The Journal of Immunology

RESEARCH ARTICLE | OCTOBER 01 2001

IL-10 Improves Skin Disease and Modulates Endothelial Activation and Leukocyte Effector Function in Patients with Psoriatic Arthritis[1] FREE

Iain B. McInnes; ... et. al

J Immunol (2001) 167 (7): 4075–4082.
https://doi.org/10.4049/jimmunol.167.7.4075

IL-10 Improves Skin Disease and Modulates Endothelial Activation and Leukocyte Effector Function in Patients with Psoriatic Arthritis[1]

Iain B. McInnes,[2,3]* Gabor G. Illei,[2]* Carol L. Danning,* Cheryl H. Yarboro,* Marianne Crane,* Takashi Kuroiwa,* Ryan Schlimgen,* Eric Lee,* Barbara Foster,† Donald Flemming,§ Calman Prussin,† Thomas A. Fleisher,‡ and Dimitrios T. Boumpas*

Psoriatic arthritis (PsA) provides an ideal disease model in which to investigate the bioactivities of potentially therapeutic cytokines at multiple sites of tissue inflammation. We investigated the effects of IL-10, an antiinflammatory cytokine, given s.c. for 28 days in a double-blind, placebo-controlled study in PsA patients. Synovial/skin biopsies, peripheral blood leukocytes, articular magnetic resonance images, and clinical disease activity scores were obtained sequentially. Modest, but significant clinical improvement in skin, but not articular disease activity scores with only minor adverse effects was observed. Type 1, but not type 2 T cell cytokine production in vitro was suppressed in human rIL-10 compared with placebo recipients. Similarly, monokine production in vitro was reduced, whereas serum soluble TNFRII levels were elevated, indicating suppression of monocyte function. Decreased T cell and macrophage infiltration in synovial tissues was accompanied by reduced P-selectin expression. Moreover, suppressed synovial enhancement on magnetic resonance imaging and reduced $\alpha_v\beta_3$ integrin expression on von Willebrand factor$^+$ vessels were observed. Together these data demonstrate that a short course of IL-10 modulates immune responses in vivo via diverse effects on endothelial activation, and leukocyte recruitment and effector function. Such biological changes may result in clinically meaningful improvement in disease activity. *The Journal of Immunology,* **2001, 167: 4075–4082.**

Interleukin-10 represents an attractive therapeutic cytokine in the management of inflammatory disorders in which dominant Th1 responses, together with high levels of monokine production, are implicated. IL-10 suppresses macrophage production of cytokines and chemokines (1–3) and enhances soluble cytokine receptor release (4, 5). It modulates Ag presentation by dendritic cells and macrophages, through reduced CD80/CD86 and MHC expression (6–8), and subsequent costimulatory interactions may be suppressed (9). Reduced IL-12 production may prejudice subsequent T cell maturation toward Th/T cytotoxic 2 phenotype, with suppression of ongoing Th/T cytotoxic 1 responses (10). Direct effects on T cells most likely occur since IL-10 can inhibit IL-2 and IFN-γ production (3, 11), can induce Ag-specific anergy in vitro (12), and may promote the emergence of regulatory CD4$^+$ T cell subsets (13). IL-10 can directly modulate endothelial cell activation and angiogenesis in vitro (14, 15). It reduces secretion of collagen (16), matrix metalloproteinases (MMP),[4] but not tissue inhibitors of MMPs (17), which together with effects on osteoclast differentiation/activation (18), indicates that IL-10 may retard inflammatory lesion extension and host tissue destruction.

Psoriatic arthritis (PsA) develops in some 5–25% of patients with psoriasis. Keratinocyte proliferation and inflammatory infiltration, with elevated cytokine and chemokine expression, characterize cutaneous psoriatic lesions. T cell activation, commensurate with an ongoing Th1 phenotype (19, 20), TCR repertoire skewing (21), and the beneficial clinical effects of T cell-directed therapies indicate that T cells, driven by local Ag recognition, play an important role in psoriasis pathogenesis. A striking feature is the relative absence of antiinflammatory moieties, including IL-10 and IL-1R antagonist (19, 22–24). Effective cutaneous therapies are often associated with reversal of the cytokine pattern to that of a type 2 phenotype (25, 26). We recently demonstrated high levels of proinflammatory cytokine expression and NF-κB activation in PsA synovium, together with relative paucity of IL-10 expression (27). Evidence for endothelial cell activation, enhanced adhesion molecule expression, coupled with proangiogenic activity through vascular endothelial growth factor, basic fibroblast growth factor, and $\alpha_v\beta_3$ integrin expression, has also been provided in both cutaneous and synovial lesions (28–30). Together these observations indicate that IL-10 administration could be therapeutically beneficial.

Clinical phase I/II studies in normal volunteers, and in rheumatoid arthritis (RA) and Crohn's disease suggest that human rIL-10

*Arthritis and Rheumatism Branch, National Institute of Arthritis, Musculoskeletal and Skin Diseases, and †Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; and ‡Department of Clinical Pathology, Warren Grant Magnuson Clinical Center, and §Department of Radiology, National Naval Medical Center, Bethesda, MD 20892

Received for publication February 6, 2001. Accepted for publication July 12, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by a Copeman Traveling Fellowship from the Arthritis Research Campaign (U.K.) (to I.B.M.) and by a Fogarty Fellowship, National Institutes of Health (to I.B.M.).

[2] I.B.M. and G.B.M. contributed equally to the present study.

[3] Address correspondence and reprint requests to Dr. Ian B. McInnes at the current address: Center for Rheumatic Diseases, University of Glasgow, Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow, G31 2ER, U.K. E-mail address: i.b.mcinnes@clinmed.gla.ac.uk

[4] Abbreviations used in this paper: MMP, matrix metalloproteinase; hpf, high power field; MRI, magnetic resonance imaging; PASI, psoriasis area and severity index; PsA, psoriatic arthritis; RA, rheumatoid arthritis; rhIL-10, human rIL-10; vWF, von Willebrand factor; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; SJC, swollen joint count.

0022-1767/01/$02.00

(rhIL-10) is well tolerated (31–33). An open-label study in psoriasis demonstrated reduction in lesion size following local administration of rhIL-10, associated with the presence of increased Th2-type cytokines in circulating lymphocytes (23). However, significant placebo effects might be anticipated in such a study design, rendering interpretation of in vivo bioactivity difficult. We have now performed a placebo-controlled, dose-escalating study investigating the safety and biological activity of rhIL-10 in PsA. We report that significant immune deviation can be safely achieved by systemic administration of IL-10 to PsA patients. Such immune effects were associated with significant clinical improvements in cutaneous, but not synovial inflammation in the short term.

Materials and Methods

Clinical study design

Twenty-nine patients were submitted to a dose-escalating, placebo-controlled, phase II study, approved by the Institutional Review Board, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health. Patients self-administered daily s.c. injections of either placebo, or 1, 5, or 10 μg/kg rhIL-10 (Schering-Plough, Madison, NJ) for 28 consecutive days. All patients were <18 years of age and provided informed consent. Entry criteria included: 1) PsA of <6 mo duration, defined as follows (5–14): classic psoriatic skin lesion with or without nail involvement; peripheral arthritis alone or in combination with spinal disease; negative rheumatoid factor and absence of s.c. nodules; and radiographic findings compatible with PsA. 2) Active arthritis with three or more swollen joints considered capable of responding to drug therapy and at least two of the following: tenderness or pain on movement of at least three joints/entheses; at least 45 min of morning stiffness (peripheral joints or axial); and erythrocyte sedimentation rate (ESR) ≥28 mm/h or C-reactive protein (CRP) <0.8 mg/dl. 3) Failure to respond to or development of intolerable side effects to at least one disease-modifying antirheumatic drug.

Low-dose glucocorticoids (≤10 mg/day of prednisone or equivalent) and nonsteroidal antiinflammatory drugs were allowed if doses were stable for at least 4 wk before randomization and were kept constant for the study duration. Disease-modifying antirheumatic drugs (methotrexate, sulfasalazine, gold, hydroxychloroquine, cyclosporin, azathioprine, cyclophosphamide, chlorambucil), retinoids, and vitamin D were discontinued ≥4 wk before randomization. Patients with seropositive/symmetric polyarthritis, systemic lupus erythematosus or other autoimmune disease, spondylitic form of PsA (spondylitis alone or in combination with shoulder and hip arthritis without evidence of peripheral arthritis), severe physical disability (RA functional class IV), serum creatinine <1.8 mg/dl or creatinine clearance >50 ml/min, preexisting malignancy, acute or chronic infections requiring antimicrobial therapy, or serious viral or fungal infections were excluded. Pregnant females, nursing mothers, or patients of childbearing age not practicing birth control were excluded.

Disease activity was evaluated on days 1, 29, 57, and 85, by the same blinded observer throughout, as follows: 1) joint count for tenderness or pain on motion for 84 diarthrodial joints graded 0–3; 2) joint swelling for 74 joints graded 0–3; 3) patient assessment of pain (100-mm linear instrument); 4) patient's general (global) assessment (100-mm linear instrument); 5) physician assessment of disease activity (100-mm linear instrument); 6) Health Assessment Questionnaire; 7) acute phase response (ESR or CRP); 8) duration of morning stiffness (min); and 9) spinal involvement, assessed by range of motion of cervical and lumbar spine, chest expansion, and sacroiliac pain. Digits with intense inflammatory changes between the joints ("sausage digits") were evaluated by assessment of erythema, warmth (both graded 1–3), and circumference. Skin disease activity was assessed by calculating the psoriasis area and severity index (PASI) (34). For calculation of this, the four main body areas were assessed: the head, trunk, and upper and lower extremities, corresponding to 10, 20, 30, and 40% of the total body area, respectively. To evaluate the severity of the psoriatic lesions, three target symptoms, namely erythema, infiltration, and desquamation, were assessed on a 0–4 scale. To calculate the PASI, the sum of the severity rating for these three main changes was multiplied with the numerical value of the areas involved and with the various percentages of the four body areas. These values were then added to obtain the PASI. The PASI varies in steps of 0.1 U from 0–72, the latter representing complete erythroderma of the highest severity.

Change in disease activity was assessed as follows: 1) significant improvement of joint disease, as defined by American College of Rheumatology (ACR) (35); 2) significant improvement in joint swelling or joint pain/tenderness (improvement by 50% or more in the number of tender/swollen joints); 3) therapeutic remission, as defined by American Rheumatism Association (ARA; now ACR); and 4) significant skin improvement defined as a 35% improvement in the PASI score.

Radiological assessment

Magnetic resonance imaging (MRI; 1.5T General Electric, Waukesha, WI; Signa Horizon operating with version 5x software) was performed on d1 and d29. Films were read simultaneously by an experienced musculoskeletal radiologist and a rheumatologist, blinded to identity, treatment assignment, and sequence of the films. Capsular distention, synovial volume, and enhancement were independently graded on a scale from 0 to 3, and the two values were averaged: 0 = normal enhancement; 1 = minimal enhancement; 2 = marked enhancement; 3 = marked enhancement with distended capsule. A difference ≥1 between the pre- and posttreatment films therefore represented clinically meaningful change.

Synovial and skin tissue evaluation

Multiple (at least 10) closed needle synovial biopsies were obtained at d1 and d29. At the same time, 3-mm punch biopsies of lesional and perilesional skin (three per site) were obtained. Tissues were Formalin fixed for H&E staining, or snap frozen for immunohistological analysis, performed as previously described (36). Murine mAbs to CD3, CD4, CD8, CD19, CD68, von Willebrand factor (vWF), ICAM-1 (all DAKO, Carpinteria, CA), VCAM-1, P-selectin, E-selectin (all Novocastra, Newcastle, U.K.), $\alpha_v\beta_3$ integrin (Chemicon, Temecula, CA), ki67 (DAKO), and cytokeratin 18 (Zymed, San Francisco, CA) were employed. Briefly, 8 μM acetone-fixed frozen sections were blocked, then incubated overnight with primary Ab. Bound Ab was detected with biotinylated goat anti-mouse Ig, streptavidin-enzyme conjugate, color substrate development, and hematoxylin counterstain (all DAKO). Alkaline phosphatase was visualized with new Fuschia and HRP with diaminobenzidine (both from Vector, Burlingame, CA). Quantification was performed by light microscopy on an Olympus BX60 optic system, as previously described (30, 36). Briefly, synovial staining was assessed in lining layer, sublining, and aggregate areas separately, as was epidermal and dermal staining in skin. Twelve to sixteen high power fields (hpf) at ×400 magnification, including at least 6 hpf of lining layer and 6 hpf of sublining layer, were analyzed for each sample by two observers independently, who were blinded to tissue origin. Skin epidermal areas were calculated in square micrometers by computer image analysis using Scion Imaging software (Gaithersburg, MD). Significant interobserver discrepancy was addressed by reevaluation and mutual consent.

Evaluation of immunological function

Analyses were performed on days 1, 15, 29, and/or 57. Circulating leukocyte subsets were estimated by FACS analysis (Becton Dickinson, San Jose, CA), using directly conjugated Abs to the following cell surface markers: CD3, CD4, CD8, CD20, CD14, CD56. Cell activation status was determined by FACS using CD28, CD40L, CD69, and HLA-DR expression. Ig subsets (IgG1–4, IgE, IgM, IgA) were measured by nephelometry. Circulating soluble P-selectin, soluble E-selectin, soluble ICAM-1, soluble VCAM-1, TNFRII, IL-18 (all R&D Systems, Minneapolis, MN), IL-12p40 (Endogen, Woburn, MA), and MMP3 (Amersham Life Science, Arlington, IL) were measured by ELISA.

Whole blood cultures were established by diluting heparinized blood 1/5 in IMDM (Life Technologies, Gaithersburg, MD). Cultures in 24-well plates were immediately stimulated with medium alone, LPS (Sigma, St. Louis, MO; 1 μg/ml for 16 h) for monokine production, or PHA (Sigma; 3 μM for 48 h) for T cell cytokine production. Preliminary dose-response and time-course experiments determined that these were the optimal time and dose requirements for the cytokines estimated. Supernatants were stored at −20°C until cytokine estimation by ELISA, according to the manufacturers' instructions (IL-2, IL-4, IL-5, IL-6, IL-10, total IL-12, IL-12p70, IFN-γ, TNF-α, and IL-1β kits from Endogen; IL-18 from R&D Systems).

Intracellular cytokine expression was measured as previously described (37). Briefly, PBMCs obtained by density gradient centrifugation were stimulated for 4 h in RPMI, 10% human serum (Life Technologies) with PMA (Sigma; 100 nM)/ionomycin (Calbiochem, La Jolla, CA; 1 μM) in the presence of monensin (Calbiochem; 2 μM). Intracellular expression of IFN-γ, IL-4, IL-5, and IL-10 (detected using conjugated Abs from PharMingen, San Diego, CA) in $CD4^+$ and $CD8^+$ T lymphocytes was measured by four-color FACS analysis (Becton Dickinson). In parallel cultures, expression of the chemokine receptor CCR5 on PBLs was determined by FACS (PharMingen).

Statistical methods

Categorical outcome measures were compared using a contingency table and a χ^2 test; Fisher's exact test was used, as appropriate. Continuous outcome measures were compared by Student's *t* test for parametric variables, and the Mann-Whitney *U* test for nonparametric variables. For pairwise comparisons, paired *t* test was used for parametric, and paired sign test for nonparametric variables. Values of $p < 0.05$ are considered significant. All statistical analyses were done with the Statview V.5 statistical software package (SAS Institute, Cary, NC).

Results

Of the 29 PsA patients, 28 received 1, 5, or 10 μg/kg rhIL-10, or placebo, by daily s.c. injection for 28 days (Table I). One patient randomized to receive 5 μg/kg IL-10 failed to return for follow-up from day 8. To determine whether such an administration protocol induced functional immune deviation, immune parameters in serum, leukocyte cultures, and synovial/skin tissues were compared in rhIL-10 and placebo recipients. Three patients received 1 μg/kg IL-10 to establish the safety of IL-10 in this disease. Because of the small number of patients in this group, they were not included in subsequent analyses.

Suppression of monocyte-derived cytokine production in vitro

Since IL-10 exerts direct effects on macrophages in vitro, we first determined cytokine production by LPS-stimulated whole blood cultures. We compared immunomodulatory effects between cohorts of rhIL-10- and placebo-treated patients. Dose-dependent suppression of TNF-α and IL-1β synthesis was observed in rhIL-10 ($p = 0.0078$; Fig. 1*a*), compared with placebo-treated patients. Significant inhibition was evident within 14 days and reversed 28 days after discontinuation of therapy (data not shown). This effect was specific since no consistent effect was observed on IL-10 synthesis in vitro (Fig. 1*c*). Serum TNFRII levels rose dose dependently in rhIL-10 recipients, commensurate with which the ratio of ex vivo TNF-α synthesis to in vivo TNFRII levels was significantly suppressed (Fig. 2, *a* and *b*). Although previous single-dose studies indicated transient monocytosis, no sustained alteration in the absolute number or proportion of circulating monocyte subsets was evident by FACS analysis at day 15 or day 29. Similarly, CD14, HLA class II, and CD64 expression was comparable in rhIL-10 and placebo groups. Together, these data indicate selective functional suppression of the proinflammatory activities of circulating monocytes in rhIL-10-, but not placebo-treated PsA patients.

Table I. *Patient characteristics*[a]

	IL-10 ($n = 19$)	Placebo ($n = 10$)
Age (years)	42 ± 11.4	44 ± 10.2
Male (%)	63	60
Duration of PsA (years)	8 ± 7.38	10 ± 8.9
Duration of psoriasis (years)	13 ± 12.3	16 ± 10.4
Previous DMARDS[b]	2.2 ± 1.1	3.0 ± 1.2
Number of tender joints	23.5 ± 11.7	26.8 ± 10.5
Number of swollen joints	19.1 ± 12.2	14.7 ± 5.2
PASI	5.4 ± 6.2	4.5 ± 4.8
Morning stiffness (min)	268 ± 312	163 ± 142
ESR	35 ± 20.3	26 ± 13.5
CRP	1.18 ± 1.03	0.73 ± 0.3
Polyarthritis (%)	100	80
Spinal involvement (%)	47	40
Enthesitis (%)	12	20
Nail involvement (%)	70	90
Erosion (%)	76	80

[a] Values are mean ± SD unless single figures.
[b] DMARDS, disease-modifying antirheumatic drugs.

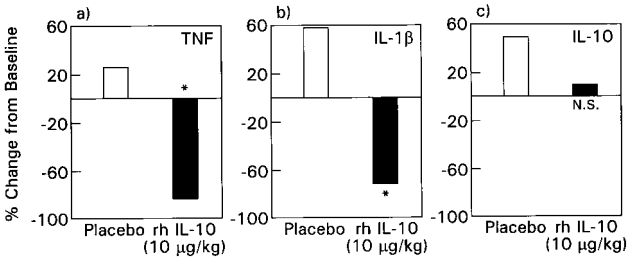

FIGURE 1. Effects of IL-10 administration on monokine production. Cytokine production to LPS (1 μg/ml) after 16-h whole blood culture was measured by ELISA. Data represent the percentage change in median cytokine production after 29 days compared with baseline (median/interquartile range (IQR) of cytokine production at baseline was as follows: TNF-α, 249 pg/ml/IQR 273; IL-1β, 586 pg/ml/IQR 736; IL-10, 546 pg/ml/IQR 492). Placebo, $n = 10$; IL-10 (10 μg/kg), $n = 8$; *, $p = 0.0078$.

Selective effects on lymphocyte function

No significant alterations on circulating lymphocytes, including $CD3^+$, $CD4^+$, and $CD8^+$ subsets, or on CD69, CD25, CD28, or CD40L expression within such subsets were observed by FACS analysis (data not shown). Inflammatory arthritis is most likely Th1 mediated (38, 39). We therefore extended these studies to investigate effects on Th1 and Th2 lymphocyte function. PHA-induced IFN-γ production in vitro was suppressed in a dose-dependent fashion by day 29 (Fig. 3, *a* and *b*), but reversed following completion of rhIL-10 administration. In contrast to placebo recipients, there was a trend toward decreased IL-2 production in IL-10 recipients. No significant effect on PHA-induced IL-4 (not shown) or IL-5 production was observed, suggesting that the effects of rhIL-10 were mediated by directly suppressing Th1 cell activity, rather than by promoting Th2 cell cytokine production (Fig. 3*c*). FACS analyses for mitogen-induced intracellular cytokine expression were also performed. No consistent effect on intracellular IFN-γ, IL-4, IL-5, or IL-10 expression was evident (Table II). Moreover, the frequency of $CD4^+$ T cells in purified PBMC that expressed $CCR5^+$, a marker of Th1 phenotype (40), was not altered through therapy. Together these data suggest that rhIL-10 mediated direct effects on the capability to synthesize cytokine, rather than modulation of the absolute frequency of circulating cells capable of Th1-type function.

We next investigated whether monocyte-mediated regulation of Th1/Th2 polarity was altered by rhIL-10 treatment. We were unable to consistently detect IL-12 production (p40 or p70) in LPS-stimulated whole blood cultures. However, plasma IL-12 (p40) levels were similar in active and placebo-treated patients and did not vary through therapy, suggesting that no major effect on IL-12 production was present (data not shown). The critical importance of IL-18, in synergy with IL-12, in regulating Th1 responses has recently been recognized (41). IL-18 production may be down-regulated by IL-10 in vitro. We therefore investigated whether IL-18 synthesis was modified by rhIL-10, analogous to its effects on TNF-α and IL-1β ex vivo. As with IL-12, however, no consistent suppression of LPS-induced IL-18 generation in whole blood cultures, nor of total serum levels, was evident in either cohort (data not shown).

Effects on B cell function

Given the B cell stimulatory effects of IL-10, it was important to monitor effects on B cell activation. No alteration in the distribution or quantification of Ig subsets or isotypes was observed, nor

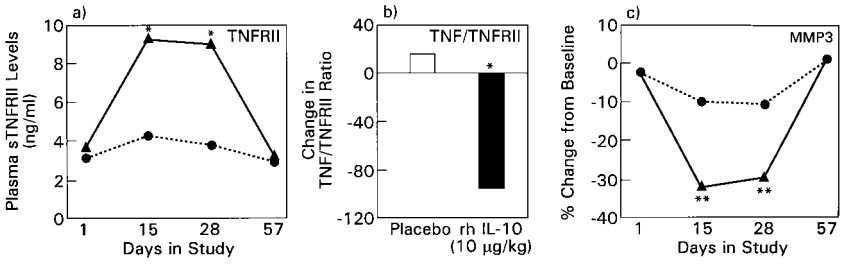

FIGURE 2. Plasma TNFRII levels were measured by ELISA at time points indicated following administration of IL-10 or placebo and are expressed as absolute values (*a*) or as a ratio comparing plasma TNFRII concentration with simultaneous LPS-induced TNF-α production in vitro in whole blood cultures (b) , as described in Fig. 1. *c*, MMP3 concentrations were measured in plasma at the time points indicated. ●, Placebo, $n = 10$; ▲, IL-10 (10 μg/kg), $n = 8$; ∗, $p < 0.007$; ∗∗, $p < 0.05$.

did titers of autoantibodies (antinuclear Ab, rheumatoid factor) change with therapy. Moreover, neither numbers of circulating B lymphocytes (by FACS analysis), nor $CD20^+$ cell infiltration in synovial or skin tissues (by immunohistochemistry) was increased (data not shown).

Modification of tissue inflammatory lesions

To determine whether these functional effects on circulating monocytes and lymphocytes were reflected by changes within inflammatory lesions, synovial membrane and skin biopsies were obtained before (day 1) and after (day 29) rhIL-10 administration.

Synovial membrane

Ten paired synovial biopsies were evaluable (four from placebo cohort; three each in 5 and 10 μg/kg cohorts). Tissues were not evaluated if biopsies were unavailable at both time points, if only fibrous tissue was obtained, or if less than 6 representative hpf per section were present. Significant reduction in the synovial $CD3^+$ cell population was detected in rhIL-10 compared with placebo recipients. This most likely reflected reduction in $CD4^+$ T subsets, since $CD8^+$ expression was unchanged (Fig. 4, *a* and *b*). Direct $CD4^+$ T cell enumeration was not possible due to coincident macrophage CD4 expression. Similarly, $CD68^+$ macrophage numbers in both the lining layer and sublining interstitium were reduced in rhIL-10-treated patients (Fig. 4, *c* and *d*). These changes could have reflected altered activation status in circulating leukocytes, since CD68 is a mature macrophage marker, or modified transendothelial migration. We therefore investigated soluble plasma and tissue adhesion molecule expression, reflecting endothelial activation. No significant alteration in circulating soluble VCAM (baseline, 559 v 548 pg/ml, and day 29, 847 v 1617 pg/ml; placebo v 10 μg/kg cohort, $p < 0.05$) or soluble ICAM-1 (baseline, 13 v 16 ng/ml, and day 29, 16 v 18 ng/ml; placebo v 10 μg/kg cohort, $p < 0.05$) was observed in active or placebo recipients. In contrast, soluble P-selectin levels in plasma were significantly suppressed by rhIL-10 therapy in a dose-dependent manner (Fig. 5*a*). This was reflected at the tissue level since the proportion of P-selectin$^+$ vessels identified in parallel sections by vWF expression was significantly reduced (Fig. 5*b*). Soluble E-selectin levels in plasma and endothelial tissue E-selectin expression, however, were similar in rhIL-10 and placebo groups (Fig. 5, *a* and *c*). Since P-selectin may selectively recruit Th1 cells (42), this represents a further mechanism whereby Th1 responses may be ameliorated by rhIL-10.

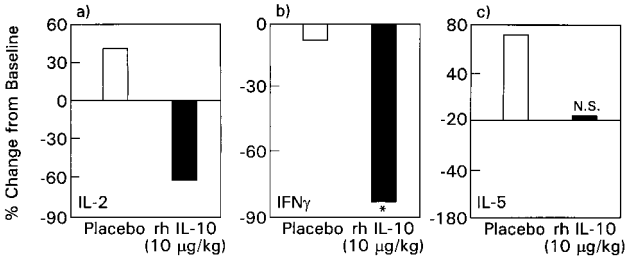

FIGURE 3. Effects of IL-10 administration on lymphokine production. Cytokine production to PHA (3 μM) after 48-h whole blood culture was measured by ELISA. Data represent the percentage change in median cytokine production after 29 days compared with baseline (median/IQR of cytokine production at baseline was as follows: IFN-γ, 141 pg/ml/IQR 409; IL-2, 48 pg/ml/IQR 85; IL-5, 31 pg/ml IQR 56). Placebo, $n = 10$; IL-10 (10 μg/kg), $n = 8$; ∗, $p = 0.0078$.

Skin biopsies

Lesional and perilesional biopsies obtained before, and on completion of, rhIL-10 administration were available from 14 patients. In contrast to observations in synovial membrane, no consistent trends in inflammatory cell subset infiltration, nor in adhesion molecule expression were observed (data not shown). This was as expected since, by study protocol, these biopsies were obtained from tissue sites that remained clinically inflamed. Nevertheless, significant reduction in the area of epidermal ki67, but not cytokeratin expression, was observed in lesional, but not perilesional skin (Fig. 6, *a* and *b*), suggesting effects on keratinocyte proliferation even in established lesions. Although these data suggest differential effects of rhIL-10 in discrete tissues, the small sample size and the inherent sampling bias in skin noted before require cautious interpretation.

Effects on MMPs and angiogenesis in vivo

A critical role for angiogenesis in inflammatory synovitis has recently been recognized (43). In addition to synovial biopsy, we performed MRI of the most inflamed, accessible joint in the 10 μg/kg rhIL-10 or matched placebo cohorts to evaluate synovial enhancement. Improvement in MRI appearances was observed in rhIL-10, but not in placebo recipients (Table III). Moreover, all rhIL-10-treated MRI responders exhibited >20% swollen joint count (SJC) improvement. Since early gadolinium uptake most likely reflects vascular flow, we sought evidence for modulation of angiogenesis by rhIL-10. Expression of the neovascular endothelial marker, $\alpha_v\beta_3$ integrin was significantly down-regulated in vWF^+ blood vessels in both 5 and 10 μg/kg cohorts (Figs. 5*d*, 6*c*, and 7). MMP production is also implicated in angiogenesis. Moreover, serum MMP3 levels are modified by anti-TNF-α therapy, suggesting a direct link between proinflammatory cytokine production, tissue remodeling, and erosion (44). Unlike placebo, administration of rhIL-10 reduced MMP3 levels and the ratio of MMP3/tissue inhibitors of MMPs ($p = 0.015$ and $p = 0.03$, respectively; Fig. 2*c*), suggesting that IL-10 may have disease-modifying properties together with its antiinflammatory effect.

Clinical effects of rhIL-10 administration

rhIL-10 administration was well tolerated, with no serious clinical adverse effects observed. As predicted by previous studies, hemoglobin concentrations and platelet counts were significantly reduced in both 5 and 10 μg/kg cohorts, but did not reach levels

Table II. *Effect of rIL-10 administration on intracellular cytokine and chemokine receptor expression in PBL*

	% Positive CD4$^+$ PBLs[a]			
	Placebo (n = 10)		IL-10, 10 μg/kg (n = 8)	
	D1	D29	D1	D29
IFN-γ	16.0 ± 9.6	14.5 ± 9	25.5 ± 11.4	27.1 ± 14.1
IL-4	1.3 ± 1.3	0.9 ± 0.8	2.4 ± 1.1	2.54 ± 1.5
IL-5	0.05 ± 0.04	0.11 ± 0.06	0.14 ± 0.09	0.11 ± 0.14
IL-10	0.03 ± 0.09	0.15 ± 0.12	0.08 ± 0.14	0.25 ± 0.05
Membrane CCR5[b]	—	—	26.9 ± 10.6	22.5 ± 10.5

[a] Values are median ± SEM.
[b] CCR5 expression on CD4$^+$ PBL was measured in a subset of IL-10-treated patients (n = 5).

associated with serious clinical consequence and did not necessitate study withdrawal. Although the present study was not adequately powered to examine efficacy, IL-10 administration induced significant improvement in the median PASI and in the number of 35% PASI responders (Table IV; Fig. 6*d*; $p < 0.02$). In contrast, IL-10 was not different from placebo in inducing clinical improvement in articular measures of disease activity (ACR20; Table IV). Since core criteria sets designed for RA may not be applicable to PsA, we also analyzed individual disease activity parameters, including tender joint count, SJC, and health assessment questionnaire, but found no consistent changes associated with rhIL-10 administration. Of interest, we found no significant correlation between clinical and immunological responders.

Discussion

Most data defining the bioactivities of IL-10 have been obtained either in murine or in vitro models or by inference from IL-10 measurement ex vivo during disease states. PsA represents an ideal model in which to assess the consequences of IL-10 administration in two distinct tissues, in which plausible target biological pathways are detected and from which biopsies may be readily obtained.

IL-10 clearly modified immune responsiveness in vivo in PsA patients. Mitogen-induced release of Th1 cytokines was reduced in peripheral blood cultures. In contrast, intracellular expression of IL-4, IL-5, IL-10, or IFN-γ was not altered. This suggests a quantitative effect on T cell cytokine production, rather than modulation of memory T cell differentiation by rhIL-10 administration. Similar T cell hyporesponsiveness has been reported in SCID patients transplanted with HLA-mismatched stem cells, who exhibit high levels of endogenous IL-10 expression (45). Although an alternate mechanism of action of IL-10 could be the generation of T regulatory 1 cells, CD4$^+$ T cells characterized by high levels of IL-10 expression (13), we did not, however, detect increased numbers of IL-10-producing CD4 cells by intracellular cytokine analysis. We did not consistently detect enhanced Th2-type responses, in contrast to a previous study in which blood Th2 cell frequency was increased after local IL-10 administration to psoriatic patients (23).

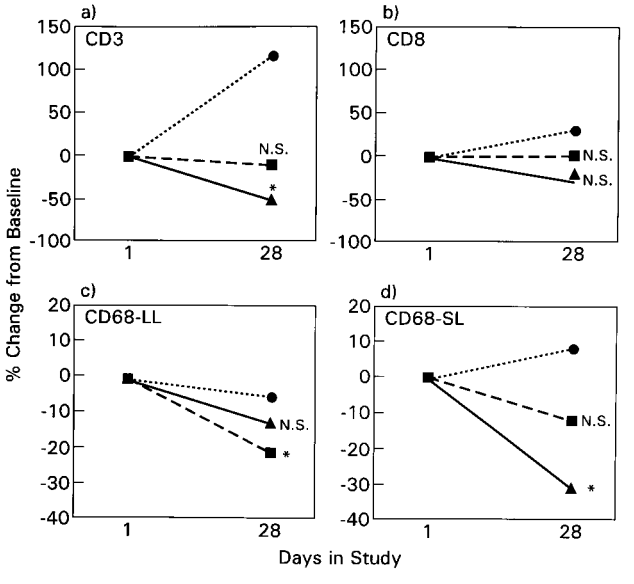

FIGURE 4. Modulation of synovial cellular infiltration. Cell-specific markers were quantified immunohistochemically in synovial biopsy tissues obtained before and after 29 days of IL-10 administration (n = 3/group; 5 μg/kg ■, 10 μg/kg ▲) or placebo (n = 4, ●). The proportion of each cell subset in lining layer (LL) and sublining layer (SL) areas was first calculated as a percentage of total nuclei/hpf, as described in *Materials and Methods* (30). Data represent the median percentage change in this proportion after 29 days compared with baseline. *, $p < 0.05$.

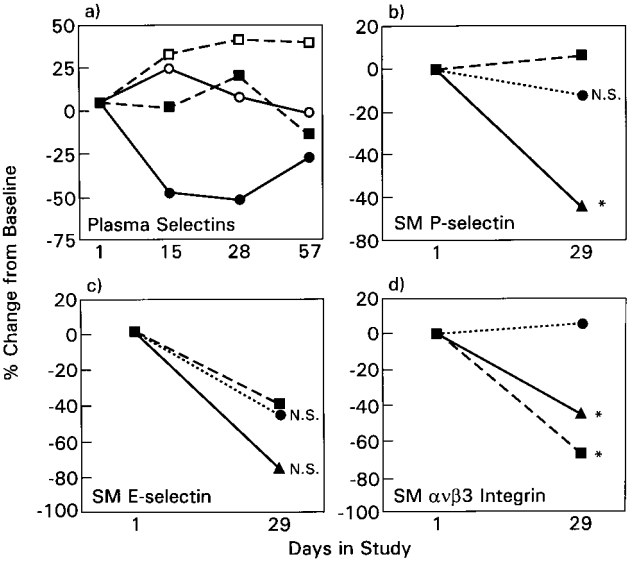

FIGURE 5. Vascular activation marker modulation by IL-10 administration. *A*, Soluble P- and E-selectin concentrations were measured by ELISA in plasma at the time points indicated (———, P-selectin; ○, placebo; ●, IL-10. – – – –, E-selectin; □, placebo; ■, IL-10). *B–D*, Expression of P-selectin, E-selectin, and $\alpha_v\beta_3$ integrin was quantified immunohistochemically, as described in the above synovial biopsy tissues (see Fig. 4). The number of adhesion molecule-positive vessels/hpf was first expressed as a proportion of the total number of vWF$^+$ vessels located on a parallel section (6 μM). Data represent the median percentage change in this proportion after 29 days compared with baseline. *, $p < 0.05$.

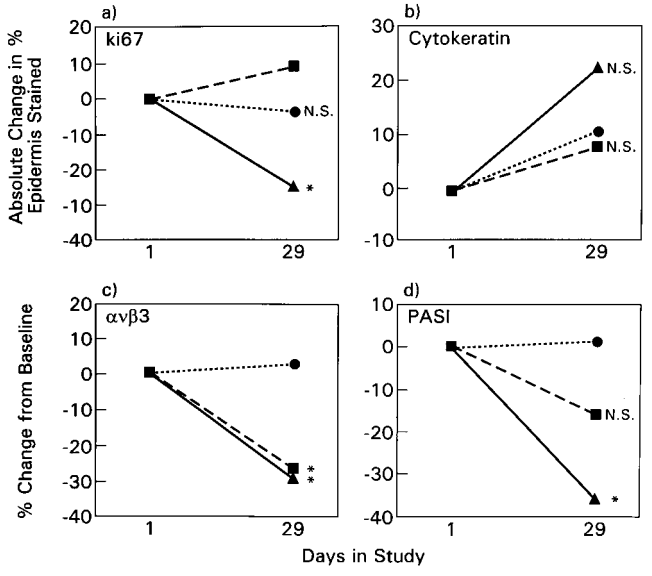

FIGURE 6. Modulation of cutaneous lesional disease markers following IL-10 administration. Data represent the median percentage change in this proportion after 29 days compared with baseline. The area of epidermis expressing ki67 (*a*) or cytokeratin 18 (*b*) was calculated by image analysis following immunohistochemical localization and expressed as square micrometers (2) before calculation of the percentage change through treatment. *c*, The number of $\alpha_v\beta_3$-positive vessels/hpf in dermis was first expressed as a proportion of the total number of vWF^+ vessels located on a parallel section (6 μM). *d*, Significant improvement in the clinical PASI score was observed in the IL-10 (10 μg/kg ▲) recipient group. IL-10 (n = 5/group; 5 μg/kg ■, 10 μg/kg ▲) or placebo (n = 4, ●). *, $p > 0.05$.

The reasons for this discrepancy are currently unclear. The present study therefore establishes that T cells can be functionally modified even in the context of ongoing autoimmune activation. Th1 responses are implicated in psoriatic pathology and with synovial inflammation (19, 22, 38, 39), suggesting that these effects are of likely significance. An important proviso lies in the lack of available data for tissue T cell responses. In some circumstances, IL-10 can promote IFN-γ production by $CD8^+$ CTL in vitro (46). Since

Table III. *Association of changes in synovial enhancement on MRI with treatment and clinical response*

	Synovial Enhancement on MRI		
	Improved	No change	Worsened
Treatment			
IL-10	5	2	0
Placebo	0	1	2
>20% improvement in SJC			
Yes	5	2	1
No	0	1	1

CD8 T cells have been implicated in PsA synovitis, it is possible that the effect of rhIL-10 administration upon synovial CD8 T cells differed from that on peripheral blood T cells in which CD4 cell responses may predominate.

rhIL-10 induced suppression of LPS-inducible monokine release, together with dose-dependent induction of TNFRII levels in serum. This provides compelling evidence for significant functional impairment of monocyte effector responses by rhIL-10, even in the context of ongoing autoimmune stimulation. Unlike previous studies, we did not see major effects on blood monocyte MHC class II expression, suggesting that this effect in circulating monocytes may not be a dominant feature during prolonged, systemic IL-10 therapy. No effect was evident by FACS on circulating costimulatory molecule expression (CD80 and CD86) by CD14 cells. However, IL-10 modifies skin-derived dendritic cell function in vitro (47), and such an effect for IL-10 therapy cannot be excluded.

An important element of the present study was the comparison of tissue responses following rhIL-10 administration. Reduced synovial infiltration by CD3 T cells and CD68 macrophages was evident in rhIL-10 compared with placebo recipients. A likely explanation for these effects was a direct effect on endothelial cell activation. Both circulating soluble and tissue P-selectin levels were reduced, providing a further potential mechanism for Th1 amelioration since P-selectin glycoprotein ligand-1 is a putative Th1 adhesin (48). No effect on E-selectin was observed, consistent with previous in vitro data (49). Suppression of the neovascular integrin marker $\alpha_v\beta_3$ was also

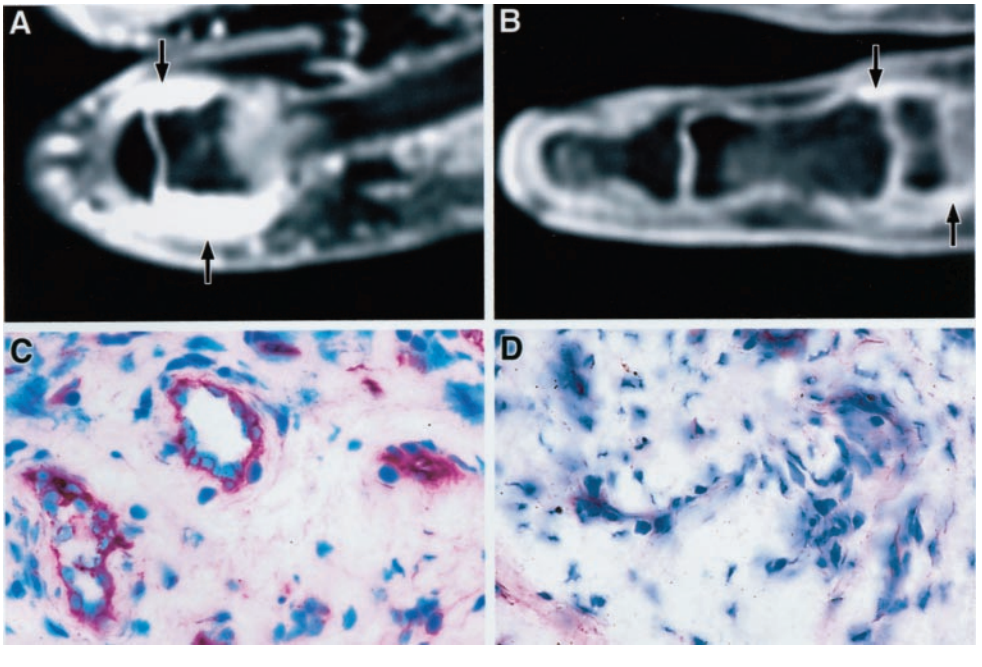

FIGURE 7. Representative MRI images with gadolinium enhancement performed before (*A*) and after (*B*) 28 days of IL-10 (10 mg/kg) administration. Arrow, Suppression of synovial enhancement. Simultaneous synovial biopsies from the same joint obtained before (*C*) and after (*D*) IL-10 administration, stained with $\alpha_v\beta_3$ integrin, demonstrate marked reduction in neovascularization.

Table IV. *Change in clinical parameters throughout treatment protocol*

Parameter	Placebo	IL-10 (μg/kg/day) 5	10
ACR 20			
Day 29	2/10 (20%)	2/7 (28%)	0/8 (0%)
>50% improvement SJC			
Day 29	5/10 (50%)	2/7 (28%)	3/8 (37%)
>30% improvement PASI			
Day 29	1/10 (10%)[a]	1/7 (14%)	6/8 (75%)[a]
Sustained improvement (>4 wk)			
>50% SJC	1/10 (10%)	1/7 (14%)	1/8 (12%)
>30% PASI	1/10 (10%)[b]	1/7 (14%)	4/8 (50%)[b]
Dose of prednisone (mg/day)[c]	2.1 ± 1.28	2.0 ± 1.29	1.62 ± 1.25

[a] Placebo vs 10 μg/kg/day; $p < 0.02$ (Fisher's exact test).
[b] Placebo vs 10 μg/kg/day; NS, $p = 0.1$ (Fisher's exact test).
[c] Mean ± SEM; no patient required change in the dose of prednisone throughout the treatment.

evident in skin and synovium. These changes correlated with clinical response and improvement on MRI in the biopsied joint. Although interpreted on small numbers of patients, these data suggest that it is possible to modify endothelial activation and neovascularization by IL-10 therapy in vivo, as previously demonstrated in vitro in tumor models (50). Indeed, systemic administration may be optimal to achieve functionally relevant bioavailability in the vasculature. Given the key role for angiogenesis in inflammatory arthritis and psoriasis, this is most likely an important observation. These data further suggest that the combination of MRI and tissue biopsy deserves validation in assessing responses to biological agents in inflammatory arthritis.

Suppressed ki67 expression in psoriatic lesions suggested some early effect on keratinocyte proliferation was manifest even in clinically established cutaneous lesions. However, most inflammatory parameters in skin were unchanged when compared with placebo. The reason for this discrepancy with synovial tissue is presently unclear. However, since by definition we biopsied ongoing inflammatory skin lesions, the lack of significant histological evidence of resolution was predictable.

IL-10 has previously been shown to improve psoriasis in open-label, uncontrolled studies (23, 51). None of these reports included PsA patients. The primary outcome of this study was therefore to establish the safety and tolerability of IL-10 in PsA. Although it was not powered to address efficacy, improvement in the PASI was detected. We believe this report is the first demonstration of clinical efficacy in psoriasis using a placebo-controlled study design. With inclusion of placebo comparison, the magnitude of cutaneous response was modest and less than that anticipated from previous studies. However, unlike previous studies, patients were recruited for this study on the basis of articular rather than cutaneous disease activity. Nevertheless, it is of interest that administration of IL-10 in the psoriatic skin/SCID transplant model does not ameliorate clinical or histological inflammatory scores (52). Further placebo-controlled studies are therefore required to properly evaluate and quantify the therapeutic potential of IL-10 in cutaneous disease.

The apparent difference in cutaneous and articular responses is intriguing and could reflect several factors. Thus, available clinical assessment tools may not operate well in PsA. The ACR20 is validated in RA and may not adequately measure PsA responses. PsA is characterized by a limited acute phase response in comparison with RA. Moreover, IL-10 may mediate direct effects on acute phase reactants independent of effects on disease activity. However, even when we examined 50% improvement in SJC a potential surrogate, we detected similar response rates in active and placebo recipients. High placebo response rates in articular disease are well recognized with parenteral administration protocols, but are usually limited to the first 2 mo of treatment. Longer-term, appropriately powered studies will be required to clarify potential efficacy of IL-10 in articular disease. It is also possible that IL-10 administration is elucidating differences in the primary disease processes in skin and articular structures. Detailed comparative studies will be required to address this possibility, particularly with respect to the functional contribution of CD8 T cells, as noted above. However, most currently available data indicate that skin and synovial lesions contain similar cytokine expression patterns, particularly the paucity of IL-10, and share T cell clonal expansions (21, 27).

That immunological responses did not correlate directly with clinical responses illustrates the requirement for further study of those immunological parameters that best reflect relevant autoimmune disease processes ex vivo. Moreover, this study clarifies the importance of a placebo control, even for measurement of immunological activities, since trends demonstrated in smaller, noncontrolled studies have not been confirmed. It is reassuring that peripheral blood and endothelium, tissues most likely to be exposed to suppressive concentrations of IL-10, exhibited consistent functional changes on subsequent analysis. In summary, the present study clearly demonstrates that significant immune modulation can be safely achieved in PsA by administration of IL-10, and as such, it represents a valuable contribution to understanding the effects of IL-10 in vivo in an autoimmune/autoinflammatory disease context.

Acknowledgments

The invaluable contributions of the following are acknowledged: nurses of the 9 East Day Hospital, Judith Starling, RPh Clinical Pharmacy; Margaret R. Brown, MT, Department of Clinical Pathology; Dr. Peter Choyke, Department of Radiology, Warren Magnuson Clinical Center; Barbara Foster, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health; and Drs. Paul Grint and Janice Wherry, Schering-Plough. We express our gratitude to the contributing patients and their referring physicians (Drs. W. Barth, M. Bennett, M. Burgoyne, C. DeVincentis, J. Ferriss, D. Francois, M. Furlong, M. Gourley, E. Jenkins, J. Laukaitis, M. Lockshin, W. Mullins, P. Nguyen, J. Pendleton, K. Price, P. Rochmis, R. Rosenberg, S. Schechter, and N. Wei).

References

1. De Waal Malefyt, R., J. Abrams, B. Bennett, C. G. Figdor, and J. E. de Vries. 1991. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med. 174:1209.*
2. Wang, P., P. Wu, M. I. Siegel, R. W. Egan, and M. M. Billah. 1995. Interleukin (IL)-10 inhibits nuclear factor κB (NFκB) activation in human monocytes: IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *J. Biol. Chem. 270:9558.*

3. Moore, K. W., A. O'Garra, R. de Waal Malefyt, P. Vieira, and T. R. Mosmann. 1993. Interleukin-10. *Annu. Rev. Immunol. 11:165.*
4. Chomarat, P., E. Vannier, J. Dechanet, M. C. Rissoan, J. Banchereau, C. A. Dinarello, and P. Miossec. 1995. Balance of IL-1 receptor antagonist/IL-1β in rheumatoid synovium and its regulation by IL-4 and IL-10. *J. Immunol. 154: 1432.*
5. Joyce, D. A., D. P. Gibbons, P. Green, J. H. Steer, M. Feldmann, and F. M. Brennan. 1994. Two inhibitors of pro-inflammatory cytokine release, interleukin-10 and interleukin-4, have contrasting effects on release of soluble p75 tumor necrosis factor receptor by cultured monocytes. *Eur. J. Immunol. 24:2699.*
6. De Waal Malefyt, R., J. Haanen, H. Spits, M. G. Roncarolo, A. te Velde, C. Figdor, K. Johnson, R. Kastelein, H. Yssel, and J. E. de Vries. 1991. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via down-regulation of class II major histocompatibility complex expression. *J. Exp. Med. 174:915.*
7. Chang, C. H., M. Furue, and K. Tamaki. 1995. B7-1 expression of Langerhans cells is up-regulated by proinflammatory cytokines, and is down-regulated by interferon-γ or by interleukin-10. *Eur. J. Immunol. 25:394.*
8. Buelens, C., F. Willems, A. Delvaux, G. Pierard, J. P. Delville, T. Velu, and M. Goldman. 1995. Interleukin-10 differentially regulates B7-1 (CD80) and B7-2 (CD86) expression on human peripheral blood dendritic cells. *Eur. J. Immunol. 25:2668.*
9. Parry, S. L., M. Sebbag, M. Feldmann, and F. M. Brennan. 1997. Contact with T cells modulates monocyte IL-10 production: role of T cell membrane TNF-α. *J. Immunol. 158:3673.*
10. Aste-Amezaga, M., X. Ma, A. Sartori, and G. Trinchieri. 1998. Molecular mechanisms of the induction of IL-12 and its inhibition by IL-10. *J. Immunol. 160: 5936.*
11. De Waal Malefyt, R., H. Yssel, and J. E. de Vries. 1993. Direct effects of IL-10 on subsets of human $CD4^+$ T cell clones and resting T cells: specific inhibition of IL-2 production and proliferation. *J. Immunol. 150:4754.*
12. Groux, H., M. Bigler, J. E. de Vries, and M. G. Roncarolo. 1996. Interleukin-10 induces a long-term antigen-specific anergic state in human $CD4^+$ T cells. *J. Exp. Med. 184:19.*
13. Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J. E. de Vries, and M. G. Roncarolo. 1997. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature 389:737.*
14. Vora, M., H. Yssel, J. E. de Vries, and M. A. Karasek. 1994. Antigen presentation by human dermal microvascular endothelial cells: immunoregulatory effect of IFN-γ and IL-10. *J. Immunol. 152:5734.*
15. Pugin, J., R. J. Ulevitch, and P. S. Tobias. 1993. A critical role for monocytes and CD14 in endotoxin-induced endothelial cell activation. *J. Exp. Med. 178:2193.*
16. Reitamo, S., A. Remitz., K. Tamai, and J. Uitto. 1994. Interleukin-10 modulates type I collagen and matrix metalloprotease gene expression in cultured human skin fibroblasts. *J. Clin. Invest. 94:2489.*
17. Lacraz, S., L. P. Nicod, R. Chicheportiche, H. G. Welgus, and J. M. Dayer. 1995. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. *J. Clin. Invest. 96:2304.*
18. Xu, L. X., T. Kukita, A. Kukita, T. Otsuka, Y. Niho, and T. Iijima. 1995. Interleukin-10 selectively inhibits osteoclastogenesis by inhibiting differentiation of osteoclast progenitors into preosteoclast-like cells in rat bone marrow culture system. *J. Cell. Physiol. 165:624.*
19. Uyemura, K., M. Yamamura, D. F. Fivenson, R. L. Modlin., and B. J. Nickoloff. 1993. The cytokine network in lesional and lesion free psoriatic skin is characterized by a T helper type 1 cell mediated response. *J. Invest. Dermatol. 101:701.*
20. Schlaak, J. F., M. Buslau, W. Jochum, E. Hermann, M. Girndt, H. Gallati, K. H. Meyer zum Buschenfelde, and B. Fleischer. 1994. T cells involved in psoriasis vulgaris belong to the Th1 subset. *J. Invest. Dermatol. 102:145.*
21. Tassiulas, I., S. R. Duncan, M. Centola, A. N. Theofilopoulos, and D. T. Boumpas. 1999. Clonal characteristics of T cell infiltrates in skin and synovium of patients with psoriatic arthritis. *Hum. Immunol. 60:479.*
22. Nickoloff, B. J., D. P. Fivenson, S. L. Kinkel, R. M. Strieter, and L. A. Turka. 1994. Keratinocyte interleukin-10 expression is up-regulated in tape stripped skin, poison ivy dermatitis and Sezary syndrome, but not in psoriatic plaques. *Clin. Immunol. Immunopathol. 73:63.*
23. Asadullah, K., W. Sterry, K. Stephanek, D. Jasulaitis, M. Leupold, H. Audring, H. D. Volk, and W. D. Docke. 1998. IL-10 is a key cytokine in psoriasis: proof of principle by IL-10 therapy: a new therapeutic approach. *J. Clin. Invest. 101: 783.*
24. Kristensen, M., B. Deleuran, D. J. Eedy, M. Feldmann, S. M. Breathnach, and F. M. Brennan. 1992. Distribution of interleukin 1 receptor antagonist protein (IRAP), interleukin 1 receptor, and interleukin 1α in normal and psoriatic skin: decreased expression of IRAP in psoriatic lesional epidermis. *Br. J. Dermatol. 127:305.*
25. Mrowietz, U., E. Christophers, and P. Altmeyer. 1999. Treatment of severe psoriasis with fumaric acid esters: scientific background and guidelines for therapeutic use. *Br. J. Dermatol. 141:424.*
26. Ockenfels, H. M., T. Schultewolter, G. Ockenfels, R. Funk, and M. Goos. 1998. The antipsoriatic agent dimethylfumarate immunomodulates T-cell cytokine secretion and inhibits cytokines of the psoriatic cytokine network. *Br. J. Dermatol. 139:390.*
27. Danning, C., C. G. Illei, C. Hitchon, D. T. Boumpas, and I. B. McInnes. 2000. Macrophage derived cytokine and NFκB p65 expression in psoriatic arthritis synovial membrane and skin. *Arthritis Rheum. 43:1244.*
28. Detmar, M., L. F. Brown, K. P. Claffey, K. T. Yeo, O. Kocher, R. W. Jackman, B. Berse, and H. F. Dvorak. 1994. Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. *J. Exp. Med. 180:1141.*
29. Creamer, J. D., and J. N. Barker. 1995. Vascular proliferation and angiogenic factors in psoriasis. *Clin. Exp. Dermatol. 20:6.*
30. Fearon, U., R. Reece, J. Smith, P. Emery, and D. J. Veale. 1999. Synovial cytokine and growth factor regulation of MMPs/TIMPs: implications for erosions and angiogenesis in early rheumatoid and psoriatic arthritis patients. *Ann. NY Acad. Sci. 878:619.*
31. Chernoff, A. E., E. V. Granowitz, L. Shapiro, E. Vannier, G. Lonnemann, J. B. Angel, J. S. Kennedy, A. R. Rabson, S. M. Wolff, and C. A. Dinarello. 1995. A randomized, controlled trial of IL-10 in humans: inhibition of inflammatory cytokine production and immune responses. *J. Immunol. 154:5492.*
32. St Clair, E. W. 1999. Interleukin 10 treatment for rheumatoid arthritis. *Ann. Rheum. Dis. 58:I99.*
33. Van Deventer, S. J., C. O. Elson, and R. N. Fedorak. 1997. Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease: Crohn's Disease Study Group. *Gastroenterology 113:383.*
34. Fredriksson, T., and U. Pettersson. 1978. Severe psoriasis: oral therapy with a new retinoid. *Dermatologica 157:238.*
35. Arnett, F. C., S. M. Edworthy, D. A. Bloch, D. J. McShane, J. F. Fries, and N. S. Cooper. 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum. 31:315.*
36. McInnes, I. B., B. P. Leung, M. Field, X. Q. Wei, F. P. Huang, R. D. Sturrock, A. Kinninmonth, J. Weidner, R. Mumford, and F. Y. Liew. 1996. Production of nitric oxide in the synovial membrane of rheumatoid and osteoarthritis patients. *J. Exp. Med. 184:1519.*
37. Elson, L. H., T. B. Nutman, D. D. Metcalfe, and C. Prussin. 1995. Flow cytometric analysis for cytokine production identifies T helper 1, T helper 2, and T helper 0 cells within the human $CD4^+CD27^-$ lymphocyte subpopulation. *J. Immunol. 154:4294.*
38. Dolhain, R., A. N. Van Der Heiden, N. T. Ter Haar, F. C. Breedveld, and A. M. M. Miltenburg. 1996. Shift toward T lymphocytes with a T helper 1 cytokine-secretion profile in the joints of patients with rheumatoid arthritis. *Arthritis Rheum. 39:1961.*
39. Simon, A. K., E. Seipelt, and J. Sieper. 1994. Divergent T-cell cytokine patterns in inflammatory arthritis. *Proc. Natl. Acad. Sci. USA 91:8562.*
40. Loetscher, P., M. Uguccioni, L. Bordoli, M. Baggiolini, B. Moser, C. Chizzolini, and J. M. Dayer. 1998. CCR5 is characteristic of Th1 lymphocytes. *Nature 391: 344.*
41. Dinarello, C. A. 1999. Interleukin-18. *Methods 19:121.*
42. Austrup, F., D. Vestweber, E. Borges, M. Lohning, R. Brauer, U. Herz, H. Renz, R. Hallmann, A. Scheffold, A. Radbruch, and A. Hamann. 1997. P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. *Nature 385:81.*
43. Storgard, C. M., D. G. Stupack, A. Jonczyk, S. L. Goodman, R. I. Fox, and D. A. Cheresh. 1999. Decreased angiogenesis and arthritic disease in rabbits treated with an $\alpha_v\beta_3$ antagonist. *J. Clin. Invest. 103:47.*
44. Brennan, F. M., K. A. Browne, P. A. Green, J. M. Jaspar, R. N. Maini, and M. Feldmann. 1997. Reduction of serum matrix metalloproteinase 1 and matrix metalloproteinase 3 in rheumatoid arthritis patients following anti-tumor necrosis factor-α (cA2) therapy. *Br. J. Rheumatol. 36:643.*
45. Bacchetta, R., M. Bigler, J. L. Touraine, R. Parkman, P. A. Tovo, J. Abrams, R. de Waal Malefyt, J. E. de Vries, and M. G. Roncarolo. 1994. High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. *J. Exp. Med. 179: 493.*
46. Santin, A. D., P. L. Hermonat, A. Ravaggi, S. Bellone, S. Pecoelli, J. J. Roman, G. P. Parham, and M. J. Cannon. 2000. Interleukin-10 increases Th1 cytokine production and cytotoxic potential in human papilloma virus specific CD8 cytotoxic T lymphocytes. *J. Virol. 74:4729.*
47. Mitra, R. S., T. A. Judge, F. O. Nestle, L. A. Turka, and B. J. Nickoloff. 1995. Psoriatic skin-derived dendritic cell function is inhibited by exogenous IL-10: differential modulation of B7-1 (CD80) and B7-2 (CD86) expression. *J. Immunol. 154:2668.*
48. Borges, E., W. Tietz, M. Steegmaier, T. Moll, R. Hallmann, A. Hamann, and D. Vestweber. 1997. P-selectin glycoprotein ligand-1 (PSGL-1) on T helper 1 but not on T helper 2 cells binds to P-selectin and supports migration into inflamed skin. *J. Exp. Med. 185:573.*
49. Vora, M., L. I. Romero, and M. A. Karasek. 1996. Interleukin-10 induces E-selectin on small and large blood vessel endothelial cells. *J. Exp. Med. 184:821.*
50. Huang, S., S. E. Ullrich, and M. Bar-Eli. 1999. Regulation of tumor growth and metastasis by interleukin-10: the melanoma experience. *J. Interferon Cytokine Res. 19:697.*
51. Asadullah, K., W. D. Docke, M. Ebeling, M. Friedrich, G. Belbe, H. Audring, H. D. Volk, and W. Sterry. 1999. Interleukin 10 treatment of psoriasis: clinical results of a phase 2 trial. *Arch. Dermatol. 135:187.*
52. Dam, T. N., S. Kang, B. J. Nickoloff, and J. J. Voorhees. 1999. 1α,25-dihydroxycholecalciferol and cyclosporine suppress induction and promote resolution of psoriasis in human skin grafts transplanted on SCID mice. *J. Invest. Dermatol. 113:1082.*