Cytokines, cytokine antagonists and soluble adhesion molecules in patients with ocular Behçet's disease treated with human recombinant interferon-α2a. Results of an open study and review of the literature

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ABSTRACT

Objective. *To elucidate the influence that interferon-α exerts on the cytokine network in active ocular Behçet's disease (BD).*

Methods. *Fifty patients with active ocular BD were treated with human recombinant interferon-α2a (rhIFN-α2a). Serum was analysed for the presence of IL-10, TNF-α, IL-8, IL-6, sIL-2R, IFN-γ, IFN-α, IL-12, IL-4, sTNFRI (p55), sTNFRII (p75), IL-1RA, G-CSF, sE-selectin, sVCAM-1, sICAM-1 and neopterin before initiation of and at several time points during IFN treatment and compared to 21 healthy controls.*

Results. *The levels of IFN-α, IL1-RA and sTNFRII were significantly increased in the patients at baseline in comparison to healthy controls. During treatment with rhIFN-α2a, when remission was achieved as defined by the scoring system used, a significant increase in levels of IFN-α, IL-2R, TNF-α, sTNF-RII, sICAM-1, sVCAM-1, neopterin in the serum was observed, with a tendency towards increased IL-1RA as well. In contrast, leuko- and thrombocyte counts and sE-selectin serum levels significantly decreased. Positive correlations were found between IFN dosage or serum levels and sVCAM-I, neopterin, sTNF-RII and sIL-2R, between sVCAM-I, sIL-2R, TNFα, sTNF-RII and neopterin, sICAM-I and sVCAM-I, sIL2-R and sTNF-RII, and, finally, between sIL2-R and sICAM-I.*

Conclusions. *IFN-α exerts diverse influences mainly on cytokine antagonists and soluble adhesion molecules. Because sTNF-RII and IL-1RAwere increased by IFN-α treatment, these might be interesting alternative treatment options in refractory BD. Some of the side-effects of IFN-α may be caused by activation of monocytes, which is reflected by an increase in neopterin serum levels.*

Introduction

Behçet's disease (BD) is a multisystem vasculitis of unknown origin. It is characterised by recurrent aphthous stomatitis, genital ulcerations, cutaneous symptoms such as papulopustules or erythema nodosum, a positive pathergy phenomenon (a papulopustule occurring 24-48 hours after intracutaneous puncture with a sterile 1 gauge needle) and ocular inflammation with retinal vasculitis. Oligoarthritis, meningoencephalitis or CNS vasculitis, deep vein thrombosis and arterial aneurysms are less common. Classification follows the International Study Group Criteria (1, 2). As new therapeutic options are required, Interferon-α was introduced for the treatment of BD by Tsambaos *et al.* (3) in 1986 because of its antiviral and anti-proliferative properties. In refractory cases, interferon-α was shown to be effective by several groups in case reports or small case series (4). Because of the promising results of a pilot study, (5,6), we performed a four-center, open, uncontrolled study, wherein 50 patients were treated for refractory sight-threatening ocular manifestations (exclusively panuveitis or posterior uveitis with retinal vasculitis). More than 90% of the patients achieved complete remission with rhIFN-α2a (7). The mechanism of action of rhIFN-α in BD is still unclear; hence, we have here analysed its influence on the cytokine network in BD patients.

Materials and methods

Fifty patients with refractory ocular BD (severe sight-threatening panuveitis, posterior uveitis or retinal vasculitis) were treated with rhIFN-α2a according to an algorithm published elsewhere (7). All patients except 3 were on prednisolone (mean 97 mg, range 0 to 1000) before initiation of IFN treatment. The immunosuppressive agents were stopped and prednisolone was tapered to a maximum of 10 mg the day before IFN was started. 52% of the patients (n = 26, one on mycophenolate mofetil, 19 on CSA, 4 on azathioprine and 2 on methotrexate) were on immunosuppressive agents directly before IFN treatment, which were stopped the day before IFN.

Forty-six of the patients fulfilled the International Study Group Criteria (1), while 4 had incomplete BD according to the O'Duffy Criteria (8). Disease activity was measured by the Behçets Disease Activity Scoring system (9) and the Posterior Uveitis Scoring System (10). "Remission" was defined as the scores being 0. Blood was drawn after informed consent and approval of the local ethics committee was obtained, before initiation of IFN-treatment, at weeks 1, 2, and every 4 weeks for 6 months, later every 8 weeks. The serum was stored at –70°C until analysis. Twenty-one healthy donors of comparable age and sex distributions served as controls. Differential blood counts and CRP were also assessed on each visit and in the controls.

The following cytokines, chemokines, soluble adhesion molecules and cytokine antagonists were analysed by chemiluminescent immunometric or sandwich ELISA techniques: IL-10; TNF-α; IL-8, IL-6; the soluble IL-2-receptor IL-2R (DPC Diagnostic Products Corporation, Los Angeles, CA, USA); IFN-γ, IFN-α (Bender Med Systems, Vienna, Austria); IL-12, IL-4, the soluble TNF-receptors sTNF-RI (p55) and sTNF-RII (p75), the IL-1-Receptor-antagonist IL-1RA, G-CSF, soluble adhesion molecules sE-selectin, sVCAM-1, sICAM-1 (R&D Systems Minneapolis, MN, USA). Neopterin was measured by ELISA(IBL, Hamburg, Germany).

A relevant influence of freezing on the results of the cytokine measurements was excluded by comparing 4 fresh samples from 4 healthy persons to samples from the same persons and time points which had been frozen and stored for 4 weeks.

There were no significant mean differences or ratios between fresh and frozen samples (geometric means for the fresh/frozen samples: IL-10: (normal range 2.0 to 24.0) all values below quantification limit (BQL), TNF-α: 95% confidence interval for the mean difference (CI.d) –0.84 to 0.54 pg/ml (normal range up to 8.1, detection limit 1.7), IL-8: all values BQL (normal range up to 70 pg/ml, detection limit 6.2), IL-6: confidence limit for the geometric mean ratio (CI.r) 0.87 to 1.15 (normal range up to 11.3 pg/ml, detection limit 1), sIL-2R: CI.r 0.74 to 1.11 IU/ml (normal range 223 to 710 IU/ml), IFN-γ: all values BQL (normal range up to 168 pg/ml, detection limit 1.5), IFN-α: CI.r 0.92 to 1.12 pg/ml (normal range up to 266.3 pg/ml, detection limit 4.8), IL-12: all values BQL (normal range up to 7.8 pg/ml, detection limit 5), IL-4: all values BQL (normal range up to 31.2 pg/ ml, detection limit 10), sTNF-RI: CI.d –63 to 16 pg/ml (normal range 749 to 1966), sTNF-RII: CI.d –108 to 50 pg/ml (normal range 1003 to 3170), IL-1RA: CI.r 0.98 to 1.05 (normal range 106 to 1552 pg/ml), G-CSF: CI.d –2.7 to 5.7 pg/ml (normal range up to 39, detection limit 10), sE-selectin: CI.d –9.1 to 6.3 ng/ml (normal range 29.1 to 63.4), sVCAM-1: CI.r 0.95 to 1.04 (normal range 395 to 714 ng/ml), sICAM-1: CI.d –10 to 17 ng/ml (normal range 115 to 306), neopterin: CI.d –0.10 to 0.060 nmol/ml (normal range up to 10).

Statistical analysis was performed using JMP4.0.5 (SAS Institute Inc., Cary, NC, USA). Data are given as mean and standard deviation (SD) or geometric mean and coefficient of variation (CV) depending on skewness. Accordingly, differences between means or multiples of geometric means of two samples were estimated, the latter by the log-transformation method. Patients and controls were assumed to have different variances. Where more than one observation per patient were considered, data were centered individually. Consequently, coefficients express the intra-individual difference, factor or correlation. Similarly, variables with many values below the quantification limit (BQL) were considered dichotomous and analysed by exact methods for paired (sign-test) and unpaired data (Fisher's exact test). For CRP levels, the rank-sum test was applied, as approximately 25% of all values were not quantified (<0.4 mg/dl), although within the normal range. Intra-individual correlations were estimated by centering variables by patient, weighing observations by the inverse number of observations on a patient, and computing the product-moment correlation coefficient, using R (version 1.5.1) (11). The number of patients involved was used in the degrees of freedom when computing the p-value. The Bonferroni-Holm procedure was used to adjust for multiple tests.

Results

Clinical parameters

We recently reported elsewhere (5) that mean BD activity score at baseline was 5.8, falling to 3.3 at week 24 and further to 2.8 at week 52. The total number of patients was 50. The median time to remission of BD activity score (score = 0) was 24 weeks (95% CI week 4 to week 36). The number of patients who achieved remission of BD activity score was 35 (of 42 who were followed up that long). In contrast to the posterior uveitis score (PUS), BD activity score did not reach remission in a considerable number of patients due to persisting oral aphthous ulcers (12).

The posterior uveitis score (PUS) of 50 patients including 79 affected eyes fell from 3.5 (week 0) to 0.4 at week 24. Remission (defined as a posterior uveitis score 0, remission of retinal inflammation) in all affected eyes of the responders (n = 71) was reached by week 24. The number of patients in complete remission of PUS was 46 (3 non-responder, one incomplete response). The median time to remission was 4 weeks (95% CI week 2 to week 4 (7). Mean Interferon dosage at remission by PUS was 4.4 (SD 1.7) x 10^6 iU/day, and at remission by BD score it was 3.6 (SD

2.2) x 10^6 iU day. Mean prednisolone dosage was tapered to a mean of 2 mg (range 0 to 10) at week 52.

Baseline cytokines and α-antagonists in comparison to healthy controls

The serum levels of IL-4, IL-12 and IFN-γ were below the detection limit in all patients at baseline as well as in all controls. Similarly, only 3 patients had IL-8 values above the quantification limit, and IL-10 was below the quantification limit in 90% of the controls and 54% of the patients. For IL-6, TNF-α, G-CSF, sIL-2R and sTNF-RI there were no differences between patients and controls. In contrast, patients had significantly higher levels of IFN-α, sTNF-RII and IL-1RA than controls already at baseline (Table I).

Baseline soluble adhesion molecules, neopterin, CRP and differential blood count

Leukocyte numbers were significantly higher in patients than in controls, but there were no significant differences in thrombocyte numbers, sE-Selectin, sICAM-1 and sVCAM-1 and neopterin concentrations (Table I). CRP at baseline was above 1 mg/dl in 15 of 47 patients (32%), whereas none of the 21 healthy controls registered above the normal range. This difference was significant ($p = 0.003$).

Cytokines and -antagonists at remission of PUS

During IFN-treatment, when remission of ocular disease (posterior uveitis score = 0) was reached (after a median time of 4 weeks), serum levels of IFN-α, IL-2R, TNF-α, and sTNF-RII were significantly increased. IL-1RAwas tendentially increased. No changes were seen for IL-6, sTNF-RI and G-CSF (Table II).

Soluble adhesion molecules, neopterin, CRP, differential blood count at remission of PUS

sICAM-1, sVCAM-1 and neopterin were found to be significantly increased. Levels of sE-selectin as well as leukocyte and thrombocyte counts were significantly reduced (Table II). Only 3 of 49 patients (6%) still had CRPserum levels above 1 mg/dl. This difference compared to CRP baseline levels is significant ($p < 0.0001$) as 11 patients with baseline CRP levels above 1 mg/dl had normal levels at remission of PUS.

Cytokines and -antagonists at remission of BD activity score

Considering the time point when complete remission of all features of BD was reached (BD score = 0), which was the case after a median treatment period of 24 weeks, the results were similar, with significant albeit lesser increases in IFN-α, TNF-α, sTNF-RII, a tendential increase in IL-1RA, and no changes in IL-6, G-CSF, sIL-2R, sICAM-1 and sTNF-RI (Table II).

Adhesion molecules, neopterin, CRP, differential blood count at remission of BD activity score

sVCAM-1 and neopterin significantly increased when compared to baseline levels, whereas a decrease in sE-Selectin (but in this case failing to reach significance), and significant decreases in leukocyte and thrombocyte counts occurred. The reductions of CRPserum levels were not significant at remission defined by BD score when compared to baseline because of the small numbers of observations made, although at remission by BD score only 1 of 31 patients (3%) still had elevated CRP levels ($p = 0.13$) (Table II).

Correlations

Positive correlations between IFN dosage or serum levels and sVCAM-I (correlation coefficient $r=0.76$, $p< 0.00001$), neopterin ($r=0.70$, $p< 0.00001$), sTNF-RII ($r=0.55$, $p= 0.00027$) and sIL-2R ($r = 0.46$, $p=0.0038$), between sVCAM-I, TNF-α, sTNF-RII, sIL-2R and neopterin ($r = 0.70$, $p < 0.00001$; $r = 0.48$, $p = 0.0022$; $r=0.60$, $p= 0.00005$; $r = 0.47$, $p = 0.0027$), sICAM-I and sVCAM-I ($r =0.51$, $p = 0.00085$), sIL2-R and sTNF-RII ($r=0.51$, $p = 0.00011$), and between sIL2-R and sICAM-I ($r = 0.48$, $p = 0.0024$) were established.

Discussion

Baseline levels

At baseline, when the patients still had active disease, IL-4, IL-8, IL-10, IL-12, and IFN-γ were not measurable in the patients'sera. TNF-α and G-CSF were not elevated when compared to the controls. IL-6 as a pro-inflammatory cytokine mostly paralleling CRP (13) only tended to be elevated at baseline.

The lack of elevation of the pro-inflammatory cytokines IL-8 and TNF-α in our patients with active ocular BD seems to contradict the results of others, who repeatedly reported increased cytokine and chemokine serum levels. Mostly, a prominent over-secretion of TH-1 pro-inflammatory cytokines in the active phase of the disease has been described (14-21). It was proposed that IL-8 is superior to CRPor ESR for monitoring the inflammatory activity of the disease and currently is considered to be of utmost importance in the pathogenesis of BD, inducing neutrophil chemotaxis and hyperactivity (22-28). It is possible that there is a difference in cytokine serum levels between patients with predominantly ocular BD and more systemic manifestations. This, of course, has to be evaluated further by comparing patients with different manifestations of BD, especially because a significant reduction of IL-8 production by IFN-α was reported *in vitro* (29-31), which would explain some of its therapeutic effects. Recently, Bardak *et al.* (32) reported on IL-6, IL-8 and TNF-α levels in patients with active and isolated ocular BD, inactive ocular BD and healthy controls. They found significantly higher IL-6, 8, and TNF-α levels for their active ocular BD patients in comparison to inactive BD and healthy controls.

The soluble adhesion molecules are shed from activated endothelium and are thought to reflect this activation (3). Elevated sICAM-1 may reflect both immune and endothelial activation, whereas VCAM-1 and E-selectin are restricted to endothelium. Both VCAM-1 and E-Selectin have a low or absent constitutive endothelial expression and are induced by pro-inflammatory cytokines (34). Although potential regulatory roles of soluble cell adhesion molecules remain to be proven, a preliminary report has shown that sICAM-1 and sVCAM-1 suppress the endothelial expression of the respective cellular

Table I. Comparison of healthy controls and patients at baseline.

Parameter	IFN-α pg/ml	TNF-α pg/ml	IL-6 pg/ml	G-CSF pg/ml	IL-1RA Pg/ml	SIL-2R U/ml	STNF-RI pg/ml	STNF-RII pg/ml	s-Selectin ng/ml	s-ICAM-1 ng/ml	s-VCAM-1 ng/ml	Neopterin ng/ml	Leukol 1000/u	Thrombo 1000/ul
(Controls)														
n	20	20	21	21	21	20	20	20	21	20	20	20	20	20
Mean	0.8	3.96	4.8	17	259	400	1216	1833	49.9	223	515.2	1.87	7.15	253
SD / CV	0	0.12	0	0.89	0.55	0.278	266	687	19.2	30.6	0.198	0.89	2.07	42.6
Patients baseline														
n	22	39	22	40	31	31	32	32	39	32	32	32	33	33
Mean	18.1	4.96	6.26	20	554	503	1336	2691	40.7	250	552.6	1.39	9.82	270
SD / CV	2.08	1.14	0.69	1.5	0.615	0.453	531	791	22.4	104	0.348	1.24	3.32	94.8
99% CI for the	10.4	0.87	0.896	0.32	1.42	0.964	-175	308	-24.6	-26	0.88	-1.28	0.69	-33.6
difference or ratio	to 49.3	to 1.96	to 1.90	to 1.5	to 3.23	to 1.64	to 415	to 1408	to 6.3	to 79	to 1.308	to 0.288	to 4.65	to 68.4
P (vs controls)	<0.0001*	0.087	0.058	0.19	<0.0001*	0.025	0.28	0.0001*	0.12	0.18	0.35	0.097	0.0007*	0.37

Mean: geometric for IL-1RA, IL-2R, IL-6, IFN_, sVCAM-1, other: arithmetic.
SD/VC: standard deviation (SD) for sTNF-RI, sTNF-RII, G-CSF, s-Selectin, sICAM-1, Neopterin, Leuko, Thrombo.

Table II. Parameters at remission by posterior uveitis score (PUS) and BD activity score in comparison to baseline values.

Parameter	IFN-α	TNF-α	IL-6	G-CSF	IL-1RA	SIL-2R	STNF-RI	STNF-RII	s-Selectin	s-ICAM-1	s-VCAM-1	Neopterin	Leuko	Thrombo
(Patients PUS remission)														
n	51	39	39	55	55	54	55	54	39	55	55	53	64	64
Mean	83.8	9.40	6.7	26.8	707	786	1297	3910	36.4	303	991	3.73	5.35	176
SD / CV	2.08	1.74	1.15	25.0	0.58	0.354	434	670	19.4	101	0.36	1.36	1.84	56
99% CI for the	3.31	1.57	0.75	-7.83	0.843	1.31	-191	792	3.34	20.	1.54	1.49	-6.28	-105
difference or ratio	to 10.9	to 2.42	to 2.04	to 12.4	to 1.64	to 1.68	to 85.8	to 1535	to 10.8	to 64.5	to 1.80	to 2.67	to -3.70	to -44
P (baseline)	<0.0001*	<0.0001*	0.26	0.55	0.202	0.0001*	0.32	<.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Patients BD remission														
n	14	22	14	23	22	23	23	22	23	23	23	23	28	28
Mean	6.05	8.99	6.05	25.1	828	716	1228	3943	35.1	301	925	3.53	5.156	173
SD / CV	0.69	1.17	0.69	19.0	0.66	0.482	358	769	20.8	133	0.429	1.67	1.84	45
99% CI for the	1.25	1.02	0.86	-11.9	0.852	0.844	-200	772	-17.5	-84.0	1.15	1	-5.77	-102
difference or ratio	to 28.7	to 2.50	to 2.51	to 26.8	to 2.52	to 1.97	to 368	to 2129	to –0.29	to 132	to 2.16	to 3.48	to -3.0	to -50
P (baseline)	<0.0001*	0.0007*	0.045	0.28	0.057	0.103	0.41	<0.0001*	0.056	0.53	0.0006*	<0.0001*	<0.0001*	<0.0001*

Mean: geometric for IL-1RA, IL-2R, IL-6, IFN-α, sVCAM-1, other: arithmetic. SD/VC: standard deviation (SD) for for sTNF-RI, sTNF-RII, G-CSF, s-Selectin, sICAM-1, Neopterin, Leuko, Thrombo
Other: coefficient of variance (CV). CI: confidence interval. *significant at the multiple level 0.05 after Bonferroni-Holm correction.
CRP is not included in the tables because rank sum test was applied (see text). The number of patients in the tables differs from the patients treated, because not for all patients the respective values were available. For PUS, individual affected eyes were evaluated, which explains why "n" in some cases is higher than the number of individuals treated.

molecules (35). It has been reported that levels of soluble adhesion molecules are also elevated in active BD (36-38). In the present study, soluble adhesion molecules were not elevated when compared to healthy controls. In more generalised BD (e.g. large vessel involvement) this might be different, as it has been reported for patients with "major" or "minor" Wegener's granulomatosis (39) and also by Haznedaroglu *et al*. for the soluble selectins in extensive BD (38).

In accord with previous reports on patients with active BD, we also found increased baseline levels of IL-1RA, sIL-2R and sTNF-RII (14, 17, 40-43). The soluble receptors ((sTNF-RI (p55) and sTNF-RII (p75), s-IL-2R) are shed from the cell-surface, whereas IL-1RA is produced by macrophages (44). In higher concentrations they antagonise the effects of the respective pro-inflammatory cytokines (45). Thus, this elevation most probably reflects regulatory anti-inflammatory mechanisms and not just disease activity.

Neopterin is produced by macrophages after stimulation by Interferons and is known to be increased in viral infections, several autoimmune diseases and after organ transplantation, where it can be used to monitor immunological complications (46,47). Baseline neopterin serum levels were comparable to those of the healthy controls.

Of note, the differences in some of the cytokine and especially chemokine levels between the present study and others could also be due to differences in methodology. A Japanese group recently reported on marked differences between whole-blood levels of IL-8 and those in plasma, which were significantly lower and often even below the detection limit (48). However, in the studies by Verity *et al.* (49) and Bardak *et al*. (32) as well as in the study by Everiklioglu *et al*. (28), serum ELISAs were used, which in some cases even were identical to those in the present study. As we could show that freezing did not influence the results of the ELISAsystems used here, the differences can neither be explained by the use of frozen or fresh samples (most of the other authors did not report if they used fresh or frozen samples).

Endogenous IFN-α was significantly elevated before exogenous treatment with this cytokine. One hypothesis explaining this phenomenon might be that IFN-α is not active enough to eliminate a persistent virus or to exert its specific influence on the cytokine network and the cellular immune system. As there are at least 13 IFN-α subtypes in man, and some are known to be more efficacious in eliminating foreign viral antigens than others (50-52), there might be a dysregulation of IFN-α genes and their respective products in patients with BD which is substituted for with exogenous recombinant IFN-α2a. Such dysregulations have already been described for hepatitis C (51), bacterial meningitis (53), in newborns (54) and in mouse models (52). Alternatively, there could be a lowered responsiveness to IFN-α signalling and other functions mediated directly or indirectly by IFN-α (as for example HLA-class-I expression and, consecutively, antigen presentation) in BD patients, which is corrected by substituting exogenous IFN-α. These hypotheses are supported by the lack of elevation of neopterin serum levels despite elevated baseline IFN-α serum levels in the patients.

Leukocytosis at baseline when compared to the healthy controls can be explained by glucocorticosteroid treatment of the BD patients before initiation of IFN-α treatment.

Changes under IFN-α treatment

During the course of IFN-α treatment, some of the cytokines and soluble cytokine receptors, namely sTNF-RII, IL1-RA, sIL-2R and TNF-α, soluble adhesion molecules (sICAM-1, sVCAM-1) and neopterin significantly increased, whereas soluble E-selectin decreased, as did the number of leukocytes and thrombocytes.

Most of these changes have been noted previously in healthy volunteers, patients with haematological or viral diseases being treated with IFNα or in *in-vitro* experiments (30, 44, 55, 56). The increase of neopterin is used for indirect estimation of the efficacy of type I IFN-treatment in other diseases such as hepatitis C and multiple sclerosis (57).

The increase of sICAM-1 and sVCAM-1 under IFN-α treatment might reflect their anti-inflammatory potential, antagonising the effects of cellular adhesion molecules when present in abundance (58). It reflects direct effects of IFN-α, which at least in the case of sVCAM-1 is underlined by its direct correlation with IFN dosage. Thus, at least in predominantly ocular BD, they are probably not useful for the monitoring of disease activity, but for prediction of response to IFN-α. This seems to contradict the results of Verity *et al*. (49), and of Saglam *et al.* (37) who reported elevated levels of soluble adhesion molecules in patients with active BD and a decrease under immunosuppressive treatment. Also in contrast to the present results, for the soluble adhesion molecules sICAM-I and sVCAM-I, decreases in responders with hepatitis C under IFN treatment were described (59). One reason for this apparent discrepancy could be that the levels of the respective soluble adhesion molecules were already elevated at baseline in Hepatitis C and in the BD patients in the studies of Saglam *et al.* and Verity *et al.*, whereas in our cohort with ocular BD they were similar to those of the healthy controls. IFN-α could exert differential effects, depending on the baseline concentration of these molecules. Another possible explanation is that the mechanism of endothelial activation in hepatitis C (viral infection) is different from that in BD and thus as soon as the causative virus is cleared from the circulation, endothelial activation ceases and the soluble adhesion molecules which were shed from it, also decrease. On the other hand, in BD, the endothelial activation may be caused more indirectly by activated PBMC and their cytokines, which is then antagonised by an increase in soluble adhesion molecules by shedding from the cell surface. The effects of IFN-α on different types of adhesion molecules are differential as well, because sE-selectin levels, in contrast to the other soluble adhesion molecules measured here, significantly decreased. Some of these changes were significantly related to remission only of PUS

(sIL-2R, sE-Selectin, sICAM-1) which occurred much earlier than remission of BD activity as a whole. This probably reflects direct effects of exogenous rhIFN-α2a, because changes were less marked later in the course of treatment, when exogenous IFN-α had been reduced or even completely tapered off, although remission of the disease was ongoing. This contention is supported by the positive correlation with IFN dosage or serum level for sIL-2R and sTNF-RII as well as for sVCAM-1, although positive correlations do not necessarily imply a causal relationship. It is now important to establish whether these markers would also reflect clinical improvement under different therapeutic regimens, such as azathioprine or CSA. If this were the case, the respective recombinant cytokine antagonists would be interesting alternative treatment options in refractory BD, as already proposed for IL-1 receptor-antagonist by BenEzra (60) and proven effective in case reports for TNF-antagonists (61).

The main limitations of the present study are the previous immunosuppressive treatment of the patients and the patient selection (predominantly refractory ocular disease). It might well be possible that the recent glucocorticosteroid treatment has influenced some of the cytokine and cytokine-antagonist levels measured here. Thus, especially the production of IL-8, IL-6 and TNF-α might have been diminished by glucocorticosteroids at baseline. In contrast, the baseline elevation of soluble cytokine receptors and IFN-α supposedly would have been even more pronounced without the recent glucocorticosteroid treatment.

The cytokine and chemokine network as well as the soluble adhesion molecules are still not completely understood concerning their influences on the immune system and their complex interactions. Furthermore, it has been shown that most cytokines and probably also chemokines are able to exert contrasting, if not opposite actions depending on their environment. The possibility for a cytokine to switch from one side to the other (from “the good guys” to “the bad guys”) was nicely illustrated by O’Shea, Ma and Lipsky in a review article in Nature Reviews Immunology (62). For ethical reasons, we were unable to examine the concentrations of the cytokines and chemokines in the affected ocular tissue, although these may differ from those in the serum and more reliably reflect what is really happening at the site of inflammation, as was recently shown for the first time in mucocutaneous lesions of BD patients by Ben Ahmed *et al.* (63). All these difficulties hamper the interpretation of the results of the present study. However, the present findings allow the following conclusions:

IFN-α probably exerts anti-inflammatory effects mainly through an increase in soluble cytokine receptors, cytokine antagonists and soluble adhesion molecules. The activation of macrophages, as reflected by an increase in TNF-α and neopterin under IFN treatment may be responsible for the major side effects of IFN such as fever, arthralgia, headache, loss of appetite and weight. The hypothesis of an inefficacy of IFN-α subtypes produced by the patients remains to be proven. Further studies on the influences of IFN-α on the cytokine network in previously untreated patients with different manifestations of BD and a combination of different methods for cytokine and chemokine measurements (*in vitro*, whole blood assays) are necessary in order to overcome the limitations of the present study.

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