

## T cell reactivity against citrullinated proteins does not differ between healthy persons and patients with inflammatory arthritis

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### Objectives:

Rheumatoid Arthritis (RA) and Spondyloarthritis (SpA) are two inflammatory auto-immune diseases. They can be distinguished by clinical presentation and the presence of antibodies to citrullinated proteins (ACPA), which is specific for RA. Production of these serological markers indicates a predominant role for B-cells in the pathology of RA. However, little is known about the effect of citrullinated proteins on T-cell reactivity.

### Methods:

PBMC were isolated from healthy volunteers (n=6), RA-patients (CCP+ (n=5) and CCP- (n=5)) and SpA-patients (n=6). The IFN $\gamma$ -production was evaluated by ELISpot analysis. PBMC (500000/100 $\mu$ L) were stimulated with *in vitro* citrullinated (*cit*) and non-citrullinated (*non-cit*) human cell extract, each at a concentration of 200 $\mu$ g/ml and 20U/ml IL2 was added. In order to identify the cells that were crucial in the citrulline-induced T cell reactivity, depletion experiments for CD4, CD8 and HLA-DR were performed according to the manufacturer's protocol (Miltenyi Biotec). In parallel, 10<sup>6</sup> PBMC were cultured for 7 days in the presence of *cit* (80 $\mu$ g/ml) or *non-cit* (80 $\mu$ g/ml). Supernatants were collected and the secretion of cytokines was evaluated by multiple ELISAs (detecting IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17a, IFN $\gamma$ , TNF $\alpha$ , G-CSF and TGF $\beta$ ).

### Results:

In the cultures of PBMCs of healthy persons, the RA and the SpA patients, the number of spot forming counts (SFC) when stimulated with *cit* was higher than when stimulated with *non cit* (significant difference = SFC (*cit*) - SFC (*non cit*) > (SD (*cit*) + SD (*non cit*))). A significant difference was seen in 5 of the 6 healthy volunteers and in all the SpA patients. Unexpectedly this phenomenon was less distinct in RA (2/5 CCP+ and 4/5 CCP-), while patients with inflammatory arthritis contain high amounts of citrullinated proteins in their joints.

After depletion of HLA DR positive cells a major drop in reactivity was observed, which illustrates the crucial role of an antigen presenting cell in the citrulline-specific T cell reactivity. Depletion experiments for CD4 and CD8 positive cells showed that both cell types were involved.

The cytokine profile, determined from the multiple ELISAs showed a great resemblance between healthy, RA and SpA patients. For IL2, IL10, IL17 and IL6 an overall greater response to *cit* compared to *non cit* was detected. On the contrary, for TGF $\beta$ , a greater response to *non cit*, compared to *cit*, was seen.

### Conclusion:

These data show that citrulline-reactive IFN $\gamma$  producing T-cells are present in the repertoire of RA patients, SpA patients and healthy people. This implicates that T cell stimulation with citrullinated proteins is a universal mechanism and that ACPA production in RA is not due to the presence of a unique set of citrulline-reactive T cells in the periphery of RA patients.