

Cytokine profiles during infliximab monotherapy in psoriatic arthritis

A. Mastroianni, E. Minutilli, A. Mussi, V. Bordignon, E. Trento, G. D'Agosto, P. Cordiali-Fei and E. Berardesca

Clinical Dermatology, San Gallicano Dermatological Institute, Via Chianesi 53, Rome, Italy

Summary

Correspondence

Enzo Berardesca MD.

E-mail: berardesca@berardesca.it

Accepted for publication

1 December 2004

Key words:

cytokines, infliximab, interleukin-6, matrix metalloproteinase-2, psoriasis, tumour necrosis factor- α

Conflicts of interest:

None declared.

Background Biological therapies are a new breakthrough in the treatment of psoriasis and psoriatic arthritis (PsA). Among these, tumour necrosis factor (TNF)- α antagonists such as infliximab and etanercept are the most promising as TNF is considered to be essential in driving cytokine cascade at sites of cutaneous and synovial inflammation in this disease.

Objectives To evaluate the time-related response of serum cytokine release during infliximab monotherapy and assess serum cytokine levels in order to provide a fast, minimally invasive tool to monitor and/or predict efficacy of anti-TNF- α therapy.

Methods Twenty patients affected by PsA with Psoriasis Area and Severity Index (PASI) score between 0.4 and 42.8 were treated with infliximab for 30–42 weeks. The assessment of arthritis severity was performed using the American College of Rheumatology (ACR) criteria and ultrasonography evaluation. The treatment schedule consisted of infliximab (5 mg kg⁻¹ intravenously) at 0, 2 and 6 weeks and every 12 weeks on an individual basis determined by therapeutic results and adverse events reported. At baseline and before every infusion blood samples were taken to assess serum cytokine levels [TNF- α , interleukin (IL-6), E-selectin, vascular endothelial cell growth factor (VEGF), fibroblast growth factor (FGF), matrix metalloproteinase (MMP-2)].

Results Eighteen of 20 psoriatic patients achieved >50% improvement and 14 of 20 patients attained >75% improvement in the PASI score at 10 weeks. All arthritic patients achieved >50% improvement (ACR-50) and 16 of 20 patients attained >75% improvement (ACR-75) at 10 weeks. TNF- α did not decrease immediately during the first part of the study. A significant decrease was detected at week 12 ($P < 0.01$). In contrast, IL-6, VEGF, FGF and E-selectin showed significant decreases after early infliximab infusions. PASI was not correlated with TNF- α in the serum but was significantly correlated with FGF, VEGF and MMP-2. Treatment was well tolerated and there were no significant adverse events in most patients, other than an urticarial reaction and an autoimmune hepatitis.

Conclusions Monotherapy with infliximab has to be considered an efficacious and safe treatment for PsA in comparison with traditional disease-modifying antirheumatic drugs. The resolution of cutaneous and synovial symptoms is not related to TNF- α serum levels in the initial phases. Apoptosis may play an important role in the modulation of the inflammatory response.

Psoriatic arthritis (PsA) is a spondylarthropathy that occurs in approximately 6–20% of patients with psoriasis which afflicts 1–3% of the world's population. Sulfasalazine, ciclosporin (CsA) and methotrexate (MTX) are the most widely used disease-modifying antirheumatic drugs in the treatment of erosive and deforming PsA, while nonsteroidal anti-inflammatory

drugs (NSAID) and local corticosteroid injections represent only a symptomatic therapy for mild PsA.¹ Combination therapy with CsA and MTX is actually a standard treatment for aggressive PsA,² even if not free from serious side-effects, especially after long-term use.^{3–7} Biological agents that neutralize cytokines and in particular tumour necrosis factor

(TNF)- α are actually the most widely used for the satisfactory therapeutic results obtained in joints and skin involvement without unexpected safety problems. The most well-known TNF- α antagonists⁸ are infliximab, which is a chimeric monoclonal antibody with murine variable regions and human IgG1 and k constant regions, and etanercept, which is a fusion protein composed of the extracellular domain of the p75 TNF receptor and the hinge and Fc domains of human IgG1. The use of TNF-antagonists is based on the demonstration that TNF is upregulated in the synovial fluid and cutaneous lesions of PsA and also that TNF levels correlate with disease activity. In fact, TNF plays a major role in the pathogenesis of psoriasis and PsA as this proinflammatory cytokine is essential to trigger the cytokine cascade which leads to the production of adhesion molecules and angiogenic factors which induce synovitis and psoriasis.^{9,10} Although both infliximab and etanercept are potent neutralizers of TNF- α bioactivity, there are fundamental differences in their molecular structures, their binding specificities and the manner in which they neutralize TNF.⁸ Besides TNF, other cytokines such as interleukin (IL-6) and growth factors [vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)] are involved in the pathogenesis of psoriasis and in the mechanisms of hyperproliferation. Serum levels of these compounds can be related to disease activity.¹¹ The purpose of this study was to evaluate the time-related response of serum cytokine release during infliximab monotherapy. Cytokines were assessed in the serum in order to provide a fast, minimally invasive tool to monitor and/or predict the efficacy of anti-TNF- α therapy.

Materials and methods

Patients

Twenty patients (16 male and four female) aged 25–60 years with a diagnosis of PsA have been included in the study. The study protocol was approved by the ethics committee and all patients provided written informed consent. Each patient had psoriatic skin lesions and joint involvement according to American College of Rheumatology (ACR)¹² criteria with persistent clinical and serological activity despite standard treatment options (NSAID, corticosteroids, antipsoriatic and antirheumatic drugs such as retinoids, CsA and MTX). Skin involvement was evaluated with the Psoriasis Area and Severity Index (PASI).¹³ Arthritis was evaluated by clinical examination of the tender and swollen joints, laboratory tests of inflammatory markers (erythrocyte sedimentation rate and C-reactive protein) and radiological examinations of joint damage. All patients were screened for recent or past tuberculosis: X-rays of the thorax and the tine-test had to be negative for study inclusion.

Exclusion criteria

Pregnant and nursing women were excluded from the study as well as any patients with the following conditions: ongoing

or recent history of severe infection (tuberculosis), prior history of malignancy, past history of autoimmune diseases (systemic lupus erythematosus), cardiac insufficiency.

Study design

Before entering the study all subjects underwent a wash-out period of 2 months for systemic active drugs both for arthritis and psoriasis. Infliximab (5 mg kg⁻¹) was infused intravenously with physiological saline (250 cm³) over a 2-h period. Vital parameters (blood pressure, pulse, temperature) were monitored until the day after the end of the infusion. Infliximab infusions were administered at weeks 0 (baseline), 2 and 6, and subsequently a single infusion every 12 weeks was administered. Laboratory analysis including blood count, liver enzymes, renal function biochemical tests, antinuclear auto-antibodies (ANA) as well as electrocardiogram (ECG) were performed before each infusion. Follow-up continued for 30–42 weeks. Patients were monitored for possible adverse events occurring during the entire treatment period.

Psoriasis was monitored clinically by calculating ACR criteria and PASI at baseline and before every infusion. PASI-50 and PASI-75 (and ACR-50 and ACR-75, respectively) were considered an improvement > 50% and > 75%, respectively, in comparison with ACR and PASI at baseline.

Serum specimens were obtained at baseline and at weeks 2 and 6 and every 12 weeks before infliximab infusion. The following cytokines were monitored: TNF- α , IL-6, E-selectin, VEGF, FGF and MMP-2.

Cytokine analysis

Blood for cytokine assessment was collected from patients in two tubes (with and without EDTA). Samples were centrifuged for 20 min at 10 000 g (5417R microcentrifuge; Eppendorf, Hamburg, Germany). Serum and plasma samples were subdivided in small aliquots to be stored at – 80 °C until tested for cytokine levels.

The different biological mediators were measured in the consecutive serum (TNF- α , IL-6, FGF) or plasma (VEGF, MMP-2) samples by quantitative enzyme immunoassays (Quantikine Immunoassays; R&D Systems, Minneapolis, MN, U.S.A.). Assays were performed following the manufacturer's instructions and each cytokine was measured in all samples in a single run. Samples and standards were analysed in duplicate and only variation coefficients < 15% were accepted.

Statistical analysis

Data were processed for normality and then analysed with ANOVA for repeated measures. Subgroup comparison was performed with the Bonferroni multiple comparison test. Correlation was calculated by Pearson's test. $P < 0.05$ was considered significant. Statistical analysis was performed with GraphPad Prism statistical software, version 4 (GraphPad Inc., San Diego, CA, U.S.A.).

Results

All patients were successfully treated according to the schedule. The treatment was discontinued in only two patients, for allergic reaction at 42 weeks in one and for an autoimmune hepatitis at 30 weeks in the second. Results are shown in Figures 1–4. Clinical improvements in PASI and ACR indexes compared with baseline (week 0) were recorded after the second and third infusions and reached the best clinical result at 10 weeks. In spite of these results, no patient discontinued infliximab therapy for clinical remission because every 12 weeks additional infusions were necessary to maintain the therapeutic results.

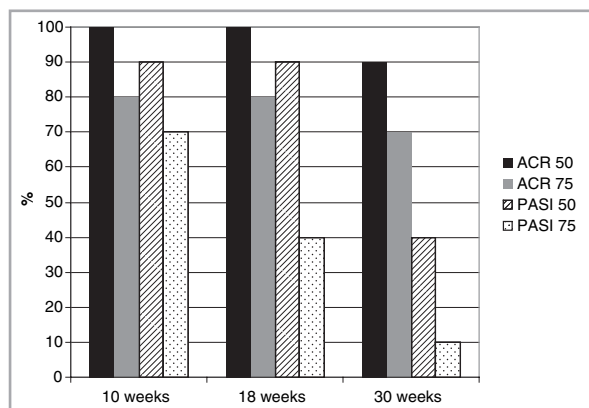


Fig 1. Long-term American College of Rheumatology (ACR) and Psoriasis Area and Severity Index (PASI) (50 and 75, respectively) response during infliximab monotherapy.

Joint involvement

At 10 weeks 16 of 20 patients (80%) attained ACR-75 responses, while all patients (100%) achieved ACR-50 results. At 18 weeks, before the first maintenance infliximab infusion, the percentage of patients with ACR-75 and ACR-50 did not vary. In contrast, at 30 weeks 14 of 20 patients (70%) attained ACR-75 responses, while 18 of 20 patients (90%) achieved ACR-50 results (Fig. 1).

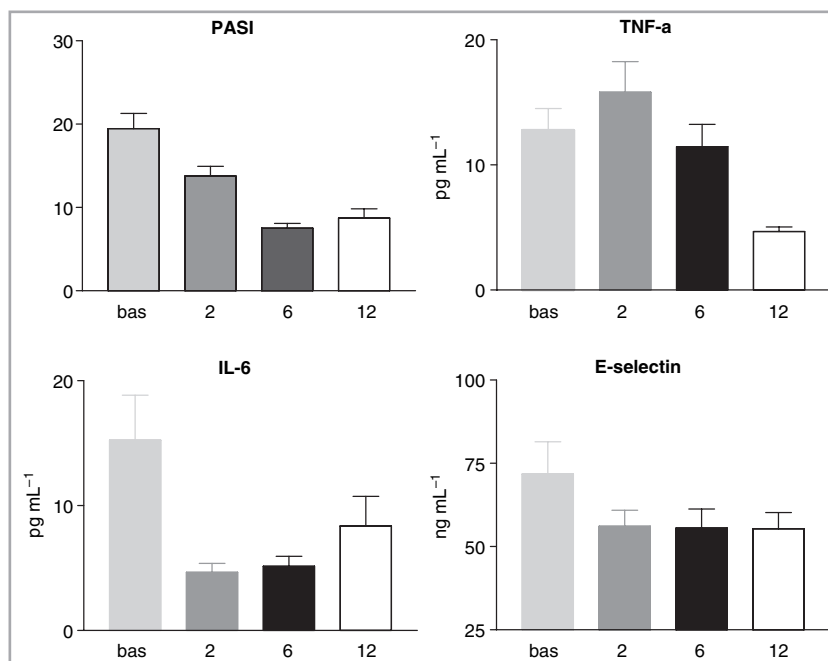
Cutaneous involvement

At 10 weeks 14 of 20 patients (70%) attained PASI-75 responses, while 18 of 20 patients (90%) achieved PASI-50 results. At 18 weeks, before the first maintenance infliximab infusion, only eight of 20 patients (40%) attained PASI-75 responses because of mild relapses for skin lesions, while the percentage of patients with PASI-50 had no change. In contrast, at 30 weeks only two of 20 patients (10%) preserved PASI-75 because of diffuse and severe relapses of psoriasis, while eight of 20 patients (40%) still maintained PASI-50 results (Fig. 1). In particular, focusing on the first weeks of treatment, PASI significantly decreased after 2 weeks ($P < 0.001$) and 3 weeks ($P < 0.001$) (Fig. 2).

Cytokine profiles

TNF- α showed a slight decrease at week 2 and a more relevant decrease at week 6 ($P < 0.01$) compared with baseline (Fig. 2). However, no significant correlation between PASI score and TNF levels during the first 12 weeks of treatment was found. In contrast, IL-6 fell significantly at week 2 ($P < 0.001$) to rise subsequently at week 12 (Fig. 2). VEGF

Fig 2. Short-term Psoriasis Area and Severity Index (PASI) and tumour necrosis factor (TNF)- α , interleukin (IL)-6 and E-selectin during infliximab monotherapy. A significant decrease of PASI score was observed (ANOVA $P < 0.0001$; baseline vs. 2 and 6 weeks, 2 vs. 6 weeks, $P < 0.001$; 6 vs. 12 weeks, ns); slight decrease of TNF- α at 6 weeks ($P < 0.05$ vs. 2) and relevant decrease at 12 weeks ($P < 0.01$) compared with baseline and week 2; serum IL-6 significantly decreased during the study (ANOVA $P < 0.0007$), baseline vs. weeks 2 and 6 ($P < 0.001$); a significant decrease is recorded for E-selectin between baseline and weeks 2, 6 and 12 ($P < 0.05$, ANOVA $P < 0.01$).



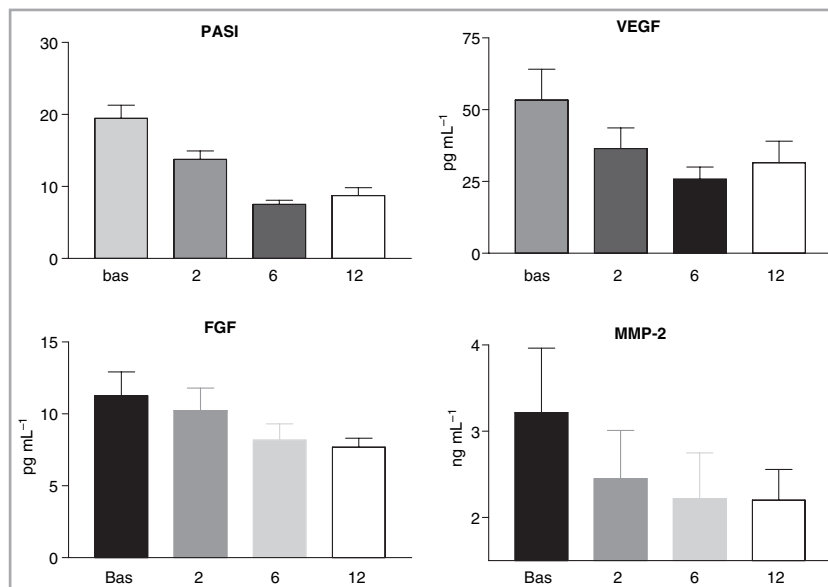


Fig 3. Short-term Psoriasis Area and Severity Index (PASI) score and vascular endothelial cell growth factor (VEGF), fibroblast growth factor (FGF) and matrix metalloproteinase (MMP)-2. PASI statistics are described in Figure 2. Serum VEGF at baseline, 2, 6 and 12 weeks. A significant decrease is shown at 6 weeks ($P < 0.001$, ANOVA $P < 0.004$); significant decrease of FGF at weeks 6 and 12 compared with baseline ($P < 0.01$, ANOVA $P < 0.007$); significant decrease of MMP-2 at weeks 6 and 12 compared with baseline ($P < 0.01$, ANOVA $P < 0.006$).

progressively decreased to reach the lowest value at week 6 ($P < 0.001$) (Fig. 3), while E-selectin decreased immediately at week 2 ($P < 0.05$), remaining at a constant level later (Fig. 2). FGF decreased significantly at week 6 ($P < 0.01$) (Fig. 3) as well as MMP-2 ($P < 0.01$) (Fig. 3). FGF, VEGF and MMP-2 were significantly correlated with PASI score during the first weeks of treatment ($P < 0.001$, $P < 0.003$ and $P < 0.002$, respectively) (Fig. 4).

Discussion

TNF- α antagonists such as infliximab and etanercept are widely used for the treatment of PsA. Infliximab is specific for TNF- α , whereas etanercept binds equally well to both TNF- α and lymphotoxin α . Infliximab binds to monomeric and trimeric forms of soluble and transmembrane TNF so that up to three infliximab molecules can bind to each TNF homotrimer, blocking all receptor binding sites on TNF.⁸ Differences of binding structure, specificity and stability between infliximab/TNF and etanercept/TNF complexes can explain the difference in clinical effectiveness of these two biological agents in patients with PsA^{14,15} and psoriasis.¹⁶

The most common adverse event can be the induction of a severe infection, especially tuberculosis;¹⁷ other possible adverse events can be infusion reactions (immediate or late), the induction of autoimmune diseases and permanent ECG alterations; more rarely, neurological disorders have been observed.¹⁸ Mild side-effects such as myalgia, asthenia, alterations of laboratory tests and ECG have been described.

In our study, the treatment was well tolerated by all subjects except for two patients who had to discontinue it for adverse effects. In the first patient an infusion reaction occurred at 42 weeks with diffuse urticaria and resolved successfully after therapy with systemic steroids. At 18 weeks, the second patient developed an autoimmune hepatitis with

elevated liver enzymes in the presence of native anti-DNA autoantibodies which resolved spontaneously after treatment suspension. All other patients continued infliximab treatment for 30–42 weeks reporting only slight side-effects such as myalgia, asthenia and laboratory abnormalities (presence or increase of ANA) and temporary ECG alterations.

The study shows that clinical responses for global (peripheral and axial) joint involvement as well as for skin lesions were particularly significant for the rapidity of improvement. Indeed, after the first or second infliximab infusion joint involvement was substantially resolved, while at 10 weeks, after the third infliximab infusion, most of the skin lesions were cleared. From a statistical point of view, the improvement of PASI was clearly significant at week 6 (Fig. 2). At 18 weeks 80% of patients preserved ACR-75 results achieved at 10 weeks and it was only at 30 weeks that this percentage decreased to 70% because of mild relapses for peripheral joint involvement. In contrast, at 18 weeks only 40% patients maintained PASI-75 results in comparison with 70% patients at 10 weeks because of premature mild relapses of skin lesions in many patients, and this percentage decreased dramatically to 10% at 30 weeks because of diffuse and severe relapses for psoriasis in most patients (Fig. 1). Cytokine profiles showed that surprisingly during the induction period (weeks 0–6) there was no decrease in serum TNF- α . This occurred significantly only at week 12 (Fig. 2, $P < 0.01$); indeed, there is no correlation between PASI and TNF- α during this time. This may indicate that the efficacy on psoriatic plaques of infliximab is due mainly to the effects exerted on cell membranes and probably on the induction of apoptosis, as recently reported both *in vitro* and *in vivo*.^{19,20} These data may also explain the differences in efficacy reported between infliximab and etanercept.^{14–16} In contrast, IL-6 significantly decreased after 6 weeks (Fig. 2, $P < 0.001$) to rise later on after the induction period. IL-6 is induced by TNF- α and these data

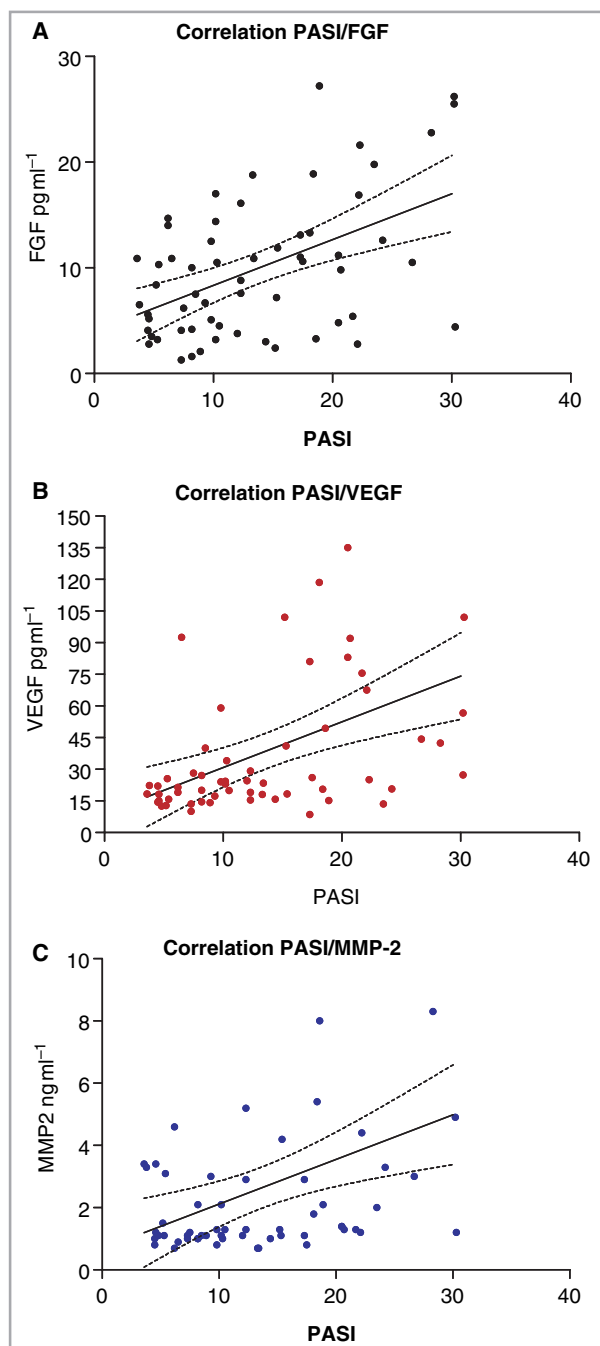


Fig 4. Significant correlations between Psoriasis Area and Severity Index (PASI) score and fibroblast growth factor (FGF) ($P < 0.001$, Pearson $r = 0.49$) (A); vascular endothelial cell growth factor (VEGF) ($P < 0.003$, Pearson $r = 0.44$) (B); and matrix metalloproteinase (MMP)-2 ($P < 0.002$, Pearson $r = 0.39$) (C).

reflect the decreased inflammatory activity related to the anti-TNF- α treatment. IL-6 in combination with TNF- α and other cytokines can be highly injurious and it has been suggested that these cytokines can be markers of disease activity.²¹

VEGF, E-selectin and FGF showed significant decreases during treatment (Figs 2 and 3). These data support the view that endothelial and vascular inflammation and proliferation can play

an important role either in the pathogenesis of the disease or in the subsequent control of its manifestations. VEGF induces angiogenesis and endothelial cell proliferation and it plays an important role in regulating vasculogenesis in several diseases, including psoriasis and rheumatoid arthritis. Keratinocytes and endothelial cells also produce basic fibroblast growth factor (b-FGF), and this cytokine is mitogenic for both cell types. Furthermore, b-FGF is stored in the basement membrane and extracellular matrix, and can be displaced from these 'buffers' by various stimuli. Displacement of b-FGF by physical stimuli, such as scratching or rubbing, could explain koebnerization in diseases such as psoriasis. Indeed, the changes in VEGF and FGF significantly correlate with PASI (Fig. 4). E-selectin belongs to the family of intercellular adhesion molecules and is stimulated by TNF- α ; it is essential in modulating the transendothelial migration of leucocytes in inflammatory conditions such as psoriasis and plays an important role in immune response and inflammation. E-selectin has also been reported to be a good marker of inflammation in synovial fluid.^{8,11,22-24}

MMPs are involved in the articular tissue destruction processes in the pathogenesis of PsA. MMP-2 is overexpressed in suprabasal keratinocytes in psoriasis.²⁵ A significant decrease (Fig. 3) ($P < 0.01$) of MMP-2 occurs after 6 weeks and is significantly correlated with PASI (Fig. 4) ($P < 0.002$). This finding is consistent with all the other data showing a reduction in the cascade of cytokine production and inflammation observed during treatment.

In conclusion, the study shows that infliximab monotherapy can be a safe and useful treatment for selected cases of psoriasis and PsA. The success of the therapy is not related initially to a reduction of serum TNF- α levels, but to an anti-inflammatory modulation of vascular reactions, keratinocyte proliferation and possibly to an induction of apoptotic phenomena.

References

- 1 Salvarani C, Cantini F, Olivieri I. Disease-modifying antirheumatic drug therapy for psoriatic arthritis. *Clin Exp Rheumatol* 2002; **20** (Suppl. 28):S71-5.
- 2 Mazzanti G, Coloni L, De Sabbata G, Paladini G. Methotrexate and cyclosporin combined therapy in severe psoriatic arthritis: a pilot study. *Acta Derm Venereol Suppl* 1994; **186**:116-17.
- 3 Scarpa R, Manguso F, Oriente F *et al.* Leflunomide in psoriatic polyarthritis: an Italian pilot study. *Arthritis Rheum* 2001; **44** (Suppl.):S92.
- 4 Liang GC, Barr WG. Long term follow-up of the use of leflunomide in recalcitrant psoriatic arthritis and psoriasis. *Arthritis Rheum* 2001; **44** (Suppl.):S121.
- 5 Griffiths CE. Immunotherapy for psoriasis: from serendipity to selectivity. *Lancet* 2002; **359**:279-80.
- 6 Ellis CN, Krueger GG. Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. *N Engl J Med* 2001; **345**:248-55.
- 7 Dinant HJ, van Kuijk AWR, Goedkoop AY *et al.* Alefacept reduces synovial inflammatory infiltrate and improves outcome in psoriatic arthritis. *Arthritis Rheum* 2001; **42** (Suppl.):S91.
- 8 Scallon B, Cai A, Solowski N *et al.* Binding and functional comparisons of two types of tumor necrosis factor antagonists. *J Pharmacol Exp Ther* 2002; **301**:418-26.

- 9 Partsch G, Steiner G, Leeb BF *et al.* Highly increased levels of tumor necrosis factor- α and other proinflammatory cytokines in psoriatic arthritis synovial fluid. *J Rheumatol* 1997; **24**:518–23.
- 10 Ettehadi P, Greaves MW, Wallach D *et al.* Elevated TNF α biological activity in psoriatic skin lesions. *Clin Exp Immunol* 1994; **96**:146–51.
- 11 Bonifati C, Ameglio F. Cytokines in psoriasis. *Int J Dermatol* 1999; **38**:241–51.
- 12 Felson DT, Anderson JJ, Lange ML *et al.* Should improvement in rheumatoid arthritis clinical trials be defined as fifty percent or seventy percent improvement in core set measures, rather than twenty percent? *Arthritis Rheum* 1998; **41**:1564–70.
- 13 Langley RG, Ellis CN. Evaluating psoriasis with Psoriasis Area and Severity Index, Psoriasis Global Assessment, and Lattice System Physician's Global Assessment. *J Am Acad Dermatol* 2004; **51**:563–9.
- 14 Mease PJ, Goffe BS, Metz J *et al.* Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet* 2000; **356**:385–90.
- 15 Antoni C, Manger B. Infliximab for psoriasis and psoriatic arthritis. *Clin Exp Rheumatol* 2002; **20** (Suppl. 28):S122–5.
- 16 Chaudhari U, Romano P, Mulcahy LD *et al.* Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet* 2001; **357**:1842–7.
- 17 Keane J, Gershon S, Wise RP *et al.* Tuberculosis associated with infliximab, a tumor necrosis factor α -neutralizing agent. *N Engl J Med* 2001; **345**:1098–104.
- 18 Mohan N, Edwards ET, Cupps TR *et al.* Demyelination occurring during anti-TNF α therapy for inflammatory arthritides. *Arthritis Rheum* 2001; **44**:2862–9.
- 19 Van den Brande JM, Braat H, van den Brink GR *et al.* Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology* 2003; **124**:1774–85.
- 20 Di Sabatino A, Ciccocioppo R, Cinque B *et al.* Defective mucosal T cell death is substantially reverted by infliximab in a caspase dependent pathway in Crohn's disease. *Gut* 2004; **53**:70–7.
- 21 Sagawa Y, Shiohara T, Imanishi K, Nagashima M. Is sustained production of tumor necrosis factor- α relevant to the development of pustular psoriasis? *Dermatology* 1993; **187**:81–3.
- 22 Mease PJ. TNF in psoriatic arthritis: pathophysiology and treatment with TNF inhibitors. *Ann Rheum Dis* 2002; **61**:298–304.
- 23 Danning CL, Illei GG, Hitchon C *et al.* Macrophage-derived cytokine and nuclear factor kappaB p65 expression in synovial membrane and skin of patients with psoriatic arthritis. *Arthritis Rheum* 2000; **43**:1244–56.
- 24 Austin LM, Ozawa M, Kikuchi T *et al.* The majority of epidermal T cells in psoriasis vulgaris lesions can produce type 1 cytokines, interferon- γ , interleukin-2, and tumor necrosis factor- α , defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. *J Invest Dermatol* 1999; **113**:752–9.
- 25 Fleischmajer R, Kuroda K, Hazan R *et al.* Basement membrane alterations in psoriasis are accompanied by epidermal overexpression of MMP-2 and its inhibitor TIMP-2. *J Invest Dermatol* 2000; **115**:771–7.