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Clastogenic Plasma Factors in Psoriasis—Comparison of Phototherapy and Anti–TNF-α Treatments

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ABSTRACT

As previously described, Psoralen plus UVA (PUVA) therapy induces chromosome damage in psoriatic patients. This study evaluates whether these effects are transitory or persistent. In addition, we studied these effects after narrowband UVB (nUVB) and anti-tumor necrosis factor (TNF)-α treatments. Among 40 responder patients, 10 received PUVA, 10 nUVB, 10 Infliximab and 10 Etanercept. Disease activity was determined with Psoriasis Area and Severity Index. Chromosomal breakage was evaluated by the clastogenic factor (CF) test. Potential clastogenic agents, malondialdehyde (MDA) and TNF-α were measured. Before treatment, the plasma-adjusted clastogenic scores (ACS) of patients were increased. During treatment, a further increase in ACS was observed in both phototherapy groups. Chromosome damage persisted for PUVA patients at week 32, while it diminished after nUVB to ACS values lower than before treatment. MDA and TNF-α values were also increased at baseline. MDA decreased during treatment in all groups, but without reaching normal levels. Plasma TNF-α remained unchanged in PUVA and nUVB but decreased in both anti–TNF-α treatment groups. Psoriasis is accompanied by CF-induced chromosomal breakage that increases during PUVA and nUVB treatments. Plasma clastogenic activity persisted in the follow-up after PUVA, while after nUVB ACS returned to values even lower than baseline. Clastogenic activity during the induction phase with anti–TNF-α remained unchanged.

INTRODUCTION

It is now generally accepted that plaque-psoriasis is a chronic, relapsing, multisystem disease with predominant skin involvement and recognized genetic predisposition (1). It affects about 2–3% of the population (2). Autoimmune reactions are said to play a role in the disease process (3). Plaque-type psoriasis, characterized by well delineated reddish and scale papules and plaques, primarily on elbows, knees and scalp, accounts for 90% of the psoriasis cases (4).

Patients with psoriasis require an individual management and long-term planning of therapeutic strategies. The therapy is chosen in accordance with skin type, clinical history, patient's age, severity of psoriasis and the response to previous treatments (5). Topical agents are in general chosen for milder forms and limited psoriasis. Phototherapy, photochemotherapy and systemic agents are necessary for moderate and severe psoriasis (6). Biological therapies are particularly used for psoriasis in patients intolerant or not responding to conventional systemic treatments (7).

Psoralen plus UVA (PUVA) therapy is associated with a high risk of squamous cell carcinomas of the skin (8). The risk of malignant melanomas also appears to be increased (9). PUVA-induced mutations in the p53 tumor suppressor gene are in line with the observed increase of malignancy (10). PUVA did not appear to be a risk factor for internal malignancy (11).

While the role of PUVA in the induction of skin tumors is undisputed, the role of UVB phototherapy in human skin carcinogenesis is less clear (12). Data investigating the carcinogenic risks of narrowband UVB (nUVB) and broad-band UVB (bUVB) are limited. In humans, nUVB seems not to be associated with a higher carcinogenic risk compared with bUVB, but the risk compared with PUVA is significantly reduced. A first long-term retrospective study of Weischer *et al*. (13) during a follow-up of 10 years supports the view that neither nUVB nor bUVB significantly increases the risk of skin cancer. Nevertheless, phototherapy must be applied with due caution and regular follow-up.

Because of the recognized role of tumor necrosis factor (TNF)-α in psoriasis, the use of anti–TNF-α biological agents had a strong impact in psoriasis treatment in recent years (14). These therapeutic approaches use recombinant monoclonal antibodies or fusion proteins with the aim to target specific steps of the immune pathways involved in the development of psoriasis. The primary concern of anti–TNF-α therapy as a long-term treatment is the chronic immune-suppression induced by these agents, with recurrent infections and possibly also predisposition to malignancy as a consequence (15,16).

Oxidative stress is implicated in psoriasis (17). Activated polymorphonuclear leukocytes are able to induce lesions in the skin tissues by releasing reactive oxygen species, and keratinocytes are also a major source of oxygen species generated in psoriatic lesions (18). Malondialdehyde (MDA) and other lipid peroxidation products are increased in the plasma of psoriatic patients, while enzymatic and nonenzymatic antioxidants are decreased (17,19).

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In a previous study with patients receiving PUVA treatment, we suggested that oxyradical-mediated clastogenic factors (CFs) present in psoriatic patients' plasma and responsible for increased chromosomal breakage might represent a risk factor in addition to photocarcinogenesis (20). For this reason it seemed important to study the persistence of the breakage phenomenon after arrest of PUVA treatment, in particular since CF induced by ionizing radiation persist many years after arrest of exposure (20). An increased risk of cancer and leukemia is indeed observed in diseases accompanied by CF formation, including systemic lupus erythematosus and scleroderma patients (21), CFs are composed of lipid peroxidation products, cytokines and other oxidants with chromosomal effects. For detailed description of CF formation and CF action see a recent review (22).

Moreover, an association between psoriasis and malignancy has been explored. A significant excess relative risk was noted for upper aerodigestive tract, esophagus, stomach, liver, pancreas, lung, kidney and bladder as well as lymphoma. The association of psoriasis and lymphoma is strongest for Hodgkin's lymphoma and cutaneous T-cell lymphoma (23,24).

So far there are no published data on the clastogenic activity in psoriatic patients receiving nUVB and biological agents treatments. Therefore, in the present study, we compared the clastogenic activity in the plasma of patients exposed to nUVB with conventional phototherapy PUVA. Since TNF-α is one of the clastogenic components of CFs (for review see 22), we included patients treated with two anti–TNF-α agents in this comparative study, shown before to be effective in chronic plaque-type psoriasis (25).

MATERIALS AND METHODS

Patients. Fifty-seven adult patients with chronic plaque-type psoriasis at exacerbation stage and 40 controls were enrolled in the study after giving informed consent. The protocol used for this cross-sectional study was approved by the local Ethics Committee. All patients were clinically and analytically studied in an active phase of the disease *i.e.* exacerbation of psoriatic lesions, before the start of therapy. The Psoriasis Area and Severity Index (PASI) (26), considered as the most validated tool for assessment of psoriasis severity in clinical trials, was applied also in this study. It combines the assessments of four body areas: head and neck, trunk, upper and lower limbs. To assess the affected body surface area, the proportion of skin affected in each area is given by a numerical score representing the proportion involved. Within each area, the severity of the lesions is assessed by three signs, erythema, thickness/induration and desquamation/scaling. Each of the three signs is assessed on a five-point scale. The PASI score ranges from 0 to 72. A PASI score below 10 defines psoriasis as mild, between 10 and 20 as moderate, and above 20 as severe (27). Current guidelines establish the primary endpoint of systemic treatment at 12 weeks. Patients are considered responders whenever there is a reduction in 75% of baseline PASI, after 12 weeks of treatment.

Fifty-seven patients were enrolled in order to obtain a total of 10 responding patients for each treatment group (PUVA, nUVB, Infliximab and Etanercept) at week 12. A total of 17 patients were excluded from this cross-over assay due to lack of clinical response, side effects or nonadherence. From these, 13 patients were excluded for lack of clinical response (3 of 13 in PUVA group, 4 of 16 in nUVB, 3 of 13 in the Infliximab group and 3 of 15 in Etanercept group). Two of 16 patients dropped out in the nUVB group and 2 of 15 patients in Etanercept group had adverse effects (see Supporting Information). A total of 40 patients were considered responders (17 women/23 men, mean age 55.1 ± 13.8). The exiguity of the number of nonresponders *versus* responders lack statistical power, therefore comparisons with responders are not possible. The mean PASI score of the 40 patients enrolled was 21.7 ± 5.8. Psoriasis had been diagnosed between 2 and 37 years earlier, and the age at onset of psoriasis was between 13 and 59 years. Ten patients were exposed to PUVA therapy and 10 to nUVB. Infliximab or Etanercept were given to another 20 patients, 10 for each treatment. The patients exposed to PUVA and nUVB were followed up to 6–8 months after the last irradiation. This follow-up was not possible for patients receiving Infliximab or Etanercept, in whom the treatment was not arrested at 12 weeks.

Psoralen plus UVA- and nUVB-treated patients were naïve for phototherapies and systemic treatments for psoriasis. Eleven of the 20 patients receiving either Infliximab or Etanercept had been treated previously with oral methotrexate, the remaining nine with nUVB in monotherapy or associated with acitretin. However, no patient had received systemic treatment or phototherapies during the 6 months (washout period) prior to anti–TNF-α treatment.

Patients presenting other skin diseases, diabetes, inflammatory or infectious diseases, cardiovascular, hepatic or renal disease, were not included in the study.

The 40 patients were compared with a control group of 40 healthy volunteers (17 women/23 men) of similar age (mean 54.7 ± 12.3) with normal hematological and biochemical data (Table 1).

Treatments. UVA irradiation (320–400 nm) was performed in an Atmos cabin comprising 48 Philips TL 09 lamps. Two hours before, 8-methoxypsoralen was administered (0.6 mg kg^{-1} body weight). The initial dose was 2–3 J cm^{-2}, according to the phototype. In every session (thrice a week) the dose was increased by 0.5 J cm^{-2}, until a maximum dose of 10 J cm^{-2} was reached.

Narrowband UVB irradiation (311 ± 2 nm) was administered using a Waldmann 7001K cabin (UVA/UVB-TL01, Waldmann Medizintechnik, Villingen-Schwenningen, Germany). The initial dose was 0.2–0.3 J cm^{-2}, dependent on the phototype of the patient. In every session (thrice a week), the dose was increased by 0.1 J cm^{-2} until a maximum dose of 2.5 J cm^{-2} was reached.

Infliximab, a chimeric monoclonal antibody, is a specific inhibitor of TNF-α (27). It was given at a dose of 5 mg kg^{-1} intravenously over a time period of 2–4 h. Additional perfusions at the same dose were administered 2 and 6 weeks later and then continued every 8 weeks.

Etanercept, a recombinant TNF-receptor fusion protein, competitively inhibits the effects of endogenous TNF-α by interaction with cell-surface receptors (28). It was administered subcutaneously twice weekly at a dose of 25 mg.

Methods. Blood samples from fasted subjects were collected to obtain plasma and serum.

Detection of clastogenic activity in patients' plasma, the CF-test. Heparinized blood was centrifuged immediately, and the plasma was handled according to established procedures for isolation of CFs (22,29). Since the clastogenic components are in the small molecular weight range, the high molecular weight molecules, which would disturb culture growth in the case of blood group incompatibilities, were eliminated by ultrafiltration through filters with a cut-off at 30,000 Da. Ultrafiltrates were kept frozen at −50°C until study on regular blood cultures. Series of samples from each study group were tested the same day on the cultures set up with the same donor's blood. Additional cultures without ultrafiltrate served for the establishment of the spontaneous chromosomal aberration rate of the donor's lymph-

Table 1. Basal characteristics of patients and controls.

	Controls, $n = 40$	Patients, $n = 40$
Age (mean ± SD)	54.7 ± 12.3	55.1 ± 13.8
Male (%)	55	57
Female (%)	45	43
PASI (mean ± SD)	–	21.7 ± 5.8
TNF-α (mean ± SD)	1.43 ± 0.31	1.91 ± 0.32*
MDA (mean ± SD)	2.8 ± 0.4	4.3 ± 0.8*
ACS (mean ± SD)	0.8 ± 1.0†	8.1 ± 4.8*
CF+/CF±/CF−	0/4/96†	20/10/10*

*Significant compared with controls; †lab standard determined by the study of 100 healthy blood donors (21).
ACS, adjusted clastogenic scores; CF, clastogenic factor; MDA, malondialdehyde is expressed in μmol; PASI, Psoriasis Area and Severity Index; TNF-α, tumor necrosis factor is expressed in pg mL^{-1}.

ocytes. This background level of aberrations was subtracted from the aberration rate of the ultrafiltrate-treated cultures. The difference of the two values was called the adjusted clastogenic score (ACS). This way of treating results is necessary, since the clastogenic activity of samples collected at subsequent dates in a clinical trial will be tested on donor blood with different background levels of chromosomal breakage. For the present study the background levels of spontaneous chromosomal aberrations in the 10 simultaneous test cultures varied between 0% and 2%. All cultures were studied by the same observer.

The culture conditions were the same as those in the first study (19), except for the quantity of ultrafiltrate, reduced to 200 μL instead of 250 μL. TCM 199 (Flow Laboratories, Paris) was the culture medium, classical in chromosome mutation studies because of the absence of free radical scavengers. The ultrafiltrates were added at time 0 and were present all over the 72 h cultivation period. Mitoses were obtained by stimulation with phytohemagglutinin and arrested in metaphase by addition of colchicine. Microscopic slides were prepared according to classical cytogenetic procedures. The chromosomes of 50 well-spread and complete metaphase plates were examined on coded slides for the presence of chromatid and isochromatid breaks, telomeric extrusions, acentric fragments or other structurally rearranged chromosomes. Gaps were not included in the aberration rate. The total number of aberrations detected on 50 mitoses was multiplied by 2 to give a numerical score referred to as a "percent" for convenience purposes.

The mean ACS rate for our laboratory, which was established by the study of 100 ultrafiltrates from healthy adults, is 0.8 ± 1.0% (21). The frequency of induced breaks was 0 or +2 for 95 samples, +3 for the remaining five samples. Samples yielding higher values were not observed with control plasma ultrafiltrates. According to these data, an ACS of 8% is considered as CF+, of 6% as CF± and of <6 as CF−. In contrast to plasma from healthy blood donors from the Transfusion Center, ultrafiltrates from persons consulting for various minor health problems (so-called "sick controls") may induce ACS up to 3.3 ± 2.1%. The ACS differences between samples taken 2 h apart from nine psoriatic patients submitted to PUVA, calculated by the same observer, were of 4.7 ± 1.4% (I. Emerit, P. Filipe, unpublished).

Biochemical assays. Lipid peroxidation was evaluated by measuring the formation of thiobarbituric acid-reactive substances in the serum of patients and controls, expressed as total MDA, according to Yagi (30).

The plasmatic levels of TNF-α were evaluated by enzyme immunoassay (Human TNF-α High Sensitivity ELISA, Bender MedSystems, Vienna, Austria).

Statistical analysis. The Statistical Package for Social Sciences (SPSS, version 16 for Windows, SPSS Chicago, IL) was used: comparison of patients and controls at baseline (WO), after treatment (W12) and follow-up was performed with the Mann–Whitney test; comparison of differences between W0, W12 and follow-up for the four treatment groups was performed with the Wilcoxon test. Measurements were expressed as mean values ± SD. A P-value of <0.05 was considered statistically significant. The correlation analysis was performed by calculating the Spearman coefficient correlation.

RESULTS

All analytical values and the statistical significance of the differences between patients and controls are shown in Table 1. There were no differences for age and sex. In 19 of the 40 patients, PASI reflected moderate psoriasis. The remaining 21 patients had severe psoriasis. Patients had significantly higher levels of TNF-α and MDA than controls. A positive correlation between the severity of the disease measured by PASI was found for MDA (Fig. 1), but not for TNF-α.

Highly significant clastogenic scores were induced with the 40 psoriasis samples. The mean ACS (8.1 ± 4.8%) represented a 10-fold increase compared with our laboratory standard for healthy subjects (0.8 ± 1.0%). The ACS observed with psoriasis ultrafiltrates were also significantly increased in comparison with our values for "sick controls" (3.3 ± 2.1%). Since our previous work had shown that

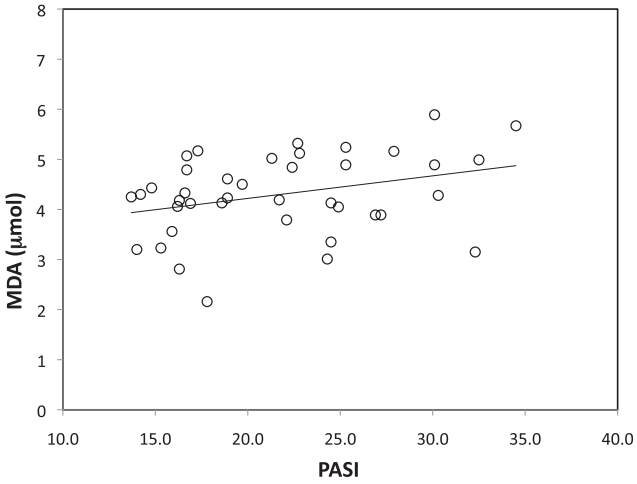

Figure 1. Correlation between MDA and PASI for patients' group (r = 0.322; P = 0.043).

normal plasma, even concentrated, does not induce chromosomal breakage, the clastogenic activity in the plasma of the 40 controls was not examined for the present study. The simultaneous cultures set up for each series of psoriasis samples without addition of ultrafiltrate ascertained that the donor's blood was suitable for the test. For six of these untreated test cultures, the ACS was 0, in four others +2. According to our definition (see Methods section), 20 of the 40 patients were CF+ (ACS 8% or higher). In another 10 patients, the ACS was 6% (CF±), while the remaining 10 patients were CF− (0–4%). High PASI scores and high clastogenic activity were correlated, when groups were formed according to CF scores (Fig. 2). A correlation could not be observed for individual results. As usual for CF-induced chromosomal breakage, the aberrations were of the chromatid type and consisted of open breaks in one or both chromatids, or they were separate fragments. There were no dicentrics, rings or other structurally abnormal chromosomes.

Table 2 summarizes the effect of treatment for the different parameters studied.

PASI

The lowest PASI values were observed after PUVA and Infliximab. After the 12 weeks treatment, PASI was reduced from 22 to 2, *i.e*. from moderate or severe to mild psoriasis. With nUVB and Etanercept, the effect was somewhat less than with PUVA and Infliximab (PASI 3.7, difference not statistically significant). The patients exposed to phototherapy were examined again at W32 after arrest of the treatment at W12. PASI had increased again, 7.3 ± 1.8 for PUVA and 10.8 ± 2.2 for nUVB.

TNF-α

For PUVA and nUVB, the TNF-α levels, which were increased before treatment, were not significantly modified. However, considerable reduction in TNF-α levels was observed in the Infliximab-treated group (0.37 ± 0.13), as well as in the group receiving Etanercept (0.66 ± 0.16), to levels significantly lower than pretreatment values and control values.

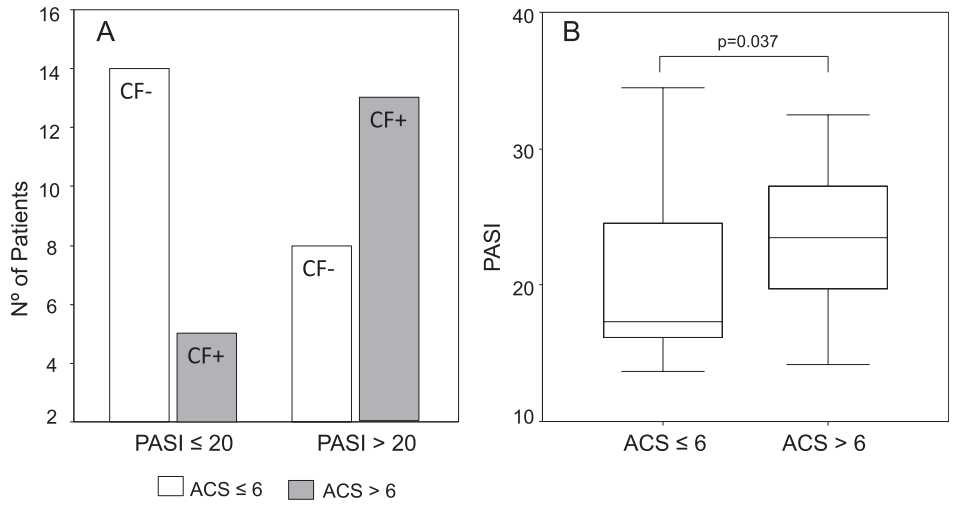

Figure 2. (A) CF+ patients were more likely to occur in patients with severe disease (PASI > 20). (B) Patients with ACS > 6 (CF+) had significantly higher PASI than the CF− patients. The box-plot graph represents medians and interquartiles.

Table 2. Mean values and standard deviations for 10 patients in each treatment group. Week 0 (W0), week 12 (W12) and week 32 (W32).

	W0	W12	W32
PUVA			
PASI	21.9 ± 6.3	2.2 ± 1.1*	7.3 ± 1.8†
TNF-α	1.77 ± 0.27	1.61 ± 0.30	1.74 ± 0.27
MDA	4.2 ± 0.7	3.5 ± 0.6*	4.1 ± 0.4†
ACS	9.0 ± 4.3	20.0 ± 9.8*	12.6 ± 2.3
CF+/CF−‡	6/4	10/0	10/0
nUVB			
PASI	21.3 ± 5.2	3.7 ± 1.2*	10.8 ± 2.2†
TNF-α	1.80 ± 0.28	1.74 ± 0.23	1.86 ± 0.18
MDA	4.2 ± 0.6	3.5 ± 0.6*	4.1 ± 0.4†
ACS	8.6 ± 4.7	14.0 ± 7.4	3.6 ± 3.0†
CF+/CF−	6/4	8/2	1/9
Infliximab			
PASI	22.3 ± 6.5	2.1 ± 0.7*	
TNF-α	1.93 ± 0.33	0.37 ± 0.13*	
MDA	4.5 ± 1.0	3.1 ± 0.4*	
ACS	6.2 ± 6.1	7.6 ± 4.5	
CF+/CF−	3/7	5/5	
Etanercept			
PASI	21.4 ± 6.1	3.7 ± 1.2*	
TNF-α	2.01 ± 0.24	0.66 ± 0.16*	
MDA	4.3 ± 1.0	2.9 ± 0.6*	
ACS	9.2 ± 3.9	5.2 ± 2.9*	
CF+/CF−	5/5	5/5	

*Significant between W0 and W12; †significant between W12 and W32; ‡CF± included in CF−.
ACS, adjusted clastogenic scores; CF, clastogenic factor; MDA, malondialdehyde is expressed in μmol; PASI, Psoriasis Area and Severity Index; PUVA, Psoralen plus UVA; TNF-α, tumor necrosis factor is expressed in pg mL^{-1}.

MDA

Levels were significantly lower at W12 after all treatments, but remained higher than control values. The lowest levels were seen at W12 after Etanercept (2.9 ± 0.6 compared with 2.8 ± 0.4 μmol in controls). At W32, after nUVB and PUVA treatment, MDA had returned to pretreatment values.

CF

The clastogenic activity in the plasma, already high before treatment, increased under PUVA treatment from 9% to 20% ($P = 0.004$). Eight months after the last exposure to PUVA (W32), the ACS remained increased compared with the values at W0 (12.6%). Compared with the overall value of CF in the whole group of 40 psoriasis patients (8.1%), this persisting clastogenesis is highly significant ($P = 0.006$). Also the four patients who had been CF− before PUVA were now CF+ and remained CF+ during the 32 weeks follow-up.

The clastogenic activity of patients' plasma increased also under nUVB irradiation, from 8.6 ± 4.7% to 14.0 ± 7.4%. Because of the important standard deviation, the difference is only at the limit of significance ($P = 0.067$). In contrast to PUVA, this increase did not persist after arrest of nUVB treatment. After the 32 weeks follow-up, the ACS were even lower than at the start of the treatment ($P = 0.014$). The value of 3.6 ± 3.0% approaches our results in "sick" controls (3.3 ± 2.1%). Nine of the 10 patients were CF− at week 32 after arrest of nUVB, while all 10 patients exposed to PUVA were still CF+.

For Infliximab, the slight increase in ACS from 6.2 ± 6.1% to 7.6 ± 4.5% was not statistically significant, given the important standard deviation. This group had lowest mean ACS compared with the other three groups before treatment, with only three CF+ among the 10 patients. Three of the seven CF− patients became CF+ under treatment, four remained CF−.

Etanercept treatment did not increase the clastogenic activity, but rather resulted in a decrease (9.2 ± 3.9 to 5.2 ± 2.9%, $P = 0.025$). The incidence of CF+ and CF− patients in the group did not change.

DISCUSSION

The present study confirms the findings of our previous article, indicating the presence of CF-induced chromosomal breakage in patients with psoriasis. The fact that the clastogenic activity in the plasma of psoriasis patients is found in the small molecular weight fraction (filter cut-off 30.000 Da) and that

the clastogenic effects are inhibited by superoxide dismutase, are arguments for similarities between CFs in psoriasis and CFs in other chronic inflammatory diseases. Increased superoxide production by activated phagocytes and immunocytes with formation of enzymatic and nonenzymatic lipid peroxidation products, release of clastogenic cytokines in particular of TNF-α, are responsible for the clastogenic effects of plasma in these diseases, a phenomenon previously described as superoxide-mediated clastogenesis (31). Since the clastogenic products in CFs have also superoxide stimulating properties—the best example is TNF-α—the vicious circle of superoxide production by CF and further production of CF by this superoxide results in self-sustaining genotoxic effects. A detailed description of the complex mechanisms of CF formation and CF action is available in a recent review (see ref. 22). Although PUVA has anti-inflammatory properties evidenced by the decreased PASI scores at week 12, there was a residual inflammatory process demonstrated by the sustained TNF-α and MDA elevated levels as compared with the control group. This minimal inflammatory process may explain the persistence of superoxide-mediated CF formation.

In addition to CF formation as a consequence of the chronic auto-inflammatory process, other sources of superoxide may contribute to the significant increase in clastogenic plasma activity after PUVA. Based on our experiments, in which we exposed normal lymphocytes to PUVA *in vitro*, an increase in CF formation was observed with minimal concentrations of psoralen and 1.0 J cm^{-2}, indicating that the CF formation is not specific for psoriasis (32).

The clastogenic scores before treatment were very similar, when the previous study group and the PUVA-treated group of the present study were compared (9.3 ± 3.5% and 9.0 ± 4.3% respectively). These baseline values were already 10 times increased compared with the controls. Starting with an increase of aberrations to 12.7% after the first irradiation, the ACS reached 18% after the last irradiation (16th exposure) for the previous study. In the present study the pretreatment ACS rose to 20%. This time it was possible to follow the patients after arrest of PUVA treatment, and an increased chromosomal aberration rate was still noted at W32 (12.6%). Not only 4 of 10 patients were CF+, but all of them. Given that PUVA treatments have to be repeated, this permanent DNA damage exceeds the repair system and represents a risk factor for the development of skin cancer and probably of other malignancies, as this is known from other diseases accompanied by chromosomal breakage (21,22). There are conflicting data on the link between PUVA treatment and risk of internal malignancy, which we hypothesize may be mediated through PUVA-induced CFs. While Gach *et al.* (11) found no link, several other epidemiologic studies demonstrated a link between PUVA treatment and an elevated risk for internal malignancy. Stern and Väkevä (33) found an identical overall risk of noncutaneous cancer in PUVA-treated patients compared with the general population, but significant increases were observed for thyroid, breast and central nervous system neoplasms. In a Swedish follow-up study increased incidence of respiratory cancer in men and women and of kidney cancer in women was reported (34). Also, patients exposed to methotrexate and PUVA had higher incidence of lymphoma (35).

The increase in clastogenic scores was also observed after nUVB, but was less important than after PUVA. Also in contrast to PUVA treatment, the chromosomal damage diminished again after arrest of treatment and ACS were even lower than at the beginning of the treatment. Only one of the 10 patients was still CF+. At W32, nUVB-treated patients still had a PASI score of 10 at the limit of mild psoriasis, and ACS corresponding to those of our "sick controls." Low incidence of skin cancer and low incidence of chromosome damage are an advantage of nUVB compared to PUVA.

In contrast to phototherapy, only minor variation in clastogenic scores was observed with the anti–TNF-α treatments. Infliximab slightly, but not significantly, increased the baseline levels of the clastogenic scores, and this was also expressed by an increase in CF+ cases in the group. A significant decrease was noted with Etanercept, but was expressed only for mean ACS values, while the total of CF+ patients did not change.

Since TNF-α is a component of CFs, we would have expected a more pronounced decrease of ACS with anti–TNF-α treatment, by which TNF-α values were reduced to values lower than the control values. However, there was no correlation between TNF-α and ACS. One has to keep in mind that TNF-α is not the only clastogenic agent in CF, and therefore anti-TNFs cannot completely counteract CF action. Also there was no correlation between ACS and the marker for lipid peroxidation, MDA. Indeed, MDA reflects only incompletely the process of lipid peroxidation, and among the lipid peroxidation products present in CFs, only the degradation product 4-hydroxynonenal is highly clastogenic (36). However, others found correlations between TNF-α or MDA with the worsening of psoriasis (37–39).

While all four treatments resulted in a successful reduction of the PASI scores after a 12 weeks treatment, only PUVA and nUVB were responsible for additional clastogenesis, which only regressed during the follow-up in the group treated with nUVB. The same therapeutic effect was obtained with the two TNF antagonists without this handicap. Further follow-up of the patients receiving Etanercept will show whether the observed decrease in ACS will be confirmed over longer treatment periods. Other side effects of these new agents such as infectious complications and predisposition to malignancy, consequences of the chronic immune-suppression, have to be taken into consideration. As much as one can say, on the basis of this limited number of patients in each group, treatment with nUVB appeared to be the most beneficial approach.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean values and standard deviations for nonresponder patients, adverse effects or nonadherence in each treatment group. Week 0 (W0) and week 12 (W12).

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