CONCISE REPORT

Lymphocyte depletion with fludarabine in patients with psoriatic arthritis: clinical and immunological effects

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Objective: To obtain preliminary information on the safety and efficacy of fludarabine in PsA and analyse its immunomodulatory effects in peripheral blood and synovial tissue.

Methods: 15 patients with active PsA who did not respond to DMARDs were randomly allocated to receive fludarabine every four weeks or placebo. Primary outcomes were the proportion of patients who met the ACR20 and the psoriatic arthritis response criteria (PsARC) at 16 weeks. Secondary outcomes were changes in tender or swollen joint counts and scores of the psoriasis area and severity index (PASI). Phenotypic analysis of peripheral blood mononuclear cells (PBMC), synovial immunohistochemistry, and functional analysis of PBMC were used to determine the immunomodulatory effects of fludarabine.

Results: At 16 weeks the ACR20 criteria were met by 3/7 (43%) fludarabine treated v 0/8 placebo treated patients ($p=0.08$); the PsARC was achieved by 4/7 (57%) fludarabine treated v 2/8 (25%) placebo treated patients; and 3/7 (43%) fludarabine treated v 0/7 placebo treated patients had $\geq 20\%$ improvement in the PASI. Marked peripheral lymphopenia involving naive ($CD4^+CD45RA^+$) and memory ($CD4^+CD45RO^+$) T cells, $CD8^+$ T cells, and B cells was seen in fludarabine treated patients.

Conclusions: In PsA fludarabine induces significant peripheral, but modest, synovial lymphopenia, and a trend towards improved clinical response.

The synovium of patients with psoriatic arthritis (PsA) is characterised by oligo- or monoclonal T cell expansion and increased levels of predominantly Th1 cytokines.^{1–3} PsA may therefore be an appropriate target for lymphocyte depleting therapy. Fludarabine is a halogenated adenosine analogue that specifically depletes lymphocytes and induces profound and prolonged immunosuppression. It also inhibits cytokine induced activation of STAT1 and STAT1 dependent transcription in resting as well as activated lymphocytes,⁴ that may contribute to its immunosuppressive effect. We conducted a randomised, placebo controlled, double blind study to obtain preliminary information on the safety and efficacy of fludarabine in psoriatic arthritis and to analyse its immunomodulatory effects in peripheral blood and synovial tissue.

MATERIALS AND METHODS

Patients

Eligible patients were 18 years of age or older who had had PsA for more than six months, and had responded inadequately to at least one disease modifying antirheumatic drug (DMARD). Patients had three or more painful or swollen joints and at least two of the following: (a)

≥ 30 minutes of morning stiffness, and (b) erythrocyte sedimentation rate ≥ 28 mm/1st h or C reactive protein > 8 mg/l. Low dose glucocorticosteroids (≤ 10 mg/day of prednisone or equivalent) and stable doses of non-steroidal anti-inflammatory drugs were allowed. DMARDs and retinoids were discontinued ≥ 4 weeks, and topical agents ≥ 2 weeks before randomisation. Patients were excluded from the study if they had seropositive arthritis, purely spondylitic PsA, or arthritis mutilans.

Study design and treatment

The protocol was approved by the institutional review board and all patients gave written informed consent. Patients were randomised at a 1:1 ratio to fludarabine (supplied by Berlex Laboratories, Inc, Richmond, CA) or placebo, given for a total of four cycles (every four weeks) as a single bolus solution of 30 mg/m² over 30 minutes according to guidelines described previously.⁵ Clinical assessment and routine blood tests were performed every four weeks. The primary efficacy outcomes were the achievement of the American College of Rheumatology criteria for improvement (ACR20)⁶ or the psoriatic arthritis response criteria (PsARC)⁷ at 16 weeks. Efficacy in psoriasis was evaluated by the psoriasis area and severity index (PASI).

Laboratory evaluations

Flow cytometry

Peripheral blood leucocyte phenotyping was performed every eight weeks using a FACScan (Becton Dickinson, San Jose, CA, USA) with Lysis II software.

ICAM-1 expression

Purified peripheral blood mononuclear cells (PBMCs) were cultured in the presence of 500 U/ml of interferon gamma (R&D Systems, Minneapolis, MN) or 1 μ g/ml of lipopolysaccharide (Sigma) for 24 hours. After stimulation, cells were washed and intercellular adhesion molecule-1 (ICAM-1) expression on monocytes was determined by FACS analysis.

Western blot for STAT1 and phosphorylated STAT1 (pSTAT1)

PBMCs were cultured in the presence or absence of 500 U/ml of interferon gamma for one hour. Western blots were prepared from protein samples containing 5×10^5 of cells equivalent. They were probed overnight with anti-phospho-STAT1 antiserum (gift from Dr David A Frank, Dana-Faber

Abbreviations: ACR, American College of Rheumatology; DMARD, disease modifying antirheumatic drug; ICAM, intercellular adhesion molecule; PBMCs, peripheral blood mononuclear cells; PsA, psoriatic arthritis; PASI, psoriasis area and severity index; PsARC, psoriatic arthritis response criteria

Table 1 Baseline characteristics and clinical responses (median (range))

	Fludarabine (n = 7)	Placebo (n = 8)	p Value‡
<i>Baseline characteristics</i>			
Age at entry	36 (33–45)	47 (31–69)	
Disease duration (years)	9 (2–20)	4 (1–15)	
Women/men	4/3	3/5	
Prednisone use at entry (No (%))	2 (29)	4 (50)	
Erosion at entry (No (%))	6 (86)	5 (63)	
<i>Baseline arthritis activity</i>			
Tender joint count	33 (23–43)	26 (21.5–39)	
Swollen joint count	19 (17–33)	20.5 (11.5–29.5)	
<i>Response in arthritis</i>			
ACR20 (No (%))			
8 Weeks	1 (14)	0	0.46
16 Weeks	3 (43)	0	0.08
PsARC (No (%))			
8 Weeks	4 (57)	2 (25)	0.31
16 Weeks	4 (57)	2 (25)	0.31
<i>Baseline psoriasis activity*</i> (n = 7)			
PASI score	3.2 (1.4–6.1)	5.35 (1.5–10.7)	
<i>Response in psoriasis (No (%))†</i>			
8 Weeks	1 (14)	1 (13)	1.00
16 Weeks	3 (43)	0	0.08

*Fourteen patients with psoriatic lesion at study entry were prospectively included for the analysis; † $\geq 20\%$ improvement in the PASI scores; ‡Fisher's exact test (two tailed p value).

Cancer Institute) and detected by peroxidase conjugated antirabbit immunoglobulins. For visualisation, the ECL system (Amersham) was used according to the manufacturer's protocol and positive immunoreactive bands were measured densitometrically. Blots from activated or inactivated cells were stripped and reprobed with anti-STAT1. Blots were further stripped and finally reprobed with anti- β -actin. Then we calculated the expression of p-STAT1/STAT1, p-STAT1/ β -actin and STAT1/ β -actin.

Synovial tissue evaluation

Multiple synovial specimens were obtained by closed needle biopsies at baseline and at 16 weeks. Tissues were formalin fixed for haematoxylin and eosin staining, or snap frozen for immunohistochemical analysis.⁸

Statistical methods

Continuous variables (percentage change from baseline) were compared between groups by the Wilcoxon rank sum test. Proportions of patients responding were compared with the χ^2 test or Fisher's exact test. All statistical analyses were done with the Statview version 5 (SAS Institute, Cary, NC).

RESULTS

Clinical response

All fifteen patients had polyarticular disease and 73% of patients had erosions (table 1). One patient in the placebo group discontinued the study for lack of efficacy. No immediate toxicities or any serious infections were observed. Three of 7 (43%) fludarabine treated patients met ACR20 criteria at 16 weeks, but none of 8 placebo treated patients ($p = 0.08$) (table 1). Similarly, four (57%) fludarabine treated patients met PsARC while only 2 (25%) of 8 placebo treated patients did ($p = 0.31$). No significant difference was observed in the individual measures of disease activity between the groups at 16 weeks. Fourteen patients had psoriatic skin lesions at study entry; 3 of 7 (43%) fludarabine

treated patients had at least 20% improvement in the PASI, while none of the placebo treated patients did ($p = 0.2$) (table 1). Response in skin disease was not concordant with that in arthritis.

Effects on the peripheral blood lymphocyte subpopulations

Both CD4⁺ and CD8⁺ T cells decreased and reached a nadir of approximately 30% of baseline at four weeks after the last treatment (figs 1A and 1B). While both memory (CD4⁺CD45RO⁺) and naïve (CD4⁺CD45RA⁺) T cells decreased, their ratio increased, indicating a greater resistance in the memory subset (fig 1C). The CD3⁺CD25⁺ subset decreased significantly with fludarabine treatment (fig 1D); but the CD3⁺HLA-DR⁺ subset did not. CD20⁺ B cells decreased profoundly (fig 1E), but the quantity or composition of serum immunoglobulins was not significantly altered.

Effects on the synovial tissue

Effects on infiltrating CD3⁺ T cells were evaluated by immunohistochemical analysis in synovial biopsy specimens (four pairs of pre- and post-treatment specimens were available from the fludarabine group and one from the placebo group). Infiltrating CD3⁺ T cells decreased in all four patients in the fludarabine group (median reduction 56%, range 21–59%), while they increased in the patient from the placebo group. Of interest, specimens from two of the three ACR20 responders in the fludarabine group comprised the two largest reductions in infiltrating CD3⁺ T cells (55.8 and 58.8%). Fludarabine had no effect on the infiltrating CD14⁺ macrophages.

Effects on endothelial cells

Effects on the expression of E- and P-selectin on the vascular endothelium in synovial biopsy specimens were evaluated (four pairs available from the fludarabine group and three from the placebo group). The proportion of E-selectin positive vessels decreased in three of four patients in the fludarabine group, whereas it increased in all three patients in the placebo group ($p = 0.0019$) (fig 2A). Specimens from two of the three ACR20 responders in the fludarabine group were evaluable, and both showed reduction in the proportions of E-selectin positive vessels (31.4 and 45.5%). Staining for P-selectin did not show a significant change from baseline in either group (fig 2B).

Effects on STAT1 expression and phosphorylation in PBMCs

No consistent change in STAT1 expression was observed in either group. In contrast, all four fludarabine treated patients showed reduction in interferon gamma induced STAT1 phosphorylation (median reduction 14%, range 9–32%), while no consistent trend was observed in the placebo treated patients (data not shown). We then evaluated the effects of fludarabine on the interferon gamma induced expression of one of the STAT1-dependent genes, ICAM-1, and found no statistically significant differences (data not shown).

DISCUSSION

We have found that a short course of fludarabine led to modest clinical improvement (ACR20) in patients with psoriatic arthritis compared with placebo ($p = 0.08$). While fludarabine significantly decreased both CD4⁺ and CD8⁺ T cells, B cells, and activated T lymphocytes (CD25⁺ T cells) in the peripheral blood, it decreased infiltrating T lymphocytes in the synovium only modestly. Furthermore, the patients who achieved ACR20 response criteria showed the largest decreases in infiltrating T lymphocytes. These results may imply that the modest clinical response of fludarabine may be

Figure 1 Effects of fludarabine on peripheral blood lymphocyte population. Mean (SEM) of the following at baseline, two months, and four months were plotted: numbers of (A) CD4⁺ T cells and (B) CD8⁺ T cells; (C) the ratio of CD4⁺CD45RO⁺/CD4⁺CD45RA⁺ cells; numbers of (D) CD25 expressing CD3⁺ cells and (E) CD20⁺ cells. Data were obtained for both the fludarabine and placebo groups.

Figure 2 Effects of fludarabine on the activation status of lymphocytes and endothelial cells. Percentage changes in the proportion of (A) E-selectin and (B) P-selectin positive vessels in seven pairs of synovial biopsy specimens.

explained by its limited effects on infiltrating T lymphocytes. Most of the infiltrating T lymphocytes are expected to be memory cells and thus may be more resistant to fludarabine, as we observed in the peripheral blood. In addition, endothelial E-selectin expression was modestly inactivated by fludarabine but that of P-selectin was not and this might have allowed continued migration of fludarabine resistant memory T lymphocytes into the synovium.⁹⁻¹³

The modest clinical response may also be due to fludarabine resistant cells, such as macrophages. In this study, fludarabine had virtually no effect on the numbers of macrophages in the synovium or on the in vitro production of monokines (data not shown). Frank *et al* recently showed that in vitro treatment with fludarabine inhibited ligand induced STAT1 phosphorylation, and that in vitro or in vivo treatment with fludarabine led to a sustained loss (up to 96 hours) of STAT1 protein and mRNA.⁴ STAT1 is essential for the biological function of several inflammatory cytokines including interferon gamma, which is considered to have a principal role in maintaining the pathology in PsA. Our study, however, showed that the effects of fludarabine on STAT1 expression and interferon gamma induced STAT1 phosphorylation in PBMCs obtained four weeks after fludarabine administration were very small. Furthermore, fludarabine did not significantly inhibit interferon gamma induced ICAM-1 expression on the PBMCs. These findings suggest that the inhibitory effects of fludarabine on STAT1 expression and phosphorylation are of limited duration.

The lack of statistical difference in treatment efficacy between the two groups may be due to a type 2 error. This prevents any definite conclusion about the efficacy of fludarabine. However, the rather limited clinical benefit, the potentially substantial adverse effects of fludarabine, and recent advances in treating PsA with tumour necrosis factor blocking agents limit the therapeutic potential of fludarabine. On the other hand, further studies are needed to identify antigens, triggers, and locations of autoreactive T lymphocytes.

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