

Reduction of Inflammatory Biomarker Response by Abatacept in Treatment of Rheumatoid Arthritis

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ABSTRACT. *Objective.* Abatacept, a soluble selective costimulation modulator, selectively modulates T cell activation via the CD80/CD86:CD28 costimulation pathway. Data from a Phase II trial showed efficacy in patients with active rheumatoid arthritis (RA) and inadequate response to methotrexate when treated with abatacept (10 mg/kg or 2 mg/kg). To determine the mechanism of action of abatacept, we analyzed changes in the serum levels of inflammatory biomarkers in the patients enrolled in this trial.

Results. Following 12 months' treatment, serum levels of interleukin 6 (IL-6), soluble IL-2 receptor, C-reactive protein, soluble E-selectin, and soluble intercellular adhesion molecule-1 were significantly lower in patients receiving abatacept 10 mg/kg versus placebo. Smaller reductions in tumor necrosis factor- α and rheumatoid factor were also observed in the abatacept 10 mg/kg group compared with the placebo group. Although there was no evidence for efficacy of the 2 mg/kg dose, small reductions in inflammatory biomarkers at this dosage support the biologic effect of this therapy.

Conclusion. These findings reveal the antiinflammatory and immunomodulatory effects of abatacept in patients with RA, and are consistent with the concept that modulating T cell activation improves clinical signs and symptoms and inhibits the progression of structural damage. These data suggest that selective modulation of the CD80/CD86:CD28 pathway with abatacept may affect several inflammatory cell types and cytokines that are involved in the proinflammatory cascade. (First Release Oct 1 2006; J Rheumatol 2006;33:2162-6)

Key Indexing Terms:

ABATACEPT

C-REACTIVE PROTEIN

INTERLEUKIN 2 RECEPTOR

INTERLEUKIN 6

RHEUMATOID FACTOR

BIOMARKER

Abatacept, a soluble selective costimulation modulator, has demonstrated significant clinical benefit in patients with rheumatoid arthritis (RA) and an inadequate response to methotrexate (MTX)¹. A fully soluble fusion protein, abatacept consists of the extracellular domain of human cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) linked to the Fc (hinge, CH2 and CH3 domains) portion of human immunoglobulin G1² that has been modified to avoid comple-

ment fixation. Mechanistically, abatacept acts by modulating the CD80/CD86:CD28 pathway², a key costimulatory pathway required for full T cell activation³. In effect, abatacept inhibits the interaction of CD80/CD86 on antigen-presenting cells (APC) with CD28 on T cells, thereby selectively modulating T cell activation. When administered in combination with MTX, abatacept has been shown to lead to significant improvements in the signs and symptoms of RA, and in health-related quality of life¹. A Disease Activity Score 28 (DAS28) of less than 2.6 was also induced in a significant proportion of patients^{1,4}: following 12 months of treatment with abatacept plus MTX, 34.8% of patients were shown to achieve this improvement versus 10.1% of placebo-treated patients ($p < 0.001$)⁵. In a similar population of patients, abatacept also demonstrated significant inhibition of structural damage progression compared with placebo, as assessed by the Genant-modified Sharp score⁶.

Multiple inflammatory mediators are elevated in autoimmune disorders such as RA⁷. In this 12-month study of patients with active RA, the biologic effect of selective costimulation modulation with abatacept on several inflammatory biomarkers [C-reactive protein (CRP), rheumatoid factor (RF), soluble interleukin 2 receptor (sIL-2R), IL-6, soluble (s)E-selectin, soluble intercellular adhesion molecule-1 (sICAM-1), tumor necrosis factor- α (TNF- α)] was assessed.

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MATERIALS AND METHODS

This was a 12-month, multicenter, randomized, double-blind, placebo-controlled, parallel-design, dose-finding Phase IIb study of the safety, efficacy, and pharmacokinetics of abatacept in patients with active RA and an inadequate response to MTX. Patients were randomized to receive a fixed dose of abatacept 10 mg/kg or 2 mg/kg, or placebo, in a 1:1:1 ratio. The design of the study has been described previously^{1,8}.

The study was approved by the Institutional Review Board (IRB) and Independent Ethics Committee (IEC) and was carried out in accord with the principles of the Declaration of Helsinki.

Treatment was administered by intravenous infusion on Days 1, 15, and 30, and then every 30 days up to and including Day 330. Patients received MTX (10–30 mg/wk) for the duration of the study. For the first 6 months, no adjustments in the dose of MTX were permitted except in the case of liver, hematologic, or pulmonary toxicity. Other disease modifying antirheumatic drugs (DMARD) were not permitted for the first 6 months. After 6 months, adjustments in DMARD were permitted at the discretion of the investigator. These were as follows: change in MTX dose (provided the dose was less than 30 mg/wk); addition of another DMARD (hydroxychloroquine, sulfasalazine, gold, or azathioprine); and adjustment in corticosteroids equivalent to prednisone \leq 10 mg/day. No adjustment of either abatacept dose was permitted.

Serum samples were obtained on each visit day prior to the infusion of study medication. Mean serum levels were computed for each visit day for the following pharmacodynamic markers: CRP, RF, sIL-2R, IL-6, sE-selectin, sICAM-1, and TNF- α . CRP and RF were measured using an immunoturbidimetric assay (catalog no. 1551922 and 1552007, respectively; Roche Diagnostics Corp., Indianapolis, IN, USA) as described previously. Serum concentrations of sIL-2R (catalog no. DR2 A00; Quantikine assay, R&D Systems, Minneapolis, MN, USA), IL-6 and TNF- α (catalog no. Q600 and QTA00, respectively; QuantiGlo chemiluminescent assays, R&D Systems), and sE-selectin and sICAM-1 (Parameter[®] human sE-selectin quantitative sandwich ELISA and Parameter[®] human sICAM-1 colorimetric sandwich ELISA, respectively; R&D Systems) were measured by ELISA according to the manufacturers' instructions.

Longitudinal analyses using a linear mixed-effects model were performed to assess the treatment effect and estimated values over time. All data for scheduled visits were used. The longitudinal analysis assumes that data were missing at random and not dependent on current or future responses. The model for each biomarker included treatment, visit day, and treatment-by-visit interaction as fixed effects. An autoregressive¹ covariance was used to account for within-patient correlation over time. Model-based estimates are presented for Days 90, 180, and 360.

RESULTS

A total of 115, 105, and 119 patients were randomized to receive abatacept 10 mg/kg, abatacept 2 mg/kg, and placebo, respectively, in addition to continued background MTX. Ninety, 74, and 71 patients, respectively, completed the study⁵. Patient demographics, clinical characteristics (as described¹), and mean levels of all biomarkers (CRP, RF, sIL-2R, IL-6, sE-selectin, sICAM-1, TNF- α) were similar across treatment groups at baseline (Table 1). With the exception of sIL-2R, all biomarkers were elevated above the normal range.

After 12 months of treatment with abatacept 10 mg/kg, the serum levels of CRP, sIL-2R, IL-6, sE-selectin, and sICAM-1 were significantly lower compared with placebo (Table 1). Mean biomarker levels for 3, 6, and 12 months are shown in Figure 1.

Inflammatory biomarker levels over 12 months Treatment with abatacept 10 mg/kg

C-reactive protein

On Day 90, CRP levels were significantly lower in the abatacept 10 mg/kg treatment group versus the placebo group [mean values 1.6 (standard error 0.2) vs 2.6 (SE 0.2) mg/ml ($p < 0.005$); Figure 1A]; these reductions were maintained through Day 360, where mean levels of CRP were 1.5 (SE 0.2) and 3.0 (SE 0.3) mg/ml for the abatacept 10 mg/kg and placebo groups, respectively ($p < 0.0001$).

Rheumatoid factor

Levels of RF were also reduced in the abatacept 10 mg/kg group through Day 360; however, these reductions were not significantly different compared with placebo [Day 360 mean values 159.3 (SE 32.0) vs 225.2 (SE 32.9) IU/ml for placebo ($p =$ nonsignificant); Figure 1B].

Soluble IL-2 receptor

Mean levels of sIL-2R in the abatacept 10 mg/kg group were significantly lower than in the placebo group on Day 90 through Day 360 [Day 90 and 360 mean values 1082.1 (SE 64.3) vs 1493.6 (SE 64.6) and 1228.3 (SE 69.0) vs 1697.1 (SE 72.0) pg/ml for the abatacept 10 mg/kg and placebo groups, respectively ($p < 0.0001$ for all comparisons); Figure 1C].

Serum IL-6

On Day 90, serum IL-6 was significantly reduced with abatacept treatment compared with placebo [Day 90 mean values 9.5 (SE 3.0) vs 23.4 (SE 3.1) pg/ml for abatacept 10 mg/kg versus placebo ($p < 0.005$)]; reductions in the abatacept 10 mg/kg group were maintained through Day 360 ($p < 0.05$; Figure 1D).

Tumor necrosis factor- α

Compared with Day 1, levels of TNF- α were reduced in the abatacept 10 mg/kg group compared with placebo through Day 360 [Day 1 and Day 360 mean values 11.7 (SE 1.5) vs 11.4 (SE 1.4) and 7.4 (SE 1.9) vs 10.3 (SE 2.1) pg/ml, respectively]; however, these differences were not statistically significant for the abatacept 10 mg/kg group versus placebo (Figure 1E).

sE-selectin and sICAM-1

On Day 90, sE-selectin was significantly reduced with abatacept 10 mg/kg treatment compared with placebo [Day 90 mean values 59.0 (SE 3.3) vs 68.9 (SE 3.4) ng/ml ($p < 0.05$)]; these reductions were maintained through Days 180 and 360, where the mean levels were 61.0 (SE 3.4) vs 70.8 (SE 3.5) ng/ml ($p < 0.05$) and 58.5 (SE 3.5) vs 72.7 (SE 3.7) ng/ml ($p = 0.005$), respectively (Figure 1F). Significant reductions were also observed for sICAM-1 on Days 180 and 360 [Day 180 and 360 mean values 360.7 (SE 14.6) vs 404.1 (SE 15.1) ng/ml ($p < 0.05$) and 351.0 (SE 15.1) vs 408.9 (SE 15.7) ng/ml ($p < 0.01$), respectively (Figure 1G)].

Table 1. Mean levels of pharmacodynamic biomarkers at baseline and Day 360.

Biomarker	Mean Levels at Baseline (SE)			Mean Levels at Day 360 (SE)			Normal Range
	Abatacept 10 mg/kg (n = 115)	Abatacept 2 mg/kg (n = 105)	Placebo (n = 119)	Abatacept 10 mg/kg (n = 115)	Abatacept 2 mg/kg (n = 105)	Placebo (n = 119)	
CRP, mg/dl	3.0 (0.2)	3.2 (0.2)	3.2 (0.2)	1.5 (0.2)*	2.1 (0.3)	3.0 (0.3)	0–0.4
RF, IU/l	290.1 (29.9)	276.0 (31.2)	223.5 (29.4)	159.3 (32.0)	261.2 (34.3)	225.2 (32.9)	0–20.0
sIL-2R, pg/ml	1426.4 (63.8)	1413.1 (66.6)	1483.6 (62.6)	1228.3 (69.0)*	1441.5 (74.2)†	1697.1 (72.0)	676.0–2132.0
IL-6, pg/ml	27.3 (2.9)	33.8 (3.1)	26.3 (2.8)	7.3 (3.7)†	15.8 (4.0)	19.9 (4.1)	0.3–14.8
Soluble E-selectin, ng/ml	68.4 (3.3)	69.1 (3.5)	68.2 (3.3)	58.5 (3.5)††	71.6 (3.7)	72.7 (3.7)	29.1–63.4
sICAM-1, ng/ml	403.4 (14.2)	397.6 (14.8)	393.5 (14.0)	351.0 (15.1)††	394.1 (16.0)	408.9 (15.7)	115.0–306.0
TNF- α , pg/ml	11.7 (1.5)	9.0 (1.5)	11.4 (1.4)	7.4 (1.9)	7.8 (2.1)	10.3 (2.1)	1.2–8.0

* $p < 0.0001$; † $p < 0.05$; †† $p < 0.01$, all versus placebo; based on a longitudinal mixed-model analysis. CRP: C-reactive protein, RF: rheumatoid factor, sIL-2R: soluble interleukin 2 receptor, sICAM-1: soluble intercellular adhesion molecule-1, TNF- α : tumor necrosis factor- α , SE: standard error.

Treatment with abatacept 2 mg/kg

Previously reported efficacy data^{1,4} for patients receiving abatacept 2 mg/kg in this study failed to support the efficacy benefit of this dose over placebo. Consistent with these results, the analyses here showed that patients in the abatacept 2 mg/kg treatment group also experienced smaller reductions in all biomarkers compared with the 10 mg/kg dose. On Day 90, sIL-2R was the only biomarker shown to be significantly lower than in patients receiving placebo; on Days 180 and 360, mean levels were significantly lower for both sIL-2R and CRP compared with placebo (Figures 1C and 1A). Small reductions in sE-selectin and sICAM-1 were also observed in the abatacept 2 mg/kg group; however, levels of these adhesion molecules were not statistically lower than those seen in patients receiving placebo (Figures 1F, 1G).

DISCUSSION

In this 12-month study of patients with active RA and an inadequate response to MTX, mean levels of CRP, sIL-2R, IL-6, sE-selectin, and sICAM-1 were significantly lower with abatacept 10 mg/kg treatment than in patients receiving placebo. The most marked reductions were seen during the first 90 days of treatment, with decreases being sustained thereafter. Patients in this group also showed reductions in levels of RF and TNF- α . The abatacept 2 mg/kg dose showed a trend toward smaller decreases in all biomarkers compared with the abatacept 10 mg/kg dose. These findings are consistent with the previously presented efficacy data for this Phase IIb study, in which the 2 mg/kg dose was reported to be suboptimal in comparison with the 10 mg/kg dose of abatacept^{1,4}.

Because the immune cascade in RA involves a number of immune and inflammatory cell types⁷, of which the activated T cell may play a central role in driving the immune response⁹, the “upstream” activity of abatacept — selectively modulating T cell activation via the CD80/CD86:CD28 pathway — has the potential to modulate “downstream” immune events.

By acting upstream in RA immunopathology, T cells may

contribute to the activation of downstream effector¹⁰ cells such as osteoclasts and chondrocytes that produce the joint inflammation and progressive destruction that is characteristic of RA. Following recognition of an antigen in the context of the major histocompatibility complex presented on the surface of an APC, T cells require costimulation for full activation to occur¹¹. One of the most well characterized costimulatory pathways is the engagement of CD80/CD86 on APC with CD28 on T cells³. Once activated, T cells proceed to activate other immune cells via direct cell–cell interactions^{12,13} and by the production of inflammatory mediators⁷. Stimulation of these other immune cells in turn facilitates the secretion of an array of inflammatory cytokines, adhesion molecules, and surface-bound receptors⁷. Data presented here support the upstream mechanism of action of abatacept at the level of T cell activation, with the potential to influence multiple downstream cell types and inflammatory mediators, as described below.

T cell activation is associated with upregulated expression of membrane-bound IL-2R and secretion of sIL-2R¹⁴. Reductions in sIL-2R observed during this 12-month study with abatacept are consistent with the mechanism of action of abatacept as a T cell costimulation modulator. When activated, T cells also interact with B cells via the CD40:CD40L pathway, facilitating B cell activation and the production of autoantibodies¹⁵. Over 12 months of treatment, abatacept 10 mg/kg reduced the levels of RF in this RA patient population with an inadequate response to MTX. The relationship between these reductions and the mechanism by which abatacept exerts its effects on B cells is unknown and remains to be determined.

In RA, T cells are also thought to form direct cell–cell interactions with synovial macrophages and fibroblast-like synoviocytes (FLS)¹³, both of which produce the proinflammatory cytokine IL-6. Among a number of effects, IL-6 regulates the development of osteoclasts and is also associated with joint damage in chronic disease^{16,17}. Activated macrophages also produce TNF- α , a pleiotropic cytokine in

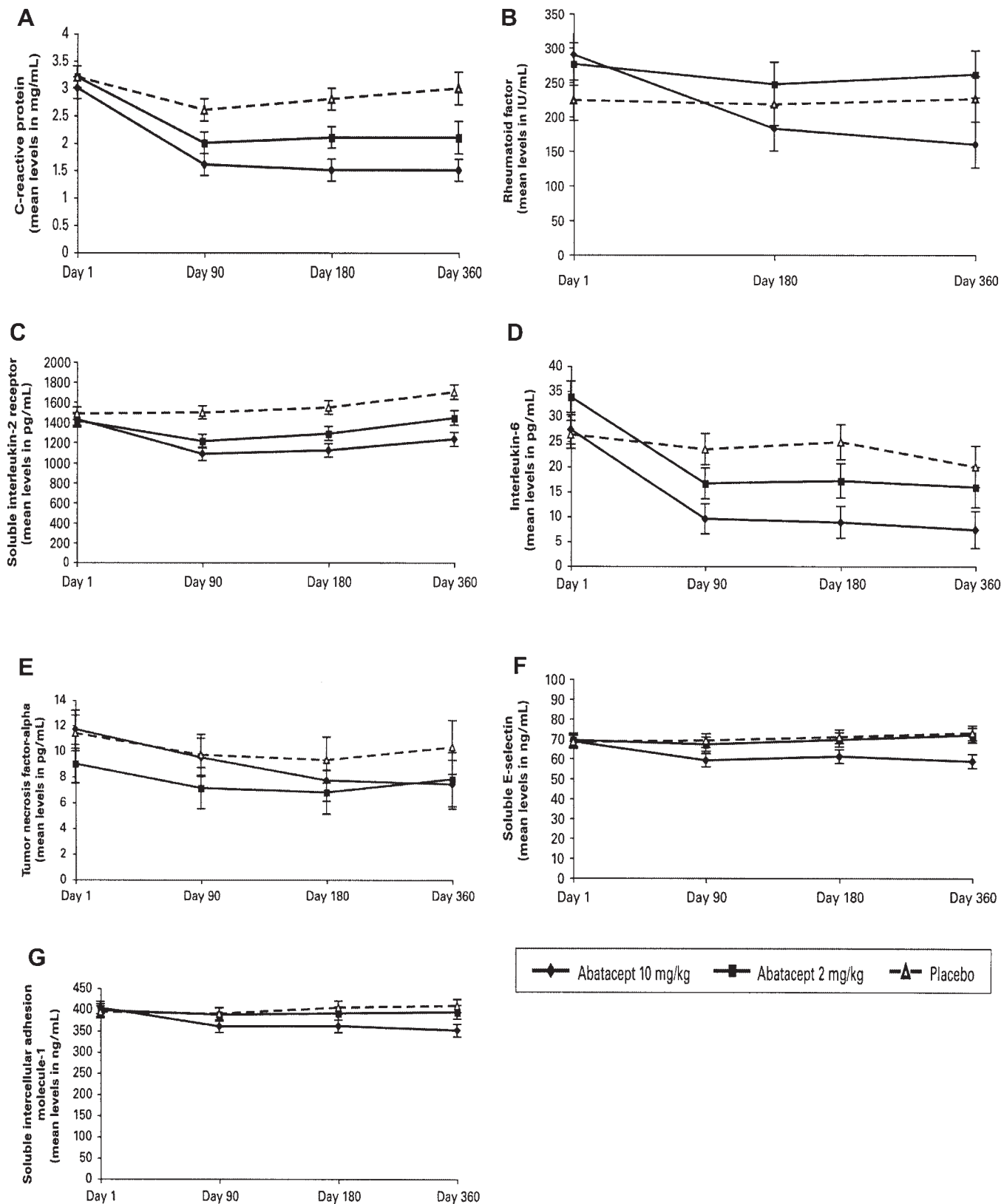


Figure 1. Mean biomarker levels through Day 360. Model-based estimates are presented at Days 90, 180, and 360 for (A) C-reactive protein, (B) rheumatoid factor, (C) soluble interleukin 2 receptor, (D) interleukin 6, (E) tumor necrosis factor- α , (F) soluble E-selectin, and (G) soluble intercellular adhesion molecule-1 in patients receiving abatacept 10 mg/kg, abatacept 2 mg/kg, or placebo. Error bars represent standard error of the mean.

RA that regulates the production of other cytokines, adhesion molecules, and inflammatory mediators¹⁸. In this 12-month study, IL-6 levels were reduced with abatacept 10 mg/kg treatment, with significant reductions in IL-6 observed at Days 90, 180, and 360. Since the main sources of IL-6 are macrophages and FLS, these data suggest that abatacept may modulate the function of these cells. As IL-6 provides activating signals to a number of other immune cells, such as T cells, B cells, and osteoclasts, this may provide another mechanism by which abatacept may reduce inflammation and destruction.

Following abatacept 10 mg/kg treatment, TNF- α levels declined more slowly than the other biomarkers analyzed. As patients in the placebo group and the abatacept 2 mg/kg group also experienced reductions in the levels of this cytokine, differences between the abatacept and placebo treatment groups were not statistically significant. The clinical significance of these changes is uncertain; studies are under way to determine the longterm effect of abatacept on TNF- α in this patient population. TNF- α is also a locally produced cytokine and thus measurement of synovial TNF- α levels may prove more relevant.

Perpetuation of chronic inflammation requires the continued recruitment of inflammatory cells into the synovial joint¹⁹ — a process facilitated by synovial endothelium activation and the subsequent upregulation of sICAM-1 and sE-selectin expression^{20,21}. In this study, serum levels of the cellular adhesion molecules sICAM-1 and sE-selectin were significantly lower with abatacept 10 mg/kg, but not with abatacept 2 mg/kg, compared with placebo. However, as this study assessed serum levels of sICAM-1 and sE-selectin, rather than the cell-surface expression, the underlying mechanism for this effect is unknown. The effect of abatacept on cell trafficking into the joint is therefore being investigated in another clinical study.

Our data provide evidence that abatacept acts at the level of the T cell in RA immunopathology by selectively modulating T cell activation. Combined with the favorable efficacy and safety data of abatacept^{1,8}, these data provide evidence for the importance of T cell activation in the inflammatory cascade in RA, and support the rationale for selective costimulation modulation with abatacept for the treatment of patients with active RA and an inadequate response to MTX.

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