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Sun exposure induces rapid immunological changes in skin and peripheral blood in patients with psoriasis

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Summary

Background Ultraviolet (UV) radiation has immunosuppressive effects and heliotherapy is a well-described treatment modality for psoriasis.
Objectives To characterize early sun-induced immunological changes both local and systemic in patients with psoriasis.
Methods Twenty patients with moderate to severe psoriasis were subjected to controlled sun exposure on Gran Canaria, Canary Islands, Spain. Psoriasis Area and Severity Index (PASI) scores were evaluated. Skin biopsies were obtained from lesional and nonlesional skin in 10 patients at baseline and on day 16 and from five additional patients on day 2. Specimens were examined with immunohistochemistry and polymerase chain reaction. Blood samples were obtained from all patients at the same time points and were examined for T-cell subsets and cytokine production.
Results Significant clinical improvement was achieved during the study period. CD4+ and CD8+ T cells in lesional skin were significantly reduced in both the epidermis and dermis. In contrast, dermal FOXP3+ T cells were relatively increased. In the peripheral blood skin homing cutaneous lymphocyte-associated antigen (CLA)+ T cells were significantly decreased after only 1 day in the sun and *in vitro* stimulated peripheral blood mononuclear cells demonstrated reduced capacity to secrete cytokines after 16 days.
Conclusions Our data show that clinical improvement of psoriasis following sun exposure is preceded by a rapid reduction in local and systemic inflammatory markers, strongly suggesting that immune modulation mediated the observed clinical effect. We cannot completely rule out that other mechanisms, such as stress reduction, may contribute, but it is extensively documented that UV irradiation is a potent inducer of immunosuppression and we therefore conclude that the observed effect was primarily due to sun exposure.

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Psoriasis is one of the most prevalent autoimmune skin diseases, affecting 2–3% of white people.[1] It is a chronic disorder with serious medical consequences and impairment of quality of life for patients.[2] Psoriatic skin lesions are sharply demarcated, erythematous, raised, scaling plaques of varying extent but the nails, joints and other organs can also be affected.[1] The primary event is widely held to be immunological with hyperstimulated antigen-presenting cells and autoreactive T cells in the dermis initiating the pathogenic process.[3,4] The developing cytokine complex attracts additional immune cells and perpetuates the inflammatory cascade, inducing the pathological findings characteristic of the disease.[5]

Tumour necrosis factor (TNF)-α has been shown to be important in driving the immune pathology in psoriasis and the use of anti-TNF agents is now a well-established treatment modality for this disease.[6] However, more recently, the focus of attention has shifted to T-helper cells producing interleukin (IL)-17 (Th17), which have been found to be central to the pathogenesis of this disorder.[7–10] IL-23, produced by dendritic cells (DCs) and overexpressed in psoriatic lesions,[11] stimulates the survival and proliferation of Th17 cells and thus may serve as a key master cytokine regulator for this disease.[12] The therapeutic efficacy of an anti-IL-12/IL-23 antibody on moderate-to-severe plaque psoriasis as well as on psoriatic arthritis was recently demonstrated.[13,14]

While therapeutic monoclonal antibodies are effective, their use is expensive and long-term data on safety are still lacking.[15] Natural sun, which has been used for decades in the treatment of psoriasis, has a potent clinical effect presumably involving immunoregulatory mechanisms.[16–18] Immunosuppression following ultraviolet (UV)B exposure has been described locally in the skin and in the systemic compartment.[19] The induction of regulatory T cells (Tregs) has been suggested to be one mechanism mediating the clinical effect of UVB treatment.[20,21] Vitamin D produced locally in the skin upon sun exposure has also been shown to contribute to the immunological changes seen in the skin upon UVB exposure.[21,22] However, most of these studies were performed in mouse models and human data are scarce. Heliotherapy is a well-established therapeutic modality for patients with psoriasis in Scandinavian countries and approximately 500 Norwegian patients with psoriasis are selected for heliotherapy annually. We wanted to study the early immunological effects of sun exposure in patients with psoriasis, both *in situ*, in nonlesional and lesional skin, and in the systemic compartment.

Materials and methods

Subjects

Twenty patients (median age 48 years, range 24–65, six female) eligible to receive heliotherapy were enrolled in the study and transported from Norway to Gran Canaria, Spain, in the month of March. The patients were evaluated by the same dermatologist for the Psoriasis Area and Severity Index (PASI)[23] score before and after sun treatment. All patients had moderate to severe plaque psoriasis, i.e. mean/median PASI before climatotherapy of 9·8/8·7, range 3·8–18·8. All patients had stopped using any medication for psoriasis at least 4 weeks prior to this study. Two of the patients had skin type II and 18 had skin type III according to the Fitzpatrick classification.[24] Patients who are considered as candidates for receiving heliotherapy are preferred to have darker skin types in order to avoid the potentially adverse effects of sun exposure. Outdoor UV radiation was measured every hour from 9.00 a.m. until 5.00 p.m. The patients kept a diary, registering time spent in the sun every day, as well as use of sunscreen and its SPF factor. Combining the calculated UV radiation, with the sun-exposure duration from the patients' diaries, UV doses were estimated for each patient after 1 day and after the 15 days of sun exposure. Further details were described by Nilsen *et al.*[25]

Before this study we performed a pilot study with UVB treatment where the concentrations and incubation times were tested and different cytokines were measured. Based on these results we calculated the appropriate sample size according to statistical methods for power and the sample size of paired data. The study followed the protocols of the Helsinki declaration and was approved by the Regional Committee of Medical Ethics. All patients gave their written, informed consent.

Skin biopsies and blood samples

Punch biopsy samples, 4 mm diameter, were collected from lesional and nonlesional skin in 10 randomly selected patients. The samples from each individual patient were obtained within the same body area on all days but at a sufficient distance to avoid a reactive inflammation from the previous biopsy. Specimens from five patients obtained at baseline and on day 16 were fixed in formalin and paraffin-embedded, whereas samples from another five patients obtained at baseline and on days 2 and 16 were snap frozen in liquid nitrogen and stored at −80 °C until they were sectioned.

Venous blood samples were collected from all 20 patients on days 0, 2 and 16. Mononuclear cells were isolated immediately for further investigations (see below).

Immunohistochemistry

Formalin-fixed and paraffin-embedded biopsies were cut at 4 μm. Immunoenzyme staining for CD1a (mouse IgG1, clone MTB1; Novocastra, Newcastle, U.K.) and CD8 (mouse IgG1, clone C8/144B; DakoCytomation, Glostrup, Denmark) on dewaxed tissue sections was performed in a Ventana NexEs IHC instrument (Ventana Medical Systems Inc., Tucson, AZ, U.S.A.) with the standardized iView DAB detection kits as recommended by the manufacturer. Two-colour immunofluorescence staining of formalin-fixed specimens was performed combining rabbit anti-CD3 (clone SP7; Thermo Fisher Scientific, Fremont, CA, U.S.A.), and mouse anti-CD4 (IgG1, clone 1F6; Novocastra) or rabbit polyclonal

anti-CD3 (DakoCytomation) and mouse anti-FOXP3 (IgG1, clone 259D/7C; BD Pharmingen, San Diego, CA, U.S.A.). The following secondary antibodies were used: Alexa 555 goat antirabbit, Alexa 488 goat antimouse, Alexa Fluor 488 goat antirabbit IgG (all from Molecular Probes, Eugene, OR, U.S.A.) and Cy3-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA, U.S.A.).

Cryosections were cut 4 μm thick and fixed in acetone prior to staining. Two-colour immunofluorescence staining was performed combining rabbit polyclonal anti-CD3 (DakoCytomation) with mouse anti-CD4 (IgG1, clone SK3; BD Pharmingen), mouse anti-CD8 (IgG1, clone SK1; BD Pharmingen) or mouse anti-FOXP3 (clone 259D/7C; BD Pharmingen). Co-staining for the apoptosis marker cleaved caspase-3 and T cells was performed using a polyclonal rabbit antibody (Asp175; Cell Signaling, Beverly, MA, U.S.A.) and anti-CD3 (IgG1, clone SK7; BD Pharmingen). Staining for Langerhans cells (LCs) was performed with anti-CD1a (clone NA1/34; DakoCytomation). The following fluorescence-labelled secondary antibodies were used: Alexa 488 goat antirabbit IgG (Molecular Probes) and Cy3 goat antimouse IgG (Jackson Immuno Research).

Stained sections were examined by light or fluorescence microscopy at × 400 magnification by the same investigator (I.H.). Cell numbers were recorded using an ocular grid (250 × 250 μm), counting positive cell profiles in the epidermis and in the papillary and reticular dermis, to a depth of 250 μm. For the grossly thickened epidermis of psoriatic lesions, cell numbers per square millimetre rather than per millimetre of surface epidermis reflects the cell density more accurately. Data for both dermal and epidermal cell counts are given as cell numbers per square millimetre, in both lesional and nonlesional skin, in order to make comparisons.

mRNA from skin biopsies

RNA was isolated from 5 × 14 μm cryosections collected in 1·5 mL Eppendorf tubes containing 500 μL TRI reagent solution (Applied Biosystems, Foster City, CA, U.S.A.) and 1 μg used for a 20 μL cDNA synthesis reaction with SuperScript III (Invitrogen, Carlsbad, CA, U.S.A.) and 20 pmol oligo dT. Primers for real-time polymerase chain reaction (PCR) were designed with primer 3 software (http://frodo.wi.mit.edu/primer3/). Sequences are given in Table 1. As template for real-time PCR 1 μL cDNA was used in Stratagene MX3000P with 0·125 U μL^{-1} HotStar Taq polymerase (Qiagen, Hilden, Germany), EvaGreen® (Biotium Inc., Hayward, CA, U.S.A.; 0·5 × recommended amount) and $MgCl_2$ concentration as indicated in Table 1. PCR conditions included 15 min at 95 °C followed by cycling conditions as follows: 95 °C, 30 s, annealing 60 °C 30 s, 72 °C 30 s. Each target mRNA was quantified by threshold crossing point (C_t) values using a pooled cDNA to generate a standard curve and related to the GAPDH mRNA level. Melting curves were monitored for the specificity of PCR products.

Flow cytometric analysis of peripheral blood T cells

EDTA-whole blood was incubated with monoclonal antibodies in the following combinations: CD3 peridinin chlorophyll protein (PerCP)/CD4 phycoerythrin (PE)/CLA-fluorescein isothiocyanate (FITC)/CD45 allophycocyanin (APC) and CD3-PerCP/CD8-PE/CLA-FITC/CD45-APC, and with isotypically matched irrelevant antibodies (all from BD Biosciences, San Jose, CA, U.S.A.). After 20 min at room temperature in the dark, erythrocyte lysis was performed. Samples were then washed in phosphate-buffered saline (PBS) and cells were examined by flow cytometry (FACSCalibur®; BD Biosciences). At least 10 000 lymphocytes were gated by forward and side scatter and CD45 expression. The collected data were analysed using CellQuest Pro software (Apple Computer, Inc., Cupertino, CA, U.S.A.).

Release of cytokines from peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) obtained from heparinized blood by isopaque-Ficoll (Lymphoprep; Nycomed

Table 1 Primer sequences used for real-time polymerase chain reaction analyses

mRNA target (gene name)	Primer sequence (5′ to 3′, forward primer written on top)	Product size (bp)	$MgCl_2$ conc. (mmol L^{-1})
hIL10	TTACCTGGAGGAGGTGATGC	148	2·0
	GGCCTTGCTCTTGTTTTCAC		
hIL12B (p40)	TACTCCACATTCCTACTTCT	85	4·0
	CGTGAAGACTCTATCTTTCT		
hIL17A	AGGCACAAACTCATCCATCC	91	2·5
	GCTCAGCAGCAGTAGCAGTG		
hIL23A (p19)	GACACATGGATCTAAGAGAA	114	3·0
	AGCAGAACTGACTGTTGTC		
hTGFB1	GTGGAAACCCACAACGAAAT	83	2·5
	CGGAGCTCTGATGTGTTGAA		
hTNF	AGCCCATGTTGTAGCAAACC	134	2·5
	TGAGGTACAGGCCCTCTGAT		
hGAPDH	TGTTCGACAGTCAGCCGCATCTTCT	161	2·0
	TGATGGCAACAATATCCACTTTACCAGAGTT		

Pharma, Oslo, Norway) gradient centrifugation were incubated in flat-bottomed 96-well trays (Costar, Corning Inc., Corning, NY, U.S.A.; 2×10^6 cells mL^{-1}, 100 μL per well) in medium alone [RPMI-1640 containing 2 mmol L^{-1} L-glutamine (Sigma Chemical Co., St Louis, MO, U.S.A.), supplemented with 100 U mL^{-1} penicillin and 5% fetal calf serum], or with phytohaemagglutinin (PHA; Murex, Dartford, U.K.; final concentration 5 μg mL^{-1}). Cell-free supernatants were harvested after culturing for 24 h and stored at −80 °C until analysis.

Cytokine measurements

Cytokine levels in cell-free supernatants were analysed using a multiplex cytokine assay (Bio-Plex Human Cytokine 8-Plex Panel; Bio-Rad Laboratories Inc., Hercules, CA, U.S.A.), according to the instructions from the manufacturer.

Statistical analysis

Paired nonparametric Wilcoxon signed rank tests were used to examine differences within the same individuals over time. Because biopsy specimens from day 2 were available from only five individuals, these *in situ* data were omitted from the statistical analyses. P-values < 0·05 were interpreted as significant.

Results

Clinical score

All patients experienced clinical improvement, and the mean reduction of PASI scores was 72·8% after 16 days of heliotherapy (Fig. 1a).

Sun exposure for 16 days induced a reduction in lesional epidermal thickness

Biopsies obtained from lesional and nonlesional skin at baseline and on days 2 and 16 of sun exposure were examined with immunohistochemistry. At baseline, nonlesional skin was normal histologically, whereas lesional skin showed severe pathology with thickened epithelium, parakeratosis, elongated papillae and inflammatory infiltrates (Fig. 2a,b). No changes in epidermal thickness were seen after sun exposure in nonlesional skin (Fig. 1b). In lesional skin 16 days of sun exposure resulted in a significant reduction in epidermal thickness (Figs 1b and 2b, c).

T-cell populations in the epidermal and dermal compartments are rapidly reduced by sun exposure

As T cells are central in driving psoriasis pathology,[3,4] we wanted to examine the early impact of sun exposure on CD4+, CD8+ and regulatory T-cell populations *in situ*. Samples (frozen or formalin-fixed) from 10 patients were examined by immunohistochemistry and positively stained cells were enumerated. Cell densities are presented as cell numbers per square millimetre for the epidermal (Fig. 3) and dermal (Fig. 4) compartments separately.

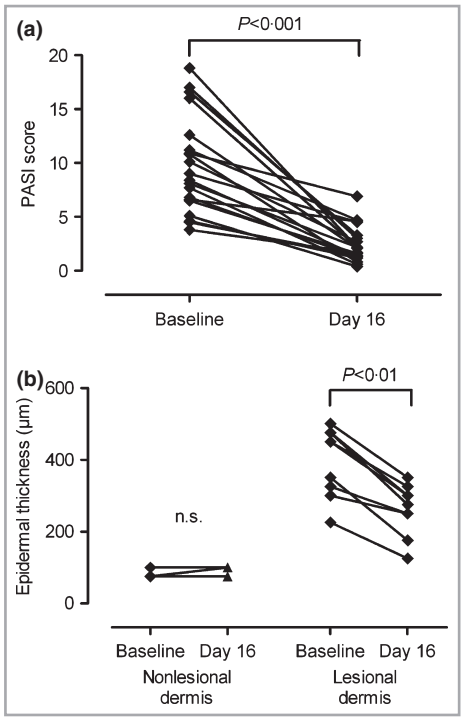

Fig 1. Psoriasis Area and Severity Index (PASI) score and reduction in epidermal thickening. Twenty patients received controlled natural sun exposure for 16 days. (a) All patients showed clinical improvement and PASI scores showed a significant reduction at day 16. (b) Epidermal thickness in specimens was unchanged in nonlesional skin after 16 days of sun exposure (n = 10), whereas in lesional skin epidermal thickness was significantly reduced (n = 10).

Epidermal compartment

Sections from nonlesional skin contained low numbers of epidermal T cells before and after sun treatment (Figs 2 left panels, and 3a, c, e). CD4+ and CD8+ T cells infiltrating the epidermis in lesional skin were significantly elevated compared with nonlesional skin ($P = 0{\cdot}0005$ and $P < 0{\cdot}0001$, respectively), but sun exposure resulted in a significant reduction for both cell types, and at day 16 the number of epidermal CD4+ and CD8+ T cells was not different from nonlesional skin (Figs 2d–i and 3a–d). A trend towards reduced numbers of CD4+ and CD8+ T cells was observed by day 2 (Fig. 3b, d).

FOXP3+ T cells (putative Tregs) were increased in lesional epidermis compared with nonlesional ($P = 0{\cdot}03$). Nonlesional epidermal FOXP3+ T cells were unaffected by sun exposure (Fig. 3e), whereas in lesional skin, they were significantly reduced by day 16 (Figs 2k, l, and 3f).

Dermal compartment

CD4+ and CD8+ T cells were significantly increased in lesional dermis at baseline compared with nonlesional dermis

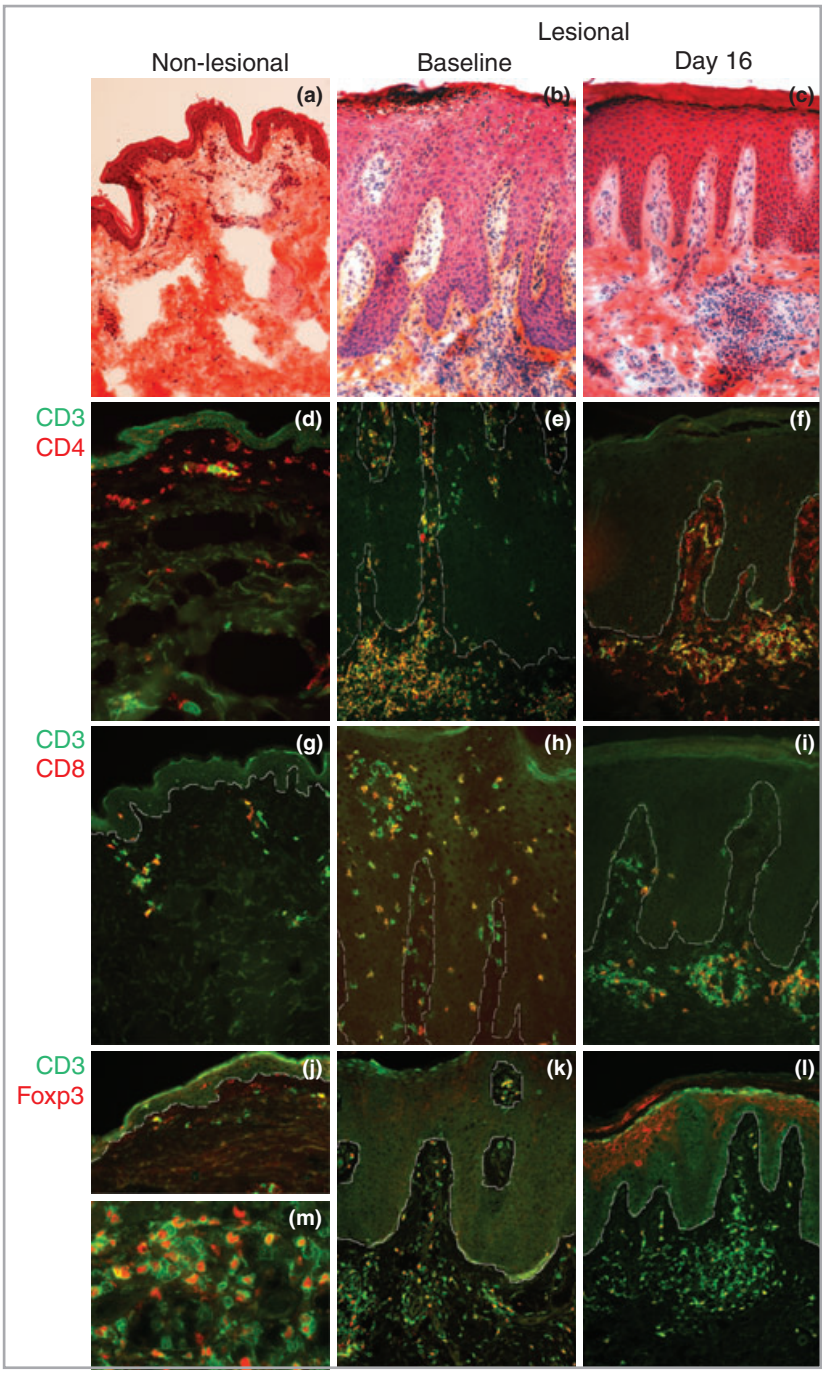

Fig 2. Histology and immunohistochemistry of (a–i) cryosections and (j–m) formalin-fixed sections. Sections shown are from nonlesional skin at baseline (left panels), from lesional skin at baseline (middle panels) and after 16 days of sun exposure (right panels). (a–c) Haematoxylin-eosin stained sections showed a reduction in epidermal thickness and of inflammatory cell infiltrates. (d–m) *In situ* phenotypic characterization of T-cell populations was performed by paired immunofluorescence staining with CD3 (Alexa 488, green) and (d–f) CD4 (Cy3, red), (g–i) CD8 (Cy3, red), and (j–m) FOXP3 (Cy3, red). Double positive cells appear yellow. The white dotted line denotes the dermoepidermal junction. Note the reduction in T-cell populations in lesional skin at day 16, especially in epidermal and papillary dermal compartments. Images (a–l) are at 200 × magnification. Panel (m) shows CD3+FOXP3+ cells in lesional dermis at 600 × magnification. (l) The anti-FOXP3 antibody showed unspecific staining of the upper epidermis in all specimens obtained after sun exposure.

($P < 0{\cdot}0001$ for both subsets, Fig. 4a–d). In nonlesional dermis, no significant changes in CD4+ or CD8+ T-cell numbers were observed after sun exposure (Fig. 4a, c). In lesional dermis, both T-cell subsets were significantly reduced on day 16 of sun exposure although the changes were less dramatic than those seen in the epidermis (Fig. 4b, d).

Dermal FOXP3+ T cells were also increased in lesional skin ($P = 0{\cdot}002$, Fig. 4e, f). In contrast with the epidermis, the number of FOXP3+ T-cells remained unchanged after sun exposure in lesional dermis (Fig. 4f), suggesting a relative increase in Tregs. No correlation between the relative change in Treg numbers and the reduction in PASI score was observed (data not shown). For all T-cell subsets studied, a redistribution of infiltrates could be observed after sun exposure, with the most dramatic reduction in cell numbers in the epidermis and papillary dermis, whereas infiltrates in the reticular dermis persisted (Fig. 2).

In order to examine whether T cells were apoptotic after sun exposure, all sections were examined for the expression of cleaved caspase-3. Only occasional CD3+ cells (0–2 per section) expressing this marker of apoptosis could be found on days 1 and 16 (data not shown).

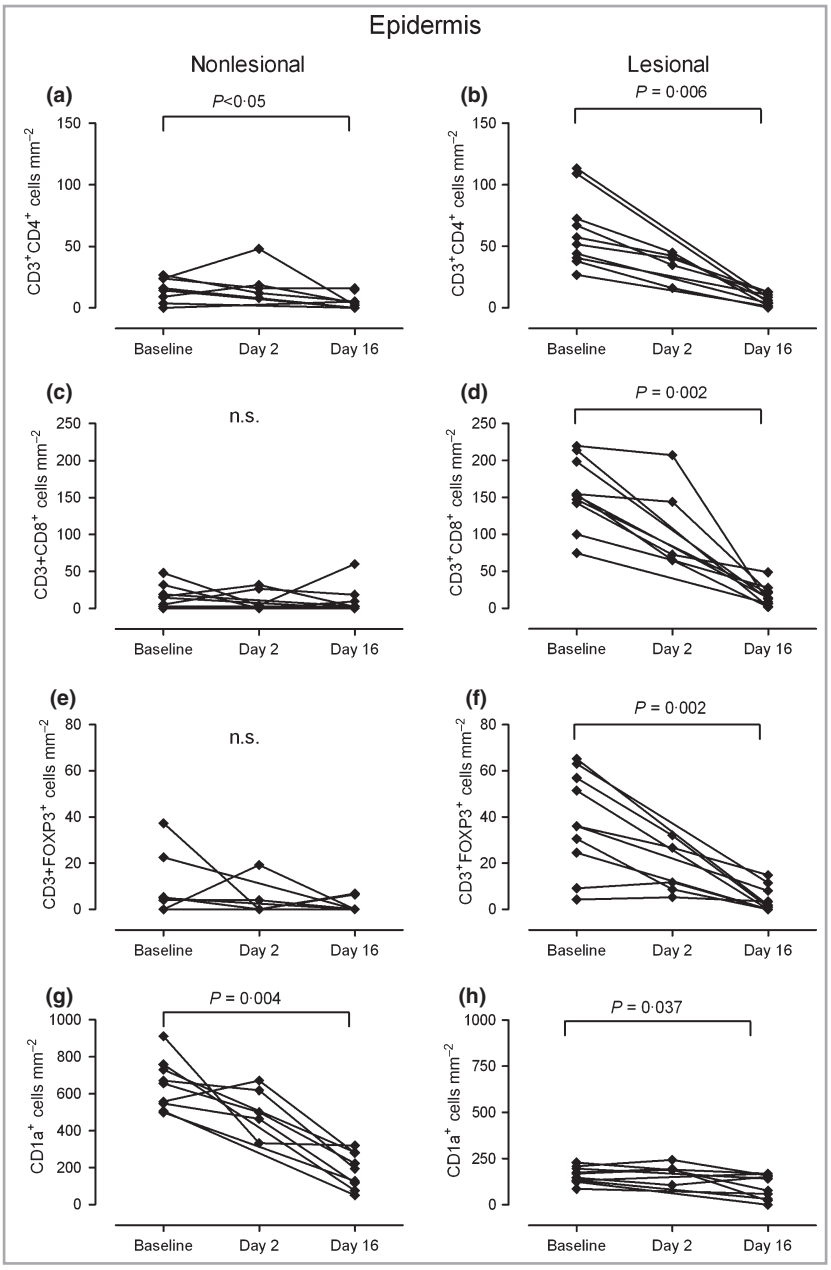

Fig 3. Numbers of immunostained cells expressed per square millimetre for the epidermal compartment in nonlesional (left panels) and lesional skin (right panels) at baseline and on days 2 and 16 of sun exposure ($n = 10$). Numbers for the following cell populations are shown: (a, b) CD3+CD4+ cells, (c, d) CD3+CD8+ cells, (e, f) CD3+FOXP3+ cells and (g, h) CD1a+ cells.

The density of Langerhans cells was decreased in lesional epidermis

In contrast to the other cell populations studied, the densities of LCs, defined as epidermal CD1a+ cells per square millimetre, were profoundly reduced in lesional skin at baseline compared with nonlesional epidermis ($P < 0{\cdot}0001$, Figs 3g,h and 5a, c). In nonlesional epidermis LCs, as expected, were dramatically reduced upon sun exposure (Figs 3g and 5a,b). In lesional epidermis, there was a slight but significant reduction in LC numbers after sun exposure (Figs 3h and 5c,d). There was no difference in dermal CD1a+ cells between nonlesional and lesional skin at baseline. The density of dermal CD1a+ cells remained unchanged in nonlesional dermis after sun exposure, whereas in lesional dermis a significant reduction was observed (Figs 4g,h and 5a–d).

Cytokine gene expression in lesional skin

In order to investigate whether sun-induced reduction in T-cell numbers was associated with reduction in cytokine gene expression, mRNA was isolated from the same frozen biopsies that were examined with immunohistochemistry. In samples from nonlesional skin, very low levels of cytokines were detected (data not shown). From lesional specimens,

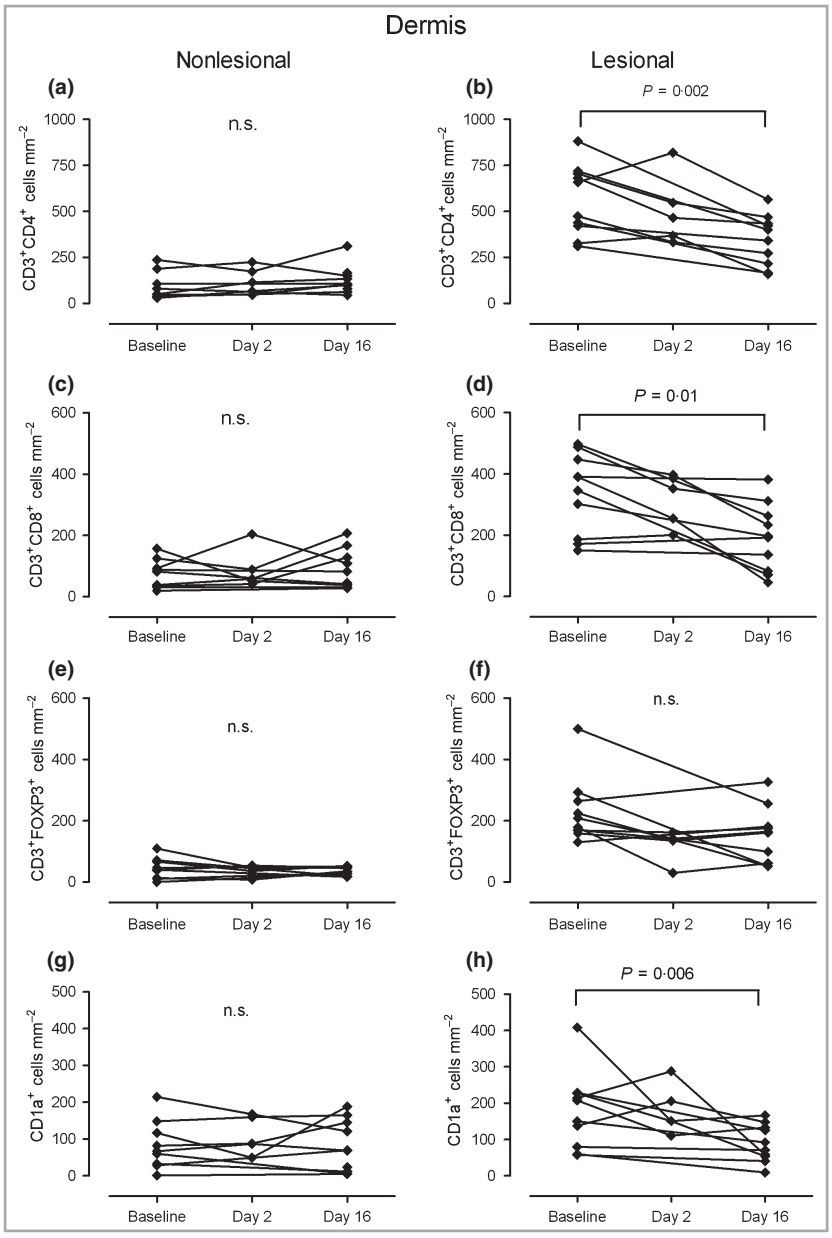

Fig 4. Numbers of dermal immunostained cells expressed per mm^2 in nonlesional (left panels) and lesional skin (right panels) at baseline and on days 2 and 16 of sun exposure (n = 10). Numbers for the following cell populations are shown: (a,b) CD3+CD4+ cells, (c,d) CD3+CD8+ cells, (e,f) CD3+FOXP3+ cells and (g,h) CD1a+ cells.

unfortunately, only samples from four individuals yielded results of satisfactory quality, so no statistics could be performed on these data. However, clear trends could be observed for some of the cytokines. The expression level of TNF-α was markedly reduced in three of four individuals on day 2, whereas it was unchanged on day 16 (Fig. 6a). IL-12p40 (also part of IL-23) was reduced in four of four individuals on day 16 (Fig. 6b). IL-23p19 and IL-17 showed markedly reduced expression levels in three of four individuals on both day 2 and day 16 of sun exposure (Fig. 6c,d). In contrast, the expression levels of the anti-inflammatory cytokine IL-10 was increased in three of four individuals on day 16 (Fig. 6e), whereas transforming growth factor (TGF)-β seemed unchanged (Fig. 6f).

The frequency of CLA+ T cells in peripheral blood was reduced on day 2 of sun exposure

To study the impact of immune function in the systemic compartment, we next examined whether sun exposure affected circulating skin homing T cells, characterized by expression of the homing marker cutaneous lymphocyte antigen (CLA). PBMCs obtained from blood samples from all 20 patients at baseline, day 2 and day 16 of sun exposure, were examined by flow cytometry. Total numbers of CD3+ T cells, CD4+ T cells and CD8+ T cells were unaffected by sun exposure (data not shown). The frequencies of both CD4+ and CD8+ CLA+ T cells as a percentage of total T cells (not shown) as well as a percentage of CD4+ and CD8+ T cells (Fig. 7a, b) were sig-

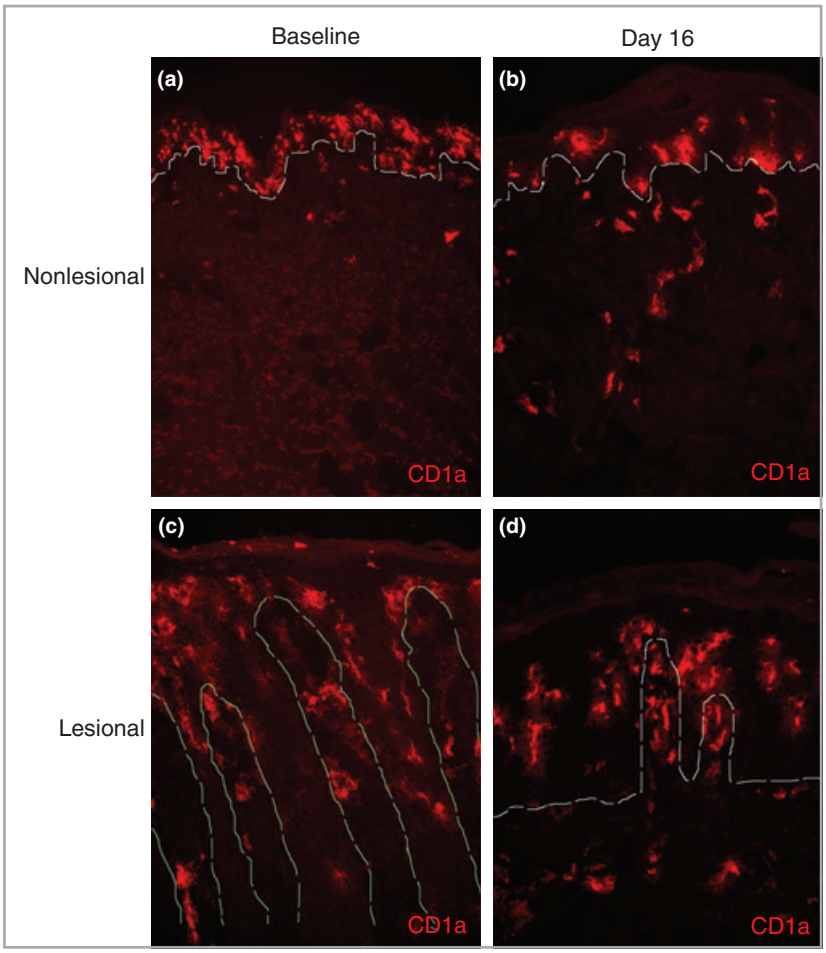

Fig 5. CD1a+ Langerhans cells (LCs) in nonlesional (upper panels) and lesional skin (lower panels) at baseline (left panels) and at day 16 of sun exposure (right panels). (a, b) The density of epidermal CD1a+ LCs was reduced in lesional skin compared with nonlesional skin. (c) After sun exposure, LCs were dramatically reduced in nonlesional epidermis, (d) whereas the reduction in lesional epidermis was significant but less pronounced.

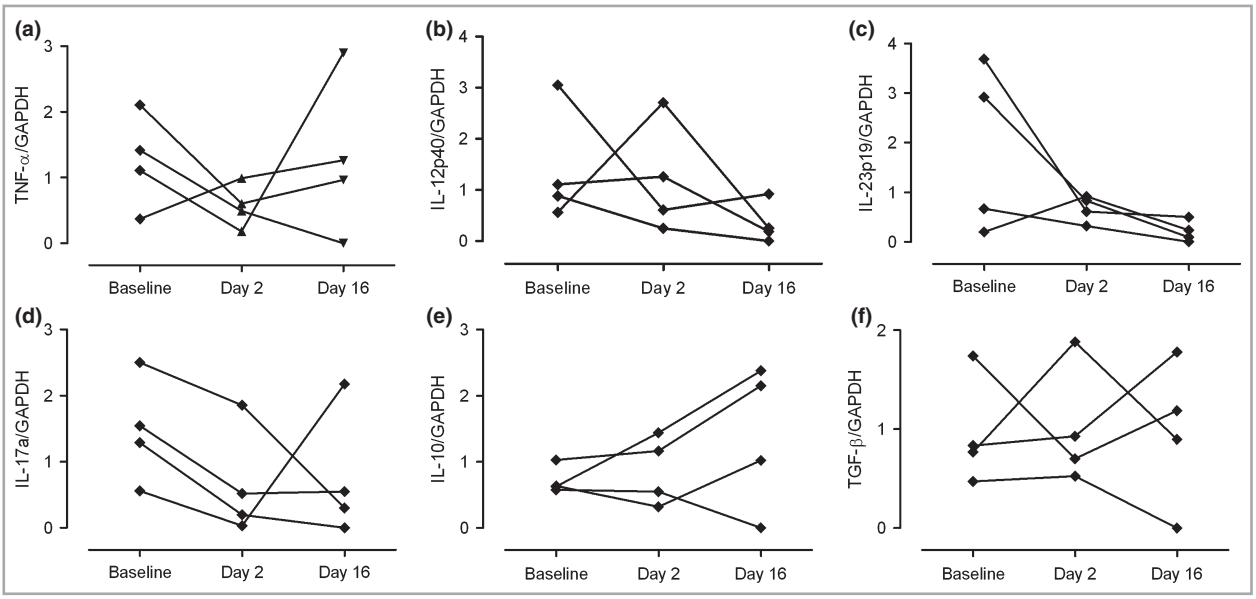

Fig 6. Cytokine mRNA in frozen biopsy specimens obtained at baseline and on days 2 and 16 after sun exposure. Unfortunately, only four specimens yielded qualitatively satisfying results, so no statistics could be performed. Real-time polymerase chain reactions were performed to investigate the expression levels of the following cytokines, (a) tumour necrosis factor (TNF)-α, (b) interleukin (IL) 12p40 (common subunit of IL-12 and IL-23), (c) IL-23p19, (d) IL-17, (e) IL-10 and (f) transforming growth factor (TGF)-β. Expression levels are normalized to GAPDH.

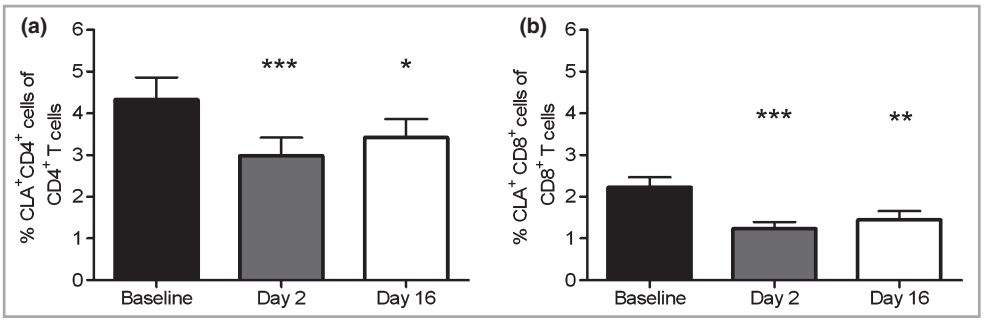

Fig 7. CLA+ T-lymphocyte subpopulations in peripheral blood at baseline and on days 2 and 16 of sun exposure analysed by flow cytometry. The percentages of (a) CD4+CLA+ cells of CD4+ T cells and (d) CD8+CLA+ cells of CD8+ T cells were significantly reduced. Data are mean ± SEM of all 20 patients. ***P < 0·001; **P < 0·01 vs. baseline.

nificantly decreased by day 2 and remained low on day 16 of sun exposure, demonstrating a selective reduction of CLA+ T cells in the peripheral blood.

Release of cytokines from peripheral blood mononuclear cells *ex vivo*

We next examined the release of cytokines from unstimulated and PHA-stimulated PBMCs obtained from all 20 patients at baseline, day 2 and day 16. The supernatants were removed after culturing cells for 24 h. In supernatants from unstimulated cells, all cytokine concentrations were below the detection limit (data not shown). There was no difference in cytokine secretion in PBMCs obtained after 2 days of sun exposure. After 16 days, PBMCs released significantly less interferon (IFN)-γ, IL-17, TNF-α and IL-10 compared with baseline levels (Fig. 8a–d), whereas the reduction in IL-12p40 release was not significant (Fig. 8e).

Discussion

This study demonstrated that 16 days of exposure to natural sun induced excellent clinical improvement in psoriatic patients. The clinical response was preceded by rapid immunological changes both *in situ* and systemically.

CD8+ T cells preferentially infiltrate the epidermis in psoriasis, but the CD4+ T cells in the dermis are believed to play a more crucial role in driving the pathogenesis of the disease.[26] Here, we found that both T-cell subsets were significantly reduced in both tissue compartments, strongly suggesting that the effect of sun treatment is mediated by its effect of pathogenic T cells in the lesion.

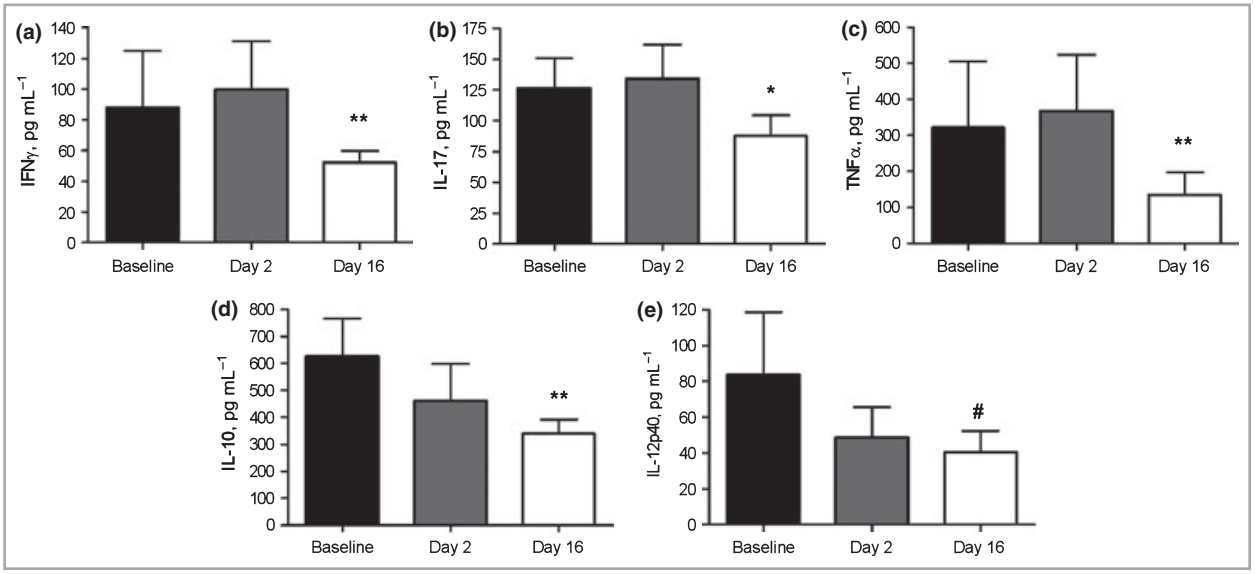

Fig 8. Cytokine release from phytohaemagglutinin-stimulated peripheral blood mononuclear cells (PBMCs) obtained at baseline and on days 2 and 16 of sun exposure. (a)The release of interferon (IFN)-γ, (b) interleukin (IL)-17, (c) tumour necrosis factor (TNF)-α, (d) IL-10 were all significantly reduced in PBMCs obtained at day 16. (e)The reduction in the release of IL-12p40 (common subunit of IL-12 and IL-23) did not quite reach statistical significance. Data are mean ± SEM of all 20 patients. **P < 0·01; *P < 0·05; #, P = 0·064 vs. baseline.

We demonstrate for the first time that the percentage of skin homing CLA+CD4+ and CLA+CD8+ T cells in peripheral blood rapidly decreases in psoriatic patients after sun exposure. Our finding accords well with a previous study showing that UVB exposure of psoriatic patients markedly reduced the expression of the skin homing molecule CLA on circulating T cells.[27] This indicates that sun exposure has a direct downregulatory effect on CLA expression of circulating T cells or that CLA+ T cells are preferentially eliminated. Either way, the rapid decrease of circulating CLA+ T cells may, at least in part, explain the rapid reduction of T cells in the psoriatic lesion. Previous studies have demonstrated that UVB irradiation induces apoptosis of T cells in psoriatic lesions whereas the effect on LCs is induction of migration rather than apoptosis.[28–30] We detected only very few T cells that expressed cleaved caspase-3; a widely used marker for apoptosis. However, this finding does not rule out the possibility that sun-induced apoptosis was involved. We only examined tissue samples on days 1 and 16, so the time points of our biopsy sampling may not have coincided with maximum expression of this molecule. Increased emigration of tissue-residing T cells, similar to LCs, could be an alternative explanation for reduction of lesional T cells. Examining regional lymph nodes for evidence of T-cell migration would be interesting, but this was not possible in the current study.

Lesional epidermal FOXP3+ cells were almost depleted after 16 days of sun treatment. Interestingly, the number of dermal FOXP3+ T cells remained unchanged whereas the total number of CD4+ T cells was significantly reduced. The great majority of FOXP3+ T cells was situated in the dermis. Thus, there was overall a relative increase in dermal Tregs, suggesting that the immunomodulatory activities of these cells could be one mechanism by which UV irradiation induces improvement in psoriasis. The dermal compartment contains the most recently recruited cells emigrating through postcapillary venules. The differential effect on FOXP3+ T-cell numbers in the two compartments may therefore suggest an increased recruitment of FOXP3+ T cells from the circulation.

Unfortunately, our mRNA data were of insufficient quality to enable us to draw conclusions, but they suggest that reductions in cell numbers are paralleled by reduction in pro-inflammatory cytokines. It has previously been shown that 3 weeks of UVB treatment reduced expression of IL-23p19[31] and that 8 weeks of ciclosporin treatment reduced IL-17 expression in psoriatic skin.[32] Together, these data are in line with recent reports[9,33,34] demonstrating that Th17 cells are central in immunopathology of psoriasis and indicate that sun exposure may downregulate the IL-23/IL-17 axis, potentially contributing to its beneficial effects in psoriatic patients. The increased IL-10 mRNA levels in lesional skin in three of four individuals on day 16 also supports the concept of a change from an inflammatory into a more homeostatic environment upon sun exposure.

The attenuated capacity of *in vitro*-cultured PBMCs to produce cytokines after sun treatment, suggests that sunlight not only has local anti-inflammatory effects but also modulates systemic immunity. This is in line with several studies showing that sunlight has generalized immunosuppressive effects.[35] Related to psoriasis the systemic effect of sun treatment may have several biological consequences. Firstly, circulating leucocytes recruited to the skin may have a higher threshold for activation, thus adding to the local anti-inflammatory effect. Reduction of adhesion molecules locally would in turn result in reduced recruitment of immune cells from the circulation. Also, it may have beneficial effects on psoriasis-related disorders in other organs, such as arthritis.

Whereas previous studies have suggested that IL-10 is important for UV-induced systemic immunosuppression, we found reduced IL-10 levels after sun exposure. This paradoxical finding could possibly be explained by disease-specific factors in patients with psoriasis. It could be speculated that PBMCs in patients with psoriasis produce more IL-10 as a counter-regulatory measure due to chronic inflammation and thus, sun exposure could result in an absolute reduction of this cytokine.

LCs have previously been found in decreased densities in psoriatic lesions.[36] We confirm this finding and our data also support previous reports of LCs having impaired migratory function in psoriatic patients.[37] LCs normally leave the epidermis and migrate to regional lymph nodes upon UV-exposure.[30] However, we found that LCs in lesional epidermis, in contrast to CD4+ and CD8+ and FOXP3+ lymphocyte populations, were only slightly reduced in numbers after sun exposure, suggesting impaired migratory function. LCs in non-lesional epidermis, as expected, were dramatically reduced after sun exposure, suggesting that this impaired migration is not a primary defect in psoriasis.

The warm climate and bathing in combination with sun exposure might reduce stress and thereby indirectly improve the psoriasis lesions in addition to the UV-induced effects. However, there is a large body of evidence to suggest that UV exposure under experimental conditions has a very strong immunosuppressive effect, which is compatible with the idea that the reduction in pathogenic T cells is mainly due to sun treatment. Interperson variability is also an aspect which must be taken into account in a study which includes relatively few patients. It is difficult to quantify the dose of UV radiation derived from the sun in terms of the minimal erythema dose which differs from person to person. However, by combining the calculated UV radiation, measured each hour, with the sun-exposure time from the patients' diaries, we consider the estimated UV doses for each patient reliable.

In conclusion, the present study shows that exposure to natural sun induces excellent clinical improvement within 16 days, associated with a rapid reduction of effector T cells in lesional skin, a relative increase in Tregs as well as a rapid reduction in skin homing T cells in peripheral blood. The concomitant change in the systemic cytokine profile favouring an immunosuppressive environment may contribute to the resolution of inflammation.

What's already known about this topic?

- T cells infiltrate psoriatic skin in great numbers and are central to the pathogenesis of this disease.
- Ultraviolet radiation exposure induces both local and systemic immunosuppression and results in the clinical improvement of several immune-mediated disorders.
- Heliotherapy is a well-established treatment modality for patients with psoriasis in Norway.
- It has not been determined how exposure to natural sun affects lesional T cells in psoriasis.

What does this study add?

- Exposure to natural sun induces rapid reductions in CD4+ and CD8+ T cells in the epidermal and dermal compartments of psoriatic skin as well as in circulating skin homing T cells, with reductions in cell numbers shown by day 2.
- Dermal FOXP3+ T cells are relatively increased after sun exposure.
- Evidence of systemic immunosuppression was shown by reduced cytokine production by peripheral blood mononuclear cells after sun exposure.
- These rapid immunological effects of sun exposure precede and presumably mediate the observed clinical improvement.

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