Concise Report

Increased expression of CD154 (CD40L) on stimulated T-cells from patients with psoriatic arthritis

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Objectives. CD40L is a costimulatory molecule and an early activation marker of T-lymphocytes. Based on the hypothesis that activated T-cells may play a role in the pathogenesis of psoriatic arthritis (PsA), we evaluated the level of CD40L expression on T-cells from patients with PsA.

Methods. We analysed 12 patients with PsA, six patients with rheumatoid arthritis (RA) and four healthy volunteers. T-cell surface expression of CD40L was evaluated using two-colour flow cytometry in (i) the resting state and (ii) following stimulation with phorbol myristate acetate/ionomycin, with or without ciclosporin (CsA)-mediated inhibition.

Results. Expression of CD40L was significantly increased on activated T-cells from patients with PsA, particularly those with active disease, when compared with normal individuals and patients with RA (mean percentages of CD3+ CD40L+ cells: 23.74, 11.59 and 9.57% for patients with active PsA, patients with RA and healthy volunteers, respectively). CsA-mediated inhibition of CD40L induction was equally effective in all study groups.

Conclusion. CD40L is overexpressed on T-cells from patients with active PsA. This may indicate a role for CD40L in PsA pathogenesis. Larger-scale studies are warranted to address these issues.

Key words: Psoriatic arthritis, CD40L, CD154, Ciclosporin, T-cells.
also had a Disease Activity Score (DAS)28 score \( \leq 2.6 \) (average 2.0), whereas all patients with active disease had a DAS28 score \( \geq 2.6 \) (average 4.7). Patients with RA fulfilled the American College Rheumatology (ACR) revised classification criteria for RA. Five patients had active disease with a DAS28 score \( \geq 2.6 \) (average 3.9). None of the patients received steroids during the last 24 h before blood was drawn. Written informed consent according to the Declaration of Helsinki was obtained from all participating individuals; the study protocol was approved by the Patras University Hospital Ethics Committee.

**Flow cytometry**

Peripheral blood mononuclear cells (PBMCs) were isolated using standard methods. Murine anti-human fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies (mAbs) used were anti-CD3-FITC, anti-CD4-FITC, anti-CD8-FITC, anti-CD40L-PE and their respective isotypic control mAbs (all from BD Pharmingen, a kind gift from Novartis Hellas, Greece). Freshly isolated PBMCs (5 \( \times 10^6 \)) were stained with saturating concentrations of mAbs or their respective isotypic control mAbs according to the manufacturer’s instructions. Two-colour flow cytometry was performed using a Beckman Coulter flow cytometer. For each sample, the percentage of cells positive for CD3 and CD40L as well as the CD40L mean channel fluorescence (MCF) for the gated CD3\(^+\)CD40L\(^+\) cells were recorded. Analysis of results was performed using the Epics-XL software.

**Short-term cell cultures**

PBMCs were incubated at 37°C for 6 h in culture medium [RPMI 1640/20% fetal bovine serum (FBS)]. PBMCs (3 \( \times 10^6 \)) from each study subject were divided into three separate wells containing (i) culture medium, (ii) culture medium containing 5 ng/ml phorbol myristate acetate (PMA) and 500 ng/ml ionomycin and (iii) culture medium with PMA, ionomycin and CsA 100 ng/ml (Novartis). The 6-h time point was optimal, based on the results of time-response curve experiments (data not shown). Evaluation of the optimal doses of CsA for maximum CD40L inhibition was made based on a dose–response curve (data not shown).

**Statistical analysis**

Data were analysed using the unpaired two-tailed Student’s \( t \)-test. \( P \)-values \( \leq 0.05 \) were considered to be statistically significant.

**Results**

**Expression of CD40L on freshly isolated peripheral T-cells from patients with PsA**

The mean CD40L MCF values of the gated CD3\(^+\)CD40L\(^+\) cells were 1.69 \( \pm 0.64 \), 1.82 \( \pm 0.79 \) and 1.58 \( \pm 0.35 \) for patients with PsA, disease-control patients and healthy volunteers, respectively. Statistical analysis revealed no significant differences between the three groups (Fig. 1A). The mean percentages of CD3\(^+\)CD40L\(^+\) cells were similar in the three different study groups as well.

We also examined whether disease activity correlated with the expression of CD40L on freshly isolated peripheral T-lymphocytes. The mean CD40L MCF was 1.52 \( \pm 0.53 \) for the active PsA group and 1.92 \( \pm 0.76 \) for the inactive group. The mean percentages of CD3\(^+\)CD40L\(^+\) cells were 1.73 \( \pm 1.19 \) and 0.65 \( \pm 0.41 \) for the active and the inactive group, respectively. No statistically significant differences were detected between the two groups indicating that in PsA, disease activity does not correlate with the baseline expression of CD40L on unmanipulated resting peripheral T-cells.

**Expression of CD40L on stimulated T-cells from patients with PsA**

We analysed CD40L induction following optimal \textit{in vitro} stimulation with PMA/ionomycin as described in ‘Patients and methods’ section. Induced expression of CD40L on CD3\(^+\) T-cells from the patients with PsA (both active and inactive) was significantly increased when compared with normal individuals (8.23 \( \pm 1.70 \) vs 5.89 \( \pm 2.37 \), respectively; \( P = 0.04 \)). When we compared PsA patients (both active and inactive) with active RA patients, we found that patients with PsA had a higher mean CD40L MCF than patients with RA, but this difference did not reach statistical significance (8.23 \( \pm 1.70 \) vs 6.95 \( \pm 2.56 \), respectively, \( P = 0.17 \)) (Fig. 1B).

However, when only the samples from active PsA patients were used in the above comparisons, statistically significant differences were detected not only with healthy volunteers (\( P < 0.01 \)) but also with active RA patients (\( P = 0.05 \)).

We also analysed the percentages of CD3\(^+\)CD40L\(^+\) cells following PMA/ionomycin-induced stimulation. The mean percentages of double-positive cells were 19.88 \( \pm 10.65 \), 11.59 \( \pm 6.27 \) and 9.57 \( \pm 6.60 \) for patients with PsA, disease-control patients and healthy volunteers, respectively. Although patients with PsA displayed higher mean percentages of CD3\(^+\)CD40L\(^+\) cells when compared with the RA patients and the healthy volunteers, differences did not reach statistical significance. Nevertheless, the percentages of CD3\(^+\)CD40L\(^+\) cells from patients with active PsA were significantly increased compared with those from disease-control patients (\( P = 0.04 \)) and normal volunteers (\( P = 0.04 \)). One such representative experiment is depicted in Fig. 2.

**CsA-mediated inhibition of CD40L induction**

The mean CD40L MCF values after \textit{in vitro} stimulation, in the presence of CsA, were 2.85 \( \pm 1.77 \), 3.62 \( \pm 2.66 \) and 2.39 \( \pm 1.57 \) and the mean percentages of CD3\(^+\)CD40L\(^+\) cells were 3.20 \( \pm 2.69 \), 2.43 \( \pm 1.87 \) and 2.46 \( \pm 2.36 \) for patients with PsA, patients with RA and normal individuals, respectively. The differences were not significant. These results indicate that CsA-mediated inhibition of CD40L induction is equally effective in patients with PsA as it is in patients with RA and healthy volunteers (Fig. 1C).

To determine whether disease activity had any effect on the inhibitory action of CsA, we compared patients with active and inactive PsA. The mean CD40L MCF values after stimulation, in the presence of CsA, were 2.56 \( \pm 1.51 \) and 3.25 \( \pm 2.22 \) for the active and the inactive group, respectively. The difference was not significant (\( P = 0.53 \)), indicating that the inhibitory capacity of CsA on CD40L induction is retained in T-cells from patients with PsA, irrespective of disease activity.

**Expression of CD40L on CD8\(^+\) cells from patients with PsA**

We also studied the expression of CD40L on CD8\(^+\) cells. The percentages of CD8\(^+\)CD40L\(^+\) cells after stimulation were remarkably low in all the three groups (mean \( \pm \) s.d.: 1.02 \( \pm 1.15 \), 0.51 \( \pm 0.30 \) and 1.01 \( \pm 0.66 \) for patients with PsA, RA and healthy individuals, respectively). Since the percentages of CD8\(^+\) T-cells expressing CD40L following \textit{in vitro} stimulation were so strikingly low and the percentage of CD3\(^+\) T-cells that were double-negative for CD4 and CD8 was negligible [ranging from undetectable to \(< 1% \) in all experiments (data not shown)], we conclude that the vast majority of CD3\(^+\) T-cells expressing CD40L after stimulation in our experiments were indeed CD4\(^+\) T-cells.

**Effect of anti-TNF-\( \alpha \) treatment on CD40L expression**

Seven out of 12 patients with PsA had been receiving anti-TNF-\( \alpha \) treatment, therefore we evaluated whether this treatment had any
effects on CD40L expression. Blood was drawn immediately before intravenous administration of infliximab on the day of scheduled anti-TNF-α infusion. We found no differences between the patients who were treated with anti-TNF-α and those who were not, in terms of percentages of CD3⁺CD40L⁺ cells and of CD40L MCF in resting, in vitro-stimulated and CsA-inhibited PBMCs.

Discussion

This is the first report indicating that T-cells from patients with PsA overexpress CD40L following in vitro stimulation, compared with normal subjects. We have also demonstrated that T-cells from patients with active PsA display higher levels of CD40L compared not only with normal subjects, but also with patients having RA as well. Nevertheless, one should take into consideration that the number of RA patients analysed was small; evaluation of a larger cohort of RA patients in future studies is desirable. We report that significant differences in CD40L expression were observed only following in vitro activation. Enhanced induction of CD40L in patients with PsA may result from a potentially lower threshold for activation of T-cells from patients with active disease, an effect that might play a role in disease pathogenesis. Since patients with active or inactive

Fig. 1. (A) Average CD40L MCF of CD3⁺CD40L⁺ resting cells. (B) Average CD40L MCF of CD3⁺CD40L⁺ cells after stimulation. Patients with active PsA display significantly higher CD40L MCF when compared with healthy donors (P < 0.01) and with patients having RA (P = 0.05). (C) Average CD40L MCF of stimulated CD3(+)CD40L(+) cells in the presence of CsA-mediated inhibition.
PsA received similar medications we propose that differences in CD40L induction are not drug-mediated. In addition, medications employed in the PsA and the RA patient groups were also similar; therefore differences in CD40L induction between these two groups cannot be explained by differences in treatment regimens.

The strong inhibitory effect of CsA on CD40L induction in normal T-cells is well-documented. In our study, we demonstrated that CsA-mediated inhibition of the inducible expression of CD40L on T-cells from patients with PsA and RA is similar to that of normal T-cells. Therefore, based on our data we propose that the inhibitory capacity of CsA is intact irrespective of disease activity. These data could probably explain in part the beneficial effects of CsA in the treatment of both PsA and psoriasis. We also had the opportunity to study one patient with PsA, before and after 12 weeks of therapy with CsA (3 mg/kg) in order to assess the effects of CsA therapy on the inducible in vitro CD40L expression. We found no differences in terms of percentages and CD40L MCF in resting, stimulated and inhibited with CsA cells, before and after CsA treatment.

Overexpression of CD40L on activated T-cells from patients with PsA reinforces the hypothesis that T-cells may play a key role in PsA pathogenesis. Since T-cell-directed biological therapies [18, 19] seem to be effective in psoriasis and in PsA, and CD40L is overexpressed in activated T-cells from patients with PsA, it is likely that a therapy that selectively targets CD40L could benefit these patients. In addition CD40-CD40L interactions are also thought to regulate a number of functions that may be important in PsA pathogenesis, such as increased interleukin-12 (IL-12) production, a key-inducer of Th1 responses by antigen presenting cells [20, 21], the induction of proinflammatory cytokines and the up-regulation of adhesion molecules [22, 23]. The results of our study suggest a potential role of the CD40-CD40L pathway in the pathogenesis of PsA. Since our study analysed small numbers of patients we propose that larger-scale studies are needed.

![Rheumatology Diagram](https://example.com/rheumatology_diagram)

**Key messages**
- Activated T-cells from patients with PsA overexpress CD40L.
- Ciclosporin A is a potent in vitro inhibitor of CD40L induction on T-cells from patients with PsA.

**Fig. 2.** CD40L expression on the surface of whole (CD3+) T-cells. Dot plots from one representative experiment analysing three study individuals, a healthy donor (left panel) a patient with RA (middle panel) and a patient with PsA (right panel). FL1 represents CD3-FITC and FL2 represents CD40L-PE fluorescence.
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References