



Expression of immunoglobulin-like transcript 4 as an inhibitory receptor in patients with psoriatic arthritis

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Abstract

Objectives: To investigate the presence of immunoglobulin-like transcript (ILT)4 and costimulatory proteins (CD40, CD80 and CD86), as well as tumour necrosis factor (TNF)- α production in antigen-presenting cells (APCs) from patients with psoriatic arthritis, before and after treatment with the antitumour necrosis factor- α therapy, adalimumab.

Methods: Peripheral blood monocytes from patients with psoriatic arthritis and healthy controls were cultured with CD40 ligand (CD40L) to stimulate differentiation to APCs. Cell-surface phenotype was analysed via fluorescence-activated cell sorting.

Results: CD40L-stimulation resulted in significantly more ILT4⁺ monocytes in cultures from control subjects ($n = 21$) than those from patients ($n = 20$). ILT4-positivity on CD40L-stimulated monocytes was negatively correlated with disease activity in patients. Adalimumab treatment resulted in significant increases from baseline in ILT4-positivity, and in decreases in CD40, CD80 and CD86-positivity in monocytes from patients.

Conclusion: The effect of adalimumab on monocyte surface phenotype may be due to modification of the inflammatory milieu associated with therapy-induced reduction of disease activity in psoriatic arthritis.

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Keywords

Antitumour necrosis factor- α , ILT4, immunoglobulin-like transcript 4, innate immunity, psoriatic arthritis, TNF- α

Introduction

Psoriatic arthritis is a chronic inflammatory autoimmune disease characterized by inflammatory arthritis and psoriasis.¹ Inhibitory immunoglobulin-like transcript (ILT) receptors have a negative influence on the stimulatory capacity of antigen-presenting cells (APCs). High levels of inhibitory ILT receptors are found on the surface of all APCs, along with inhibition of nuclear factor- κ B activation and CD40-induced upregulation of costimulatory proteins, with a consequent effect on T-cells.² Although inhibitory ILTs appear to be upregulated in patients with rheumatoid arthritis, little is known about their expression in psoriatic arthritis.³

The aim of the present study was to investigate the presence of ILT4 and costimulatory proteins (CD40, CD80 and CD86), as well as tumour necrosis factor (TNF)- α production, in peripheral blood monocyte-derived APCs from patients with psoriatic arthritis, before and after treatment with the anti-TNF- α therapy, adalimumab.

Patients and methods

Study population

The study recruited adult patients (>18-years-old) with moderate-to-severe psoriatic arthritis (diagnosed according to CASPAR criteria⁴) who were attending the Rheumatology Unit, University of Rome "Tor Vergata", Rome, Italy, between April 2013 and March 2014. Patients were required to be naïve to biological therapies and be eligible for adalimumab treatment. All patients received 40 mg adalimumab via subcutaneous injection every other week.

Blood samples were taken and disease activity was assessed by measuring the disease activity score (DAS)⁵ immediately before the first adalimumab dose and at week 12. A group of matched healthy control subjects were recruited from blood donors attending the University Hospital "Policlinico Tor Vergata", Rome, Italy.

The study protocol was approved by the ethics committee of the University of Rome "Tor Vergata". All patients and healthy subjects provided written informed consent prior to enrolment.

Materials

Soluble trimeric CD40L was purchased from R&D system, San Diego, CA, USA. Lipopolysaccharide (LPS) from *Escherichia coli* 0111/B4 was purchased from Sigma Chemical Co. St. Louis, MO, USA. All compounds and media used in this study were analysed for endotoxin contamination using the limulus amoebocyte lysate test (QCL-1000, BioWhittaker, Inc, Walkersville, MD, USA). All samples were free of endotoxin contamination (<0.1 EU/ml).

Cell culture

Peripheral blood (from controls or patients) was enriched for peripheral blood mononuclear cells (PBMC) by centrifugation at 300g for 30 min at 20°C. over Ficoll Hypaque (GE Healthcare, Life Sciences, Uppsala, Sweden). PBMC were either immediately analysed via fluorescence-activated cell sorting (FACS; see below) or cultured in RPMI-1640 medium supplemented with 20% heat inactivated fetal calf serum (FCS), 2 mM L-glutamine,

50 IU/ml penicillin, 50 µg/ml streptomycin and 500 ng/ml CD40L (complete medium). Cells were plated in 96-well V-bottom plates at a concentration of 5×10^5 cells/well in 250 µl of complete medium and incubated for 72 h at 37°C in a humidified atmosphere of 5% carbon dioxide in air, in the presence or absence of 0.5 µg/ml CD40L, then analysed by FACS.

To evaluate the ability of sera from patients with psoriatic arthritis to modulate ILT4 positivity, buffy coat monocytes were obtained from healthy subjects by elutriation. Elutriated monocytes were plated at 5×10^5 cells/well in 250 µl complete medium without FCS, and cultured for 72 h with the addition of 20% serum from each patient (obtained at baseline just before the first administration of adalimumab) or control, or 20% FCS (negative control). Cells were then analysed by FACS. To evaluate intracellular cytokine production the cells were washed in PBS (pH 7.4) at the end of the incubation period, then incubated with fresh complete medium containing 1 µg/ml of the protein transport inhibitor brefeldin A, with or without 100 ng/ml LPS.

FACS

Cells were washed once with washing buffer (3% FCS and 0.1% sodium azide in phosphate buffered saline [PBS, pH 7.4]), then 2×10^5 cells were incubated for 30 min at 4°C in the dark with: R-phycoerythrin-cyanine (PC5)-conjugated anti-CD14 (Immunotech, Marseille, France), phycoerythrin (PE)-conjugated anti-ILT4 (Immunotech; clone: PN A22334), fluorescein isothiocyanate (FITC)-conjugated anti-CD40, anti-CD80 and anti-CD86 (BD Biosciences, San José CA, USA) (all diluted 1:10). For the determination of intracellular cytokine production, cells were washed with PBS, pH 7.4, then stained with anti-CD14 and anti-ILT4 as described above. Cells were then permeabilized in Cytotfix/Cytoperm™ solution

(BD Pharmingen, San Diego, CA, USA), stained with anti-TNF-α (R&D systems, San Diego, CA, USA; diluted 1:10) for 30 min at room temperature, then analysed by FACS. The negative range and staining specificity for each cell-surface marker and cytokine was established using a corresponding isotype-matched control antibody, conjugated with the same fluorescent dye. Flow cytometry was performed using a FACScan™ flow cytometer and analysed with CellQuest™ software (Beckton Dickinson, Franklin Lakes, NJ, USA). For each analysis, 10^4 events were gated on CD14 expression and a light-scatter gate designed to either include only viable cells (surface markers determination) or exclude cellular debris (determination of intracellular cytokine production).

Statistical analyses

The Kolmogorov–Smirnov goodness-of-fit test was used to assess normality of distribution. Comparisons between distributions of two variables for a single group were performed by Student's unpaired *t*-test or Mann–Whitney *U*-test. Correlations were evaluated using Pearson's parametric test for univariate analysis. Statistical analyses were performed using SPSS® version 10.0 (SPSS Inc., Chicago, IL, USA) for Windows®, and *P* values <0.05 were considered statistically significant.

Results

The study included 20 patients with psoriatic arthritis (six male/14 female; mean age 47.3 ± 11.5 years; age range 23–67 years) and 21 healthy control subjects (11 male/10 female; mean age 44.7 ± 15.7 years; age range 28–59 years). Demographic and clinical characteristics of the study population are given in Table 1.

There was no significant difference between patients and controls in the number of ILT4⁺ monocytes in freshly isolated

Table 1. Demographic and clinical characteristics of patients with psoriatic arthritis and healthy control subjects included in a study investigating the effect of antitumour necrosis factor- α therapy (adalimumab) on the cell-surface phenotype of monocytes

Characteristic	Patients <i>n</i> = 20	Controls <i>n</i> = 21
Age, years	47.3 \pm 11.5	44.7 \pm 15.7
Sex, male/female	6/14	11/10
Disease duration, years	7.2 \pm 6.3	NA
DAS44	4.3 (3.2–5.1)	NA
CRP, mg/l	3.8 (0.1–15.3)	<0.1
ESR, mm/h	38.0 \pm 31.6	<20
NSAIDs	5 (16.8)	NA
Corticosteroids	8 (39.7)	NA
DMARDs	13 (70.2)	NA

Data presented as mean \pm SD, *n* (%) of subjects, or median (interquartile range).

DAS, disease activity score;⁵ NA, not applicable; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NSAIDs, nonsteroidal anti-inflammatory drugs; DMARDs, disease-modifying antirheumatic drugs.

PBMCs. CD40L-stimulation resulted in significantly more ILT4⁺ monocytes in cultures from control subjects than those from patients ($P < 0.005$; Figure 1a). There was no significant increase in ILT4⁺ monocyte numbers following CD40L-stimulation of PBMCs from patients.

Disease activity score was negatively correlated with ILT4⁺ monocyte numbers in CD40L-stimulated cultures from patients ($r = -0.582$, $P = 0.02$; Figure 1b).

When CD40L-stimulated cells were sorted for ILT4, significantly more ILT4⁻ than ILT4⁺ monocytes were positive for CD40, CD80 and CD86 ($P < 0.05$ for each comparison; Figure 1c). In addition, there were significantly more CD40, CD80 and CD86-positive cells in ILT4⁻ monocytes from patients than controls ($P = 0.0048$, $P = 0.0011$ and $P = 0.0074$, respectively; Figure 1c).

When spontaneous TNF- α production was analysed at single-cell level in the total

monocyte population and ILT4⁺ and ILT4⁻ monocyte subsets from patients and controls (Figure 1d), TNF- α production was found to be restricted to ILT4⁻ monocytes. There was no significant difference between patients and controls in TNF- α production by ILT4⁻ monocytes.

Adalimumab treatment resulted in a significant increase in ILT4 positivity and decreases in costimulatory molecule-positive cell numbers compared with baseline ($P < 0.05$ for each comparison; Figure 1e). There was a significant positive correlation between the ILT4-positivity at week 12 and change in DAS44 ($r = 0.571$, $P = 0.02$; Figure 1f).

Discussion

The present study found that CD40L-stimulation resulted in significantly more ILT4⁺ monocytes in PBMC cultures from control subjects than those from patients with psoriatic arthritis. APCs expressing high levels of ILT4 are known to anergize allospecific CD4⁺ CD45RO⁺ CD25⁺ T-cells and convert them into regulatory T (Treg) cells, propagating the T-cell suppression cascade.⁶ The regulatory properties of ILT4 are mediated by downregulation of the costimulatory proteins CD40, CD80 and CD86, hindering their ability to activate CD4⁺ cells.⁷ Thus, the downregulation of ILT4 and upregulation of costimulatory proteins on monocytes from patients with psoriatic arthritis may suggest that these cells acquire an activated/nontolerogenic phenotype that may contribute to abnormally high APC activation. Inappropriate APC activity in affected joints, together with the accumulation of activated T-cells, may drive the initiation and persistence of T-cell activation.⁸

In conclusion, 12 weeks' treatment with adalimumab resulted in a significant increase in ILT4-positive cells and reduced numbers of CD40, CD80 and CD86-positive cells in monocytes from patients with

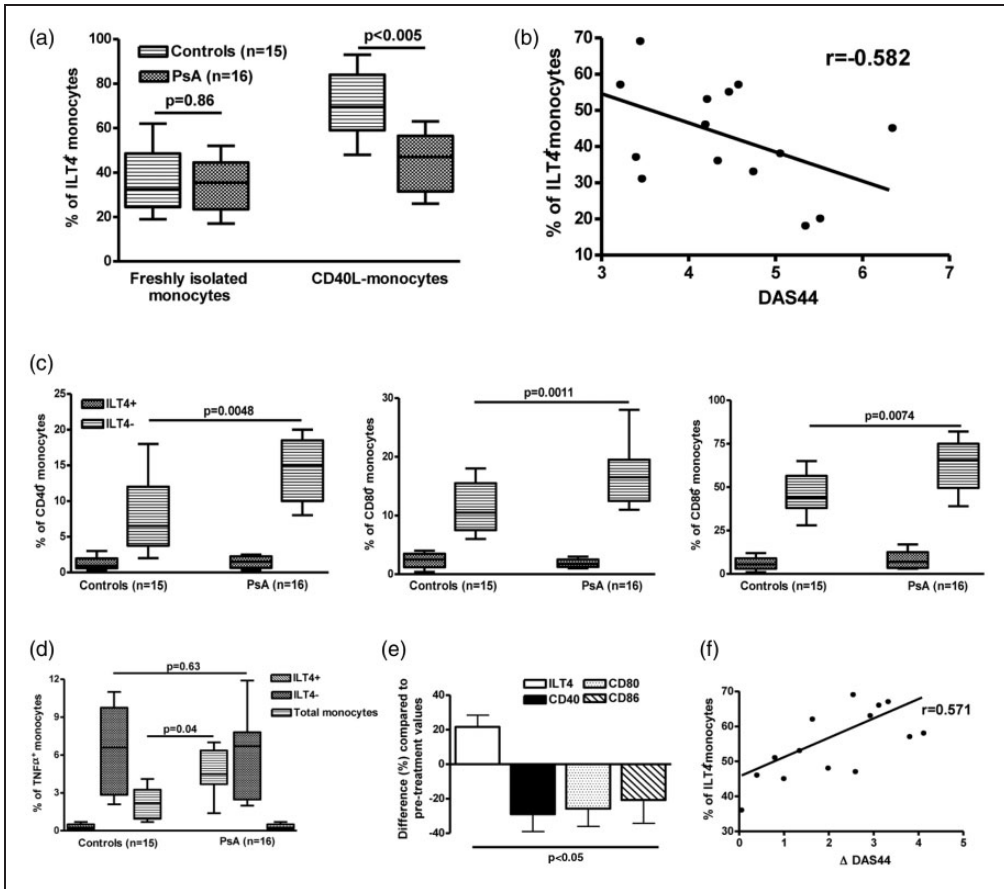


Figure I. (a) Expression of immunoglobulin-like transcript 4 (ILT4) in monocytes from patients with psoriatic arthritis (PsA; $n = 20$) and healthy control subjects ($n = 21$), before and after in vitro treatment with CD40 ligand (CD40L). (b) Correlation between monocyte ILT4 positivity and psoriatic arthritis disease activity score (DAS44⁵). (c) CD40, CD80 and CD86-positivity of ILT4⁺ and ILT4⁻ CD40L-stimulated monocytes from patients and controls. (d) Spontaneous tumour necrosis factor (TNF)- α production by total monocyte population and ILT4⁺ and ILT4⁻ monocyte subsets from patients and controls. (e) Surface phenotype of CD40L-stimulated monocytes from patients after 12 weeks' antitumour necrosis factor- α therapy with adalimumab, compared with baseline phenotype. (f) Correlation between monocyte ILT4 positivity and change in DAS44 after 12 weeks' adalimumab therapy.

psoriatic arthritis. This effect may be related to the ability of adalimumab to block the TNF- α effect in vivo, which may then inhibit ILT4 production by APCs.⁹ However, the direct relationship we found between ILT4-positivity and the change in DAS44 after treatment suggests that the effect of adalimumab on monocyte surface phenotype

may be due to modification of the inflammatory milieu associated with therapy-induced reduction of disease activity.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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