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Abnormal T-cell function in patients with psoriatic arthritis: evidence for decreased interleukin 2 production

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Summary. Psoriatic arthritis (PSA) is an inflammatory arthritis associated with psoriasis. Although not considered an autoimmune process, there is evidence for humoral and cellular immune abnormalities similar to autoimmune diseases such as rheumatoid arthritis and systemic lupus (SLE). We investigated mitogen-induced proliferation and interleukin 2 (IL-2) production by peripheral blood mononuclear cells in patients with PSA. Both IL-2 production and proliferation were significantly decreased in PSA patients when compared to controls. Increased arachidonic acid metabolism has been reported in skin and peripheral mononuclear cells of patients with psoriasis and PSA. We therefore also investigated the effect of indomethacin and prostaglandin E_2 (PGE_2) on IL-2 production. Addition of indomethacin to cultures did not significantly change IL-2 production in patients with PSA, but did so in controls. PGE_2 produced a significant reduction in IL-2 production in PSA and in controls.

Key words: Psoriatic arthritis – Interleukin 2 – Prostaglandins

Introduction

Psoriatic arthritis (PSA) is an inflammatory arthritis that occurs in 5–10% of patients with psoriasis [1]. Although generally not considered an autoimmune disorder, there are features of this disease that suggest such a process. Serum immunoglobulins are elevated and circulating immune complexes have been detected in PSA [2, 3]. An increase in antinuclear antibodies has also been reported in these patients [4]. These findings suggest hyperactive B-cell function similar to that found in other autoimmune disorders such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

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There is also evidence for abnormal cell-mediated immune function. Blastogenic responses to mitogenic stimuli have been shown to be decreased in patients with PSA, but not in patients with psoriasis alone [5]. Moreover, alterations in T-cell subpopulations have been noted in patients with psoriasis and those with PSA [6, 7].

Several studies have documented impaired interleukin 2 (IL-2) production by peripheral mononuclear cells (MNC) in patients with autoimmune disorders such as RA and SLE [8–10]. We investigated IL-2 production in patients with PSA to determine any similarity with other autoimmune diseases. We also studied the effect of indomethacin and prostaglandin E_2 (PGE_2) on IL-2 production.

Materials and methods

Patient selection. Patients had evidence of psoriasis associated with an inflammatory peripheral arthritis and met criteria for the diagnosis of PSA [11]. All patients were taking nonsteroidal anti-inflammatory agents (NSAIDs) but none were being treated with steroids or immunosuppressants. Controls consisted of 12 healthy individuals ranging in ages from 24 to 50 years. Statistical analysis was performed using a two-tailed Student's *t*-test.

Cell cultures. MNC were separated from whole blood by Ficoll-Hypaque centrifugation. MNC suspensions were adjusted to 1×10^6 viable cells/ml in culture medium containing RPMI 1640 with 10% heat-inactivated fetal calf serum. Cells were cultured in 24-well tissue culture plates (Gibco, New York). Concanavalin A (Con A; Sigma, St. Louis, Mo.) was included at initiation of cultures at a final concentration of 5 μg/ml. Preliminary studies suggested that this was the optimal concentration. Control wells in which no Con A was added were also prepared. Indomethacin (Sigma) was dissolved in 100% ethyl alcohol, diluted in culture medium and added at initiation of cultures at a concentration of 5 μg/ml to separate wells containing Con A. PGE_2 (Sigma) was diluted in complete medium and added at initiation of cultures at a concentration of 1 μg/ml to separate wells containing Con A. Cell cultures were incubated in humidified 5% CO_2 at 37° C. Supernatants were harvested daily for 4 days, filtered and stored at − 60° C.

Lymphocyte proliferation. One hundred microliters of MNC were cultured in 96-well plates (Falcon, N. J.) with 150 μl of complete

medium containing Con A alone or with indomethacin (IND) or PGE_2 at the indicated concentrations. Proliferation was measured by incorporation of ^{125}I-labeled iododeoxyuridine (1 μCi) (ICN Biomedical, Calif.) which was added to wells 4 h before harvesting onto glass fiber filter paper and counted in a Beckman Gamma 4000 gamma counter. Results are expressed as net increase in counts per minute (cpm in stimulated cells – cpm in unstimulated cells).

IL-2 measurement. Culture supernatants were assayed for IL-2 using the IL-2-dependent cell line, CTLL. CTLL cells were washed free of growth medium and resuspended in complete medium at a viable cell concentration of 2×10^5/ml. Fifty microliters of cell suspension was added to 96-well plates containing 50 μl of doubling dilutions of test samples and controls. Plates were incubated for 24 h and pulsed with ^{125}I-labeled iododeoxyuridine (0.5 μCi) prior to harvesting. IL-2 units were calculated by probit analysis. Units of activity were calculated using Con A-stimulated BALB/c mouse spleen cell supernatant designated as 100 units/ml of IL-2 activity [12, 13].

IL-4 measurement. Culture supernatants from Con A only stimulated MNC from controls and PSA patients were assayed for the presence of IL-4 using an ELISA kit (Genzyme, Cambridge, Mass.). Four controls and five PSA patients were tested in duplicate. The method is capable of detecting 0.045-3 ng/ml of natural IL-4/ml.

Table 1. T-cell-subset analysis on patients with psoriatic arthritis (% of cells stained)

Patient	CD8	CD4	CD4/CD8	Tac CD8	Tac CD4
1	14	51	3.6	0	1
2	6	53	8.6	0	0
3	19	60	3.2	0	1
4	8	52	6.5	0	2
5	15	54	3.6	0	4
6	25	48	1.9	0	2
7	14	53	3.8	0	0
8	17	46	2.7	0	0
Mean	14.6 ± 5%[1]	52.1 ± 4%[2]	4.2 ± 2[3]	0[4]	2 ± 1.2%[4]

[1] Mean CD8 population significantly lower than controls ($P < 0.01$)
[2] Mean CD4 population not significantly different from controls
[3] CD4/DC8 ratio significantly higher than controls ($P < 0.01$)
[4] In controls, Tac+ cells were < 1%. Tac+ cells identified by two-color flow cytometry

T-cell-subset analysis. Aliquots of MNC were centrifuged and washed using phosphate-buffered saline (PBS) with 0.1% bovine serum albumin. Cells were then incubated with fluorescein and/or rhodamine-conjugated monoclonal antibodies to the following antigens: CD8, CD4, CD3 (Becton Dickinson, Calif.) and Tac (T-cell Sciences, Mass.). Controls included irrelevant monoclonal antibodies of the same immunoglobulin class and evaluation of contamination with monocytes/macrophages using anti-human Leu-M3 antibodies (Becton Dickinson). Analysis was performed by flow cytometry (Epics V FACS, Coulter).

Results

T-cell-subset analysis (Table 1)

Patients with PSA demonstrated a significant decrease in the percentage of CD8-positive cells with respect to controls ($P < 0.01$). CD4-positive cells were not significantly different from controls. The CD4/CD8 ratio was significantly greater in patients with PSA ($P < 0.01$). Five of the eight PSA patients tested (63%) demonstrated IL-2 receptor-positive T-cells (CD4-positive population only).

Proliferative responses (Table 2)

Mean net cpm in Con A only stimulated cultures was significantly lower in patients with PSA during all 4 days of culture when compared to controls ($P < 0.01$ at 24–72 h; $P < 0.02$ at 96 h). The addition of IND to PSA cultures induced a significant increase in net cpm at 48 h ($P < 0.01$), 72 h ($P < 0.05$) and 96 h ($P < 0.02$) when compared to Con A only PSA cultures. A similar effect was also noted in control cultures. When comparing control and PSA cultures with IND, PSA patients still had lower proliferation than controls. The addition of PGE_2 to PSA cultures produced a significant decrease in proliferation at 24 h ($P < 0.05$), 48 h ($P < 0.02$) and at 72 h and 96 h ($P < 0.001$) when compared to Con A only cultures. This effect was not noted in controls (except at 24 h). When comparing control and PSA cultures with PGE_2, proliferation was lower in the PSA group.

Table 2. Proliferative responses (mean change in cpm ± standard deviation)

Culture conditions	Culture time			
Controls ($n = 12$)	24 h	48 h	72 h	96 h
Con A[1]	3325 ± 1556	14 355 ± 2635	16 436 ± 4045	14 391 ± 5843
Con A + IND[2]	2295 ± 1227	19 017 ± 2471	19 799 ± 4881	21 211 ± 8118
Con A + PGE[3]	1076 ± 411	13 377 ± 1639	16 104 ± 3304	11 761 ± 6192
PSA ($n = 14$)				
Con A[1]	2106 ± 826	10 102 ± 1290	12 560 ± 3218	12 093 ± 3108
Con A + IND[4]	1985 ± 564	13 675 ± 3097	14 564 ± 3565	14 985 ± 2908
Con A + PGE[5]	1931 ± 254	8076 ± 2780	10 090 ± 2186	9099 ± 2105

Con A, Concanavalin A; IND, indomethacin; PGE, prostaglandin E; PSA, psoriatic arthritis
[1] Significant difference between Con A in controls vs PSA at 24–72 h ($P < 0.01$) and at 96 h ($P < 0.02$)
[2] IND significantly increased proliferation compared to Con A only at 48 h ($P < 0.01$), at 72 h ($P < 0.05$) and at 96 h ($P < 0.02$)
[3] PGE did not significantly reduce proliferation compared to Con A only except at 24 h
[4] IND significantly increased proliferation at 48 h ($P < 0.01$), 72 h ($P < 0.05$) and at 96 h ($P < 0.01$)
[5] PGE significantly decreased proliferation at 24 h ($P < 0.05$), 48 h ($P < 0.02$) and at 72 and 96 h ($P < 0.001$)

Table 3. Effect of indomethacin and PGE_2 on mean interleukin 2 (IL-2) production (units/ml) in patients with PSA and controls

Culture time	Experimental conditions		
PSA ($n = 14$)	Con A[1]	Con A + IND[2]	Con A + PGE[3]
24 h	4.0 + 3.1	2.9 + 2.0	0.4 + 0.1
48 h	10.5 + 4.0	13.8 + 6.2	1.4 + 0.1
72 h	6.8 + 5.5	7.6 + 3.1	1.6 + 1.1
96 h	2.0 + 1.1	3.2 + 2.5	0.2 + 0.1
Controls ($n = 12$)			
24 h	4.5 + 2.1	4.4 + 2.0	0.5 + 0.4
48 h	14.8 + 3.4	20.4 + 4.7	1.8 + 0.7
72 h	48.7 + 8.7	53.9 + 6.4	4.1 + 1.3
96 h	33.0 + 4.3	55.1 + 8.9	4.5 + 1.2

[1] Mean IL-2 production in controls was significantly higher than in PSA ($P < 0.001$) except at 24 h

[2] In patients with PSA, the addition of IND did not significantly enhance IL-2 production. In controls, IND produced a significantly higher IL-2 ($P < 0.01$) except at 24 h

[3] In patients with PSA, the addition of PGE_2 led to a significant decrease in IL-2 production ($P < 0.001$); in controls, a similar reduction was noted ($P < 0.001$)

IL-2 production (Table 3)

IL-2 production in control MNC was greatest at 72 h. In patients with PSA, IL-2 production was greatest at 48 h with a mean of 10.5 ± 4 units/ml, a value significantly lower than peak IL-2 in controls ($P < 0.001$). IL-2 production was significantly lower in PSA patients at all times except 24 h ($P < 0.001$).

Effect of IND and PGE_2 on IL-2 production (Table 3)

In controls, addition of IND resulted in a significant increase in IL-2 production ($P < 0.01$) except at 24 h. This increase was also significant when comparing peak IL-2 production under both experimental conditions ($P < 0.02$). The addition of PGE_2 to controls produced a significant reduction in IL-2 production at all times ($P < 0.001$). In patients with PSA, the addition of IND did not result in a significant increase in IL-2 production. However, the addition of PGE_2 to PSA cultures did cause a significant reduction in IL-2 production ($P < 0.001$) during all culture times.

IL-4 production (data not shown)

IL-4 in cells stimulated by Con A alone was not detected in either controls or patients with PSA.

Discussion

The pathogenesis of PSA is not understood. Studies suggest the presence of B- and T-cell abnormalities similar to other autoimmune disorders. Diminished IL-2 production has been reported in many autoimmune disorders including SLE and RA. Our patients with PSA also demonstrated decreased IL-2 production. Decreased IL-2 production by MNC could be due to several factors. Failure to adequately respond to mitogenic stimulation could lead to reduced IL-2 production and significantly decreased proliferation was observed in patients with PSA. However, proliferation appeared to be less affected than IL-2 production. It is not clear, therefore, that the decreased IL-2 production observed could be entirely explained on the basis of decreased proliferation.

In vivo activation of T-cells could lead to an inability to respond adequately to a new mitogenic stimulus such as Con A. The early peak in IL-2 production seen in our patients suggested preactivation. We investigated the presence of IL-2 receptors as a marker for T-cell activation. Five of the patients studied demonstrated IL-2 receptors; however, the percentage of such cells was small. Thus, preactivation is unlikely to have caused the observed decrease in IL-2 production. A possible explanation for the observed effect could be that T-cells from patients with PSA may preferentially express low-affinity IL-2 receptors, similar to that reported in RA and SLE [14, 15].

IL-4 was investigated because of its ability to inhibit IL-2-dependent activation of T cells. Although we did not determine T-helper phenotypes, the increased presence of TH_2/TH_0 cells that produce IL-4 may have accounted for our findings. We failed to detect the presence of IL-4 in either control or PSA cultures. This is consistent with other published data on low-level production of IL-4 in polyclonally stimulated T cells [16, 17].

T-cell-subset analysis on our patients demonstrated a significant decrease in the percentage of CD8-positive cells. Similar finding have been reported in other autoimmune disease. The loss of CD8 cell number and/or function is hypothesized to lead to autoantibody production characteristic of autoimmune diseases. Since CD8 cell function has not yet been studied in patients with PSA, the significance of this finding is unclear. It does suggest a possible explanation for the B-cell hyperactivity seen in PSA.

Abnormal regulation of arachidonic acid metabolism both in skin and in peripheral MNC of patients with psoriasis has been reported. These abnormalities consist of increased levels of PGE_2 and lipoxygenase products indicating a general stimulation of arachidonic acid metabolism in skin [18]. All of our patients were being treated with NSAIDs which alter eiconsanoid metabolism and thus may effect cytokine production such as IL-2 in our patients and under experimental conditions [19, 20].

PGE has a number of effects on cell cultures including significant inhibition of T-lymphocyte proliferation and IL-2 production following stimulation with phytohemagglutinin [21–23]. Thus, the decreased IL-2 production noted in our PSA patients may be on the basis of increased prostaglandin production. On the other hand, IND can enhance IL-2 production in unfractionated cell cultures following phytohemagglutinin stimulation. This effect may be mediated by a decrease in PGE production by monocyte/macrophages [23].

As expected, the addition of IND to Con A-stimulated control cells increased proliferation and IL-2 production. However, the addition of IND to PSA cultures significantly increased proliferation, but did not significantly increase IL-2 production. This lack of IL-2 response may suggest excessive PG production by monocytes/macrophages as a possible cause. However, all patients were already on NSAID therapy and in vitro inhibition of PG synthesis by MNC has been demonstrated at physiological concentrations of some but not all NSAIDs [24]. It is possible that higher concentrations of IND were required to significantly increase IL-2 production. However, preliminary experiments suggested 5 μg/ml as the optimal concentration under our experimental conditions. Thus, the inability to significantly increase IL-2 production in PSA cultures in the face of inhibition (by NSAIDs) of PG production suggests another mechanism for the decrease in IL-2 production. Variables for which we could not control, such as serum concentration of NSAID, could also have affected the results and their interpretation. The dichotomy between proliferation and IL-2 production noted in PSA cultures could be due to other factors including the fact that IL-2 levels may have increased to sufficient levels, but not statistically significant levels, to induce the greater proliferation noted.

As expected, the addition of PGE_2 to MNC cultures from patients with PSA and controls produced a significant reduction in IL-2 production. This would indicate that MNC from patients with PSA are not more sensitive to the effects of PGE_2 than those from controls. On the other hand, proliferation was significantly reduced in the PSA group but not in controls. It is unclear why proliferation was not significantly reduced in controls.

We have demonstrated both decreased CD8 cells and decreased IL-2 production in patients with PSA. These T-cell findings have been described in classical systemic autoimmune diseases and suggest that PSA is also an autoimmune process. It also confirms the non-specific nature of these changes and implies common underlying immune effector mechanism abnormalities.

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