Rapid Interaction Between CTLA4-Ig (Abatacept) and Synovial Macrophages from Patients with Rheumatoid Arthritis

To the Editor:

We have demonstrated that macrophages could be considered one of the main direct target cells for treatment with CTLA4-Ig (abatacept) in patients with rheumatoid arthritis (RA), in mixed cultures of macrophages and activated T cells, or in primary single cultures of RA synovial macrophages\textsuperscript{1,2,3}. Previous studies showed that a significant downregulation of tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin 1\(\beta\) (IL-1\(\beta\)), and IL-6 was evident for cultured human macrophages treated with CTLA4-Ig, through direct interaction with B7 molecules on the surface of RA synovial macrophages at 24 h\textsuperscript{1}. The interaction between CTLA4-Ig and B7 molecules (CD80/CD86) masked their expression on RA synovial macrophages\textsuperscript{1}.

From those results, we carried out further evaluations of cytokine production and modulation in RA synovial macrophage primary cultures at the gene expression level and after different short-term CTLA4-Ig treatments (3 and 12 hours), to further investigate the timing of the interaction of CTLA4-Ig and synovial macrophages. As well, we analyzed transforming growth factor-\(\beta\) (TGF-\(\beta\)) gene expression and production.

Synovial macrophages were obtained, with informed consent, from 6 patients with RA (5 women, 1 man; mean age 50 ± 2 yrs; Disease Activity Score-28 > 5.2) who underwent therapeutic arthroscopic synoviectomy. Synovial macrophages were cultured with or without (controls) CTLA4-Ig at concentrations of 100 and 500 \(\mu\)g/ml. Synovial macrophages preincubated with blocking anti-CD86 antibodies were cultured with or without CTLA4-Ig (100 and 500 \(\mu\)g/ml) as an additional control.

After 3, 12, and 24 h, cytokine gene expression for TNF-\(\alpha\), IL-1\(\beta\), and IL-6, and TGF-\(\beta\) was investigated by quantitative real-time PCR (qRT-PCR). After 24 h cytokines were evaluated by immunocytochemistry and Western blot.

At 3 h after treatment, CTLA4-Ig (100 and 500 \(\mu\)g/ml) induced a significant decrease of IL-6 (\(p < 0.05\) both concentrations) and TNF-\(\alpha\) expression (\(p < 0.05\) and \(p < 0.001\), respectively) versus controls, as evaluated by qRT-PCR. After 12 h a further downregulation for IL-6, IL-1\(\beta\), and TGF-\(\beta\) versus controls was observed (\(p < 0.001\) both concentrations). No further changes from treatment were observed at 24 h (Figure 1).

In addition, at 24 h, CTLA4-Ig 100 \(\mu\)g/ml induced a significant downregulation of IL-1\(\beta\) (\(p < 0.01\)); and CTLA4-Ig 500 \(\mu\)g/ml significantly downregulated all cytokines (\(p < 0.001\)) and TGF-\(\beta\) (\(p < 0.01\)) compared to CTLA-4-untreated controls as evaluated by immunocytochemistry (Figure 2). Further immunocytochemistry tests for the 2 most...
sensitive cytokines (IL-6 and TNF-α) showed no downregulation after 24 hours in the condition of pretreatment with blocking anti-CD86 antibodies, confirming that the effects on synovial macrophages were really due to the CD86/CTLA4-Ig binding.

Western blotting confirmed the results, and in particular showed decreased cytokine synthesis in RA synovial macrophages treated with CTLA4-Ig 500 µg/ml compared to controls.

Thus, in a short time, through direct interaction with B7 molecules on the surface of RA synovial macrophages, CTLA4-Ig seemed to downregulate TNF-α, IL-1, IL-1β, and TGF-β inflammatory cytokine gene expression and production.

Previous studies have shown that CD86 expression in the RA synovium was strongly inhibited after treatment with CTLA4-Ig, because of its link with the CD86 molecule on antigen-presenting cells, resulting in inhibition of not only T cells but also B cells and macrophages; this likely plays a role in the therapeutic efficacy of abatacept in RA.

Our study describes rapid in vitro downregulation exerted by CTLA4-Ig on gene expression for TNF-α, IL-6, IL-1β, and TGF-β in RA synovial macrophages. Indeed, the transcriptional effects on cytokines were already significantly evident between 3 and 12 h from the CTLA4-Ig binding to macrophages. The influence exerted by CTLA4-Ig on a wide range of synovial target cells might suggest a multilevel modulation of synovitis in controlling clinical manifestations in patients with RA in both early and advanced phases of the disease.

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