days after the antigenic challenge. The LN cells were cultured in vitro in the