IN VITRO DIFFERENTIATION OF HUMAN TH17 CELLS

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Background: Th17 cells represent a subset of T helper lymphocytes that produce several inflammatory cytokines, including interleukin-17A, -17F, -21, -22, and tumor necrosis factor. Increased Th17 cell differentiation and IL-17 production have been observed in rheumatoid arthritis (RA) and in several other autoimmune diseases. IL-17 contributes to development of inflammation and promotes osteoclast differentiation in RA. We have studied the differentiation of Th17 cells.
Methods: CD4 positive T cells were separated by magnetic method from peripheral blood mononuclear cells (PBMC) of healthy volunteers. The cells were treated for 5-10 days with anti-CD3 and anti-CD28 antibodies and with TGF_{β} (2,5ng/ml), IL-6 (25ng/ml) and IL-1 (10ng/ml) cytokines, and with anti-IL-4 (10µg/ml) and anti-IFNγ (10µg/ml) blocking antibodies. The IL-17 production was measured by ELISPOT and ELISA, the RORc expression was measured by real-time PCR and by western blot methods, cell viability was monitored by Trypan blue staining and by Annexin V binding.
Results: Anti-CD3/CD28 treatment increased the IL-17 production, but did not alter the RORc expression. The anti-CD3/CD28, IL-1, IL-6 and TGF_{β} induced RORc expression was further increased by the anti-IL-4 and anti-IFNγ antibody treatment, without affecting cell viability.
Conclusion: Our data suggests that IL-4 and IFNγ blockade promote the T-cell activation and cytokine treatment induced Th17 cell differentiation.