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\(^1\). 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\(^1\). The interaction between CTLA4-Ig and B7 molecules (CD80/CD86) masked their expression on RA synovial macrophages\(^1\).

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 µg/ml. Synovial macrophages preincubated with blocking anti-CD86 antibodies were cultured with or without CTLA4-Ig (100 and 500 µg/ml) as an additional control.

At 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 µg/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 µg/ml induced a significant downregulation of IL-1\(\beta\) (p < 0.01); and CTLA4-Ig 500 µg/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

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**Figure 1.** Gene expression analysis by quantitative real-time PCR for interleukin 6 (IL-6), tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), IL-1\(\beta\), and transforming growth factor-\(\beta\) (TGF-\(\beta\)) in cultures of rheumatoid arthritis synovial macrophages. A. Cultures that were treated with CTLA4-Ig (abatacept) 100 µg/ml after 3, 12, and 24 h, or not treated (control). B. Cultures that were treated with CTLA4-Ig 500 µg/ml after 3, 12, and 24 h, or not treated (control). Expression values of target genes are indicated as fold expression (fold increasing) compared to those of the untreated cells (calibrator), conventionally indicated as 1. *p < 0.05; ***p < 0.001.

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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 RA4.

A report described synovial immunopathological changes that occurred after CTLA4-Ig treatment in patients with severe refractory psoriatic arthritis, with regression of synovial cell infiltration and downregulation of Th type 1 and proinflammatory cytokines, notably IL-63. Consequently, CTLA4-Ig appears to have different cell targets in modulating the immune/inflammatory response, primarily by competing with CD28 for binding to CD80/CD86 molecules (T cell activation), but also by exerting direct modulatory effects on other antigen-presenting cells, including macrophages (as shown here), osteoclasts, and B cells4,6.

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 disease7.

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