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IL-6 blockade reverses the abnormal STAT activation of peripheral blood leukocytes from rheumatoid arthritis patients

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Abstract Considering the interplay of multiple STATs in response to cytokines, we investigated how IL-6 and its blocking affect STAT signaling in rheumatoid arthritis (RA). Leukocytes obtained from RA patients before and after tocilizumab treatment and healthy donors (HDs) were cytokine-stimulated and STAT phosphorylation was analyzed by cytometry. RA patients had significantly fewer pSTAT1+, pSTAT3+, and pSTAT6+ monocytes and pSTAT5+ lymphocytes than HDs. After 24 weeks of treatment, percentages of IFNγ-induced pSTAT1+ and IL-10-induced pSTAT3+ monocytes in RA patients increased, reaching levels comparable to HDs. pSTAT1+ and pSTAT3+ cells correlated inversely with RA disease activity index and levels of pSTAT+ cells at baseline were higher in patients with good EULAR response to tocilizumab. IFNγ-induced pSTAT1+ cells correlated inversely with memory T cells and anti-CCP levels. IL-10-induced pSTAT3+ cells correlated with Treg/Teff ratio. Our findings suggest that IL-6 blocking reduces the inflammatory mechanisms through the correction of STAT1 and STAT3 activation status.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune systemic disease characterized by a poly-articular synovitis [1]. In the

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RA joint, synovium is infiltrated by macrophages, lymphocytes, plasma cells, and osteoclasts. Most macrophages are activated and work together with proliferating synovial fibroblasts to destroy local cartilage and bone. Cytokines, particularly TNFα, IL-1, IL-6, IL-12, IL-15, IL-18, IFNγ, and GM-CSF, are central regulators of this cellular activation and synovial inflammation [2]. Increased levels of IL-6 have been found in synovial fluid and in serum of RA patients [3,4]. IL-6 is a pleiotropic cytokine that was originally identified as a B cell differentiation factor produced by activated mononuclear cells [5]. However, recent research has shown that it also plays a role in regulating inflammation and the immune response [6]. IL-6 acts via receptor complexes containing at least one gp130, an almost ubiquitously signal-transducing receptor. In hepatocytes, monocytes, macrophages, neutrophils and some lymphocytes, IL-6 first binds to the IL-6 membrane receptor (mIL6R). The complex IL-6/mIL-6R then signals to gp130. A soluble form of the IL-6 receptor, sIL6R, has also been found in certain body fluids, in a process known as trans-signaling. This soluble receptor can form an IL-6/sIL-6R complex to signal cells that only express gp130 [7].

Cytokines signal through JAK/STAT pathways to influence cell-fate decisions during differentiation of naïve T cells to Th1, Th2, Treg and Th17. IL6, in particular, exerts its biological functions through the activation of STAT1 and STAT3 [8], transcription factors involved in regulating Th17 differentiation and controlling cell infiltration in inflamed joints [9]. It is therefore not surprising that in the synovia in RA patients and in experimental arthritis, STAT1 expression is elevated and both STAT1 and STAT3 are in an activated state [10–12].

The physiologic role of STAT1 in RA remains unclear. Gene expression studies have shown a marked heterogeneity in the level of STAT1 expression and in the gene pathway induced by STAT1 [13]. Furthermore, STAT1 has the capacity both to promote and to inhibit inflammation. On one hand, it mediates the inflammatory effects of IFNγ, while on the other, it plays a role in the apoptosis resistance of RA synoviocytes and other non-inflammatory effects [14]. As well as in gene expression studies, this direct relationship between STAT1 and bone formation and destruction has been shown in mouse models [15,16].

Like STAT1, STAT3 is involved in a wide variety of physiological processes and it directs apparently contradictory responses in RA [15,17]. It is the transducer of the IL-10 inhibitory signal, but IL-10 also signals through STAT1 and STAT5. Another cytokine, IL-6, induces the formation of homo- and hetero-dimers of STAT1 and STAT3, with a preference for STAT3 homo-dimers [8]. This stimulation of IL-6 through STAT3 phosphorylation is used by regulatory T cells and effector cells [18]. IL-27, a cytokine promoting Th1 differentiation, also signals via STAT3 [19]. These findings show that JAK/STAT pathways have a pronounced plasticity during cellular responses to cytokine stimuli. The deletion of one STAT does not necessarily lead to unresponsiveness to the corresponding cytokine but to changes in the activation program. It is therefore tempting to speculate that the excess of certain cytokines in RA can affect the global signaling machinery of peripheral blood leukocytes. If this were so, cytokine blockage by current treatments – such as tocilizumab – would profoundly alter the signaling of other cytokines in these cells. Tocilizumab (TCZ) is a recombinant humanized antihuman IL-6 receptor monoclonal antibody (148 kDa) that inhibits the binding of IL-6 to m-IL-6R or sIL-6R, blocking the IL-6 activity [20]. To study our hypothesis, first, we compared the phosphorylation pattern of STAT1, STAT 3, STAT 5 and STAT 6 in peripheral leukocytes from RA patients and healthy donors. Second, we analyzed changes in the phosphorylation pattern of these STATs in the patients after tocilizumab treatment. To understand the role of the activation status of STATs in the leukocytes from RA patients, we then studied the association between the phosphorylation levels of STATs and the clinical and immunological parameters in these patients.

2. Material & methods

2.1. Patient samples and study design

Peripheral blood samples were obtained from 16 patients meeting the American College of Rheumatology diagnostic criteria for RA [21]. Table 1 gives an overview of the clinical activity and laboratory parameters in patients who received tocilizumab and completed the study (N = 14). In all patients, RA was refractory to standard treatment with disease-modifying anti-rheumatic drugs (DMARDs), including methotrexate. Tocilizumab treatment was begun following European and Spanish guidelines [22,23]. The study was approved by the ethics committee at Hospital de la Santa Creu i Sant Pau and written consent was obtained from all patients before entering the study, according to the Declaration of Helsinki.

Heparinized blood samples and clinical data were collected prior to infusion at baseline and at weeks 4, 12 and 24 after initiating treatment. Patients were treated with tocilizumab 8 mg/kg every four weeks. Laboratory analysis at all visits included a hemogram, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), immunoglobulins (IgG, IgA and IgM), rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies. Clinical data collected at each visit were DAS28, SDAI, CDAI and EULAR response criteria [24].

Table 1 Demographic and clinical characteristics of RA patients at baseline.

Age; years; mean (range)	54.8 (36–70)
Gender; % women	100
Years of RA; mean (range)	9.6 (3–22)
Positive RF and/or CCP; %	92.9
DAS28; mean (SD)	5.2 (1.0)
SDAI; mean (SD)	26.8 (10)
CDAI; mean (SD)	25.8 (10)
ESR; mm/h; mean (SD)	34 (24.3)
HAQ; mean (SD)	1.5 (0.8)
Previous DMARDs; mean (SD)	2.5 (0.9)
Previous biological therapies; mean (SD)	1.9 (1.3)
Concomitant corticoids; %	64.3
Monotherapy; %	57.1
Concomitant methotrexate; %	28.6
Concomitant leflunomide; %	14.3