Toll-like receptor (TLR) 2 is upregulated on peripheral blood monocytes of patients with psoriatic arthritis: a role for a gram-positive inflammatory trigger?

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Abstract

Toll-like receptor (TLR) 2 and TLR4 are able to activate innate immune cells in response to gram-positive and gram-negative bacteria, respectively. Psoriatic arthritis (PsA) is a chronic inflammatory joint disease and gram-positive streptococcus *may have a role in its pathogenesis, suggesting the importance of TLR2 stimulation in PsA.*

Objective

To assess TLR2 and TLR4 expressions on innate immune cells of PsA patients, relating to clinical disease activity.

Methods

Forty-five patients with peripheral joint manifestations of PsA were included and disease activity was assessed by Disease Activity Score of 28 joint counts (DAS28). 32 healthy subjects constituted the control group. Membrane-bound TLR2 and TLR4 expressions were assessed on peripheral blood monocytes and neutrophils by flow cytometry.

Results

Twenty-seven patients had active PsA (DAS28 higher than 2.6) and 18 had inactive disease. TLR2 was significantly upregulated on monocytes in both active and inactive PsA group, comparing to healthy controls. TLR4 was similarly expressed in all tested groups.

Conclusion

TLR2 is overexpressed by PsA monocytes, suggesting that gram-positive exposure could induce higher inflammatory responses in this disease.

Key words

psoriatic arthritis, toll-like receptors, innate immunity, monocytes, neutrophils

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Introduction

Spondyloartritis (SpA) are a group of chronic inflammatory diseases characterised by axial and peripheral arthritis and enthesitis in close association with HLA-B27 (1). Its pathogenesis remains largely unclear, but an important clue is provided by the relation of environmental triggers and genetic background. In reactive arthritis, a subtype of SpA, chronic disease is clearly triggered by a precedent gastrointestinal or urogenital bacterial infection (2), and germ-free state prevents the development of SpA features in the HLA-B27 transgenic rat model (3). It is proposed that microbial products are presented to CD8+ lymphocytes in the context of HLA-B27+ antigen-presenting cells, initiating the inflammatory response (4).

Additionally, besides T cell antigen recognition, human innate immune cells may act in direct response to bacterial components through a system that primarily comprises Toll-like receptors (TLR) (5). TLR2 and TLR4 are extracellular receptors that recognise, respectively, gram-positive and gram-negative bacterial lipid-based structures (lipoteichoic acid and lypolysaccharide, in that order) (6). Toll-like receptor activation on monocytes and neutrophils ultimately results in cellular activation and production of several stimulating cytokines (7, 8), which would contribute to the development and maintenance of the pro-inflammatory background of some chronic inflammatory diseases.

Psoriatic arthritis (PsA) is a subtype of SpA and there is subtle evidence of its link to gram-positive bacteria exposure. Vasey *et al*. proposed that, if some bacteria are important in triggering PsA, they may be those comprising skin flora, and found that levels of antibodies against streptococcal exotoxin are higher in patients with PsA comparing to healthy controls and patients with rheumatoid arthritis (9). These findings were further confirmed: higher levels of antibodies to different streptococcal cell wall components were also found in patients with PsA and cutaneous psoriasis (10). Regarding innate immune stimulation by bacterial components, it was observed that TLR2 expression is upregulated in psoriatic skin lesions(11). TLR2 was also found to be overexpressed on immature dendritic cells obtained from patients with active PsA (12). Thus, it has been hypothesized that inappropriate gram-positive overstimulation on the innate immune system may have a role on PsA pathogenesis. To reaffirm this hypothesis and correlate these findings with disease activity, we intended to assess the expressions of TLR2 and TLR4 on peripheral blood monocytes and neutrophils from patients with active and inactive PsA, comparing to healthy controls.

Methods

Patients and controls

Our study group included PsA patients who were followed at the SpA outpatient clinic at the Rheumatology Division of the Hospital das Clinicas, the teaching hospital of the University of Sao Paulo, Brazil. All patients met CASPAR criteria for the diagnosis of PsA (13). Only patients with peripheral disease were included (RA-like, asymmetric oligoarthritis, distal interphalangeal arthritis or destructive arthritis). Patients with isolated axial disease were not included. The Disease Activity Score of 28 joint counts (DAS28) was used to assess clinical disease activity (14, 15). Active PsA was considered when DAS28 value was higher than 2.6. Demographic and medication data were also recorded. Healthy volunteers recruited from laboratory and hospital staffs were included as a control group. Our study design was consistent with the principles of the Declaration of Helsinki and received institutional ethics committee approval (CAPPesq, protocol number 0533/07). Written informed consent was obtained from all subjects prior to study commencement.

Assessment of membrane-bound expressions of TLR2 and TLR4

Venous blood samples were taken from PsA patients and normal controls in EDTA tubes. Two hundred microlitres of each sample was placed in tubes containing 2 ml of 0.1% azide in phosphate-buffered saline (PBS), centrifuged at 2000 rpm for 3 minutes and mixed with 50 μl of 2% foetal calf serum (FCS) and

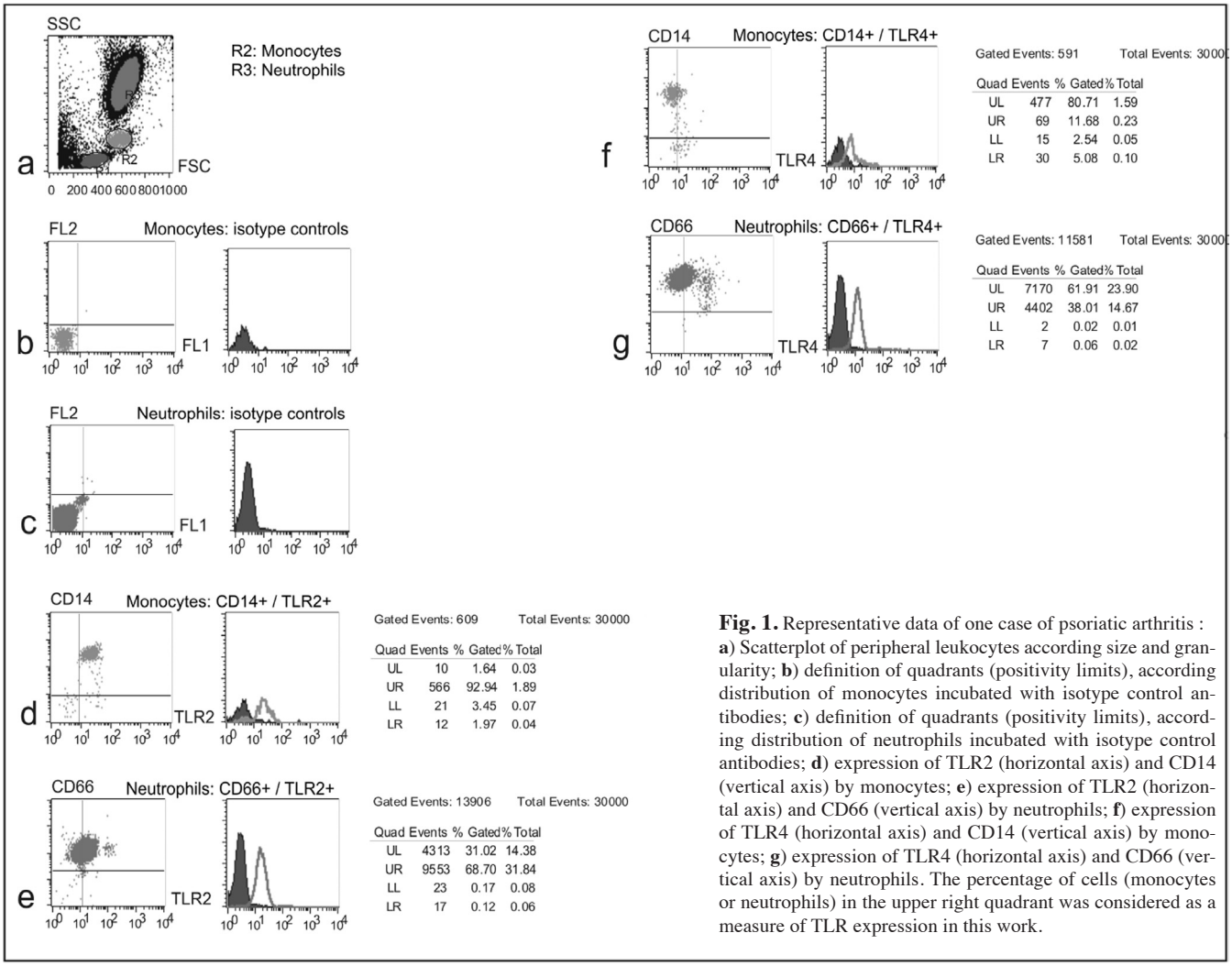

Fig. 1. Representative data of one case of psoriatic arthritis : **a**) Scatterplot of peripheral leukocytes according size and granularity; **b**) definition of quadrants (positivity limits), according distribution of monocytes incubated with isotype control antibodies; **c**) definition of quadrants (positivity limits), according distribution of neutrophils incubated with isotype control antibodies; **d**) expression of TLR2 (horizontal axis) and CD14 (vertical axis) by monocytes; **e**) expression of TLR2 (horizontal axis) and CD66 (vertical axis) by neutrophils; **f**) expression of TLR4 (horizontal axis) and CD14 (vertical axis) by monocytes; **g**) expression of TLR4 (horizontal axis) and CD66 (vertical axis) by neutrophils. The percentage of cells (monocytes or neutrophils) in the upper right quadrant was considered as a measure of TLR expression in this work.

0.1% azide-PBS. Afterwards, cell samples were incubated for 20 minutes with unconjugated anti-human Fc Receptor (FcR Block Reagent, eBioscience, San Diego, CA, USA) to avoid subsequent unspecific adhesion of labelled monoclonal antibodies (mAb) to Fc receptors on innate immune cells. Following the manufacturer's instructions (BD Biosciences, San Jose, CA, USA), cells were stained with the following mAbs: phycoerythrin (PE) conjugated mouse anti-human CD66 mAb, as a specific marker of PMN (FL2), PerCP-Cy5.5 conjugated mouse anti-human CD14 mAb (FL3) and Alexa-Fluor® conjugated mouse anti-human TLR2 or biotinylated mouse anti-human TLR4 mAb (added to streptavidin labelled with fluorescein isothiocyanate – FITC) or isotype control antibody for 20 minutes. Erythrocytes were lysed with appropriated lysing solution (BD Biosciences), and stained cells were washed twice in 0.1% azide-PBS solution and fixed in 1% paraphormaldeyde prior to flow cytometric analysis. Ten thousand cells from each sample were then analysed in a three-fluorescence detector FACScalibur™ device with CellQuest™ software (BD Biosciences). PMN and monocyte regions were gated in the forward scatter/side scatter chart. In each specific region, PMNs and monocytes were defined as CD66+ or CD14+ cells, respectively. After that, the percentage of cells expressing TLR2 or TLR4 was counted, as the number of cells in the upper right quadrant. Positivity limits for dot plot chats were defined using isotype antibody controls. Representative data of one case are presented in Figure 1.

Statistical analysis

All analyses were performed with the SPSS 15.0 for Windows® (SPSS, Chicago, IL, USA) statistical software package. Comparisons between groups were carried out using the Mann-Whitney U-test and results are expressed as median ± interquartile range, consistent with a non-parametric distribution as assessed by the Shapiro-Wilk test. *P*-values under 0.05 were considered significant.

Results

Forty-five patients with PsA (22 women, 23 men, mean age 52±13 years, mean disease duration 15±10 years) were included. Twenty-seven patients were considered with active disease (mean DAS 28 score 3.75) and 18 patients had inactive PsA (mean DAS 28 score 1.76). Four patients with active PsA and two patients with inactive PsA were in use of anti-TNF-α agents. Only one patient was in remission state without medication and the oth-

Table I. Expressions of TLR2 and TLR4 by circulating monocytes and neutrophils from patients with PsA , active PsA, inactive PsA and controls.

	TLR 2		TLR 4	
	Monocytes (%)	Neutrophils (%)	Monocytes (%)	Neutrophils (%)
PsA *vs*. controls	89 ± 17 *vs*. 71 ± 49 *p*=0.002*	6 ± 29 *vs*. 18 ± 31 *p*=0.07	2 ± 9 *vs*. 1 ± 3 *p*=0.23	2 ± 9 *vs*. 1 ± 3 *p*=0.48
Active PsA *vs*. controls	90 ± 17 *vs*. 71 ± 49 *p*=0.001*	5 ± 39 *vs*. 18 ± 31 *p*=0.15	2 ± 4 *vs*. 1 ± 3 *p*=0.33	2 ± 7 *vs*. 1 ± 3 *p*=0.69
Inactive PsA *vs*. controls	86 ± 18 *vs*. 71 ± 49 *p*=0.04*	8 ± 24 *vs*. 18,50 ± 31 *p*=0.10	2 ± 15 *vs*. 1 ± 3 *p*=0.29	2 ± 11 *vs*. 1 ± 3 *p*=0.42
Active PsA *vs*. inactive PsA	90 ± 17 *vs*. 86 ± 18 *p*=0.10	5 ± 39 *vs*. 8 ± 24 *p*=0.74	2 ± 4 *vs*. 2 ± 15 *p*=0.83	2 ± 7 *vs*. 2 ± 11 *p*=0.66

PsA: psoriatic arthritis. All *p*-values obtained by Mann-Whitney U-test.

ers were in use of traditional disease-modifying anti-rheumatic drugs, some of them in combination (DMARDs) and non-steroidal anti-inflammatory drugs (NSAIDs): 23 on methotrexate (15 active and 8 inactive PsA), 4 on sulphasalazine (3 active and 1 inactive PsA), three on leflunomide (all active PsA) and one on cyclosporine (active PsA). Three patients with active PsA and five patients with inactive PsA were in use of NSAIDs without DMARDs. Thirty-two healthy subjects were included as a control group.

Table I demonstrates the expression of TLR2 and TLR4 on peripheral monocytes and neutrophils from PSA patients and controls. Expression levels of TLR2 was significantly higher on monocytes from PsA patients than on monocytes from healthy controls (89%±17% *vs*. 71%±49%, *p*=0.002). Both active PsA (90%±17% *vs*. 71%±49%, *p*=0.001) and inactive PsA patients (86%±18% *vs*. 71%±49%, *p*=0.004) revealed higher TLR2 expression on monocytes than controls. There was no significant difference between active and inactive PsA groups regarding TLR2 expression on circulating monocytes.

In contrast TLR4 was similarly expressed on monocytes of all groups of patients and controls. Furthermore on neutrophils, both TLR2 and TLR4 were similarly expressed in patients and controls. All data are presented in Table I.

Discussion

Promising recent reports point out to the potential role for TLRs in a variety of inflammatory disorders (16-18). Studies in psoriasis have shown that normal epidermal keratinocytes constitutively express TLR2 (19), while psoriatic skin lesions overexpress this receptor in comparison to normal skin (11). Because gram-positive bacteria such as *streptococcus* may have a relevant role in inducing PsA (9, 10), skin overexpression of TLR2 may be involved in the mechanism linking bacterial exposure and cutaneous inflammation in this disease.

In PsA, Candia *et al*. (12) reported that TLR2 expression (but not TLR4) was upregulated in immature dendritic cells obtained on cultured monocytes from active PsA patients, thus suggesting that gram-positive bacterial triggering and TLR2 overexpression also specifically contribute to the imunopathogenic process involved in joint disease related to psoriasis. On the other hand, in ankylosing spondilitis (AS, the prototype form of SpA) both TLR2 and TLR4 were found to be upregulated in peripheral blood monocytes (20). Thus, one can suggest that while PsA inflammation is specifically exacerbated by gram-positive stimulation via TLR2, AS inflammation may be boosted by gram-negative stimulation through TLR4. This hypothesis makes sense considering the cutaneous source of bacterial stimulation in psoriasis, mainly gram-positive (9), while in AS gut inflammation and increased gut permeability to gram-negative intestinal flora may play a role in its pathogenesis (21).

Our results give additional support to this hypothesis, revealing that only TLR2 is upregulated on circulating monocytes of PsA patients irrespective of disease activity status. This could provide a preferential gram-positive overstimulation in PsA, which would lead to inflammatory activation. Probably Candia *et al*. (12) did not observe TLR2 overexpression in circulating monocytes (but only in immature dendritic cells) due to the small number of patients evaluated (only ten patients were considered in their study.

Our data and those from Candia *et al*. suggest that TLR2 overexpression in PsA may be a preferential route for bacterial triggering of the innate immune system (12). Reinforcing this idea, De Rycke et al. observed that anti-TNF-α blockade sharply abolishes TLR2 and TLR4 hyperexpression in AS patients (20). It suggests that TLR overexpression may have in fact a role in the inflammatory responses observed in these diseases. In our study, anti-TNF-α agents were similarly used in 4/27 active and 2/18 inactive PsA patients and did not influence our results.

In conclusion, the demonstration of TLR2 overexpression on circulating monocytes from patients with PsA may suggest a role of gram positive microroganisms triggering inflammatory responses in this disease. Functional studies are clearly warranted to clarify the pathological significance of TLR stimulation in PsA.

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