# Ultraviolet Light Induces Increased Circulating Interleukin-6 in Humans

Agatha Urbanski, M.Sc., Thomas Schwarz, M.D., Peter Neuner, M.D., Jean Krutmann, M.D., Reinhard Kirnbauer, M.D., Andreas Köck, and Thomas A. Luger, M.D.

Department of Dermatology II (AU, PN, RK, AK, TAL), University of Vienna and Ludwig Boltzmann Institut für Dermatovenerologische Serodiagnostik, Laboratory of Cellbiology, Vienna, Austria (AU, TS, PN, JK, RK, AK, TAL); Department of Dermatology, Hospital Vienna-Lainz (TS), Vienna, Austria

Although the clinical effects of acute exposure to ultraviolet (UV) light — such as cutaneous inflammation, malaise, somnolence, chills and fever — have been appreciated many years, the underlying mechanisms mediating these effects are poorly understood. Interleukin-6 (IL-6) is a potent cytokine with a wide variety of biologic activities, including induction of fever and acute phase response. Because IL-6 is produced by keratinocytes in vivo and in vitro and because the release is enhanced by UV light, the present study was performed to investigate the effect of a single UV dose eliciting moderate to severe sunburn reaction on the production of IL-6 in vivo. Therefore, plasma of UV-treated human subjects was evaluated for IL-6 activity by testing its capacity to induce the proliferation of an IL-6-dependent hybridoma cell line (B9). In contrast to plasma samples obtained before UV exposure, post-UV-specimens contained significant levels of IL-6 peaking at 12 h after UV irradiation. Plasma IL-6 activity was neutralized by an antiserum directed against recombinant human IL-6, and upon HPLC gel filtration exhibited a molecular weight of around 20 kD. Moreover, plasma IL-6 levels correlated remarkably with fever course followed by an increase of acute phase proteins such as C-reactive protein. These data indicate that IL-6, which is released by keratinocytes following UV exposure, may gain access to the circulation and via its pyrogenic as well as acute phase-inducing effect may function as an important mediator of systemic sunburn reaction. *J Invest Dermatol 94:808–811, 1990*

Severe sunburn reaction does not only include local dermal changes but also systemic effects such as fever, chills, malaise, headache, vomiting, drowsiness, leukocytosis, and increased release of acute-phase proteins [1]. Although the systemic changes caused by acute UV exposure have been appreciated for many years, the underlying mechanisms mediating these effects are poorly understood.

Currently, cytokines, which are important mediators of immunologic and inflammatory reactions, have gained interest for their role in the host response to injurious stimuli, such as UV light [2]. Interleukin-6 (IL-6) is a recently discovered multifunctional cytokine which through the multiplicity of its biologic activities has turned out as an essential mediator involved in acute-phase and immunologic response during host defense [3,4]. This is supported by recent findings demonstrating significantly elevated IL-6 serum levels in patients with burns [5], severe psoriasis [6], septicemia [7], undergoing surgical procedures [8], or after administration of endotoxin to human volunteers [3].

Manuscript received July 3, 1989; accepted for publication December 11, 1989.

This work was supported by the "Medizinisch-Wissenschaftlicher Fonds des Bürgermeisters der Bundeshauptstadt Wien" and "Fonds der Austria Tabakwerke."

Reprint requests to: Agatha Urbanski, M.Sc., Department of Dermatology II, Laboratory of Cellbiology, University of Vienna, Alserstrasse 4, A-1090 Vienna, Austria.

Abbreviations:
CRP: C-reactive protein
IL-1: interleukin 1
IL-6: interleukin 6
MED: minimal erythema dose
UV: ultraviolet

By virtue of their anatomic location, keratinocytes represent the main target for UV light, and keratinocytes have been demonstrated to be a potent source of various cytokines such as IL-1, IL-3, and colony-stimulating factors [9]. Recently, it was shown that epidermal cells also release IL-6 and that in vitro IL-6 production is significantly enhanced after UV exposure of long-term cultured keratinocytes or keratinocyte cell lines [10,11]. Therefore, the present study was performed to investigate the effects of UV light on the production of IL-6 in vivo and to study the possible involvement of this cytokine in the pathogenesis of systemic effects following extensive UV exposure.

## MATERIAL AND METHODS

**Patient Studies** Six human volunteers (male caucasians; mean age, 33 ± 3.0 years) were included in the study. The skin types were evaluated according to Pathak et al [12]. One patient had fair skin which burnt easily and never tanned (type I), three probands could be allocated to skin type II (usually burn, sometimes tan), and two volunteers had skin type III (sometimes burn, always tan). None had a tan when the study began; excessive sun exposure three weeks before and during the study was not allowed.

As a light source, an unfiltered bank of six blue light lamps (Dr. Hönle, Munich, FRG) was used, emitting a linear spectrum between 250 and 600 nm with emission peaks at around 300 nm and 360 nm. The output at 297 nm measured with a UV-meter (SGI, Greiter, Vienna) was 25 W/m², and at 365 nm measured with an UVA-meter (Waldmann, Schwenningen, FRG) was 120 W/m². As UV exposure lasted less than 120 sec, the long wave (UVA) dose delivered was minimal and biologically irrelevant; therefore the relevant action spectrum was within the UVB range (290–320 nm). At least 5 d before study begin the individual minimal erythema dose (MED) of each proband was determined, as described previously [13]. The individual MED varied from 190 J/m² to 450

**Table I.** Effect of Heat Inactivation on Plasma IL-6 Activity

| Reciprocal Dilution | Pre-UV[a] | | Post-UV (12 h)[a] | |
|---|---|---|---|---|
| | Normal | Heat Treated | Normal | Heat Treated |
| 4 | 155 | 3,099 | 34 | 9,421 |
| 8 | 202 | 2,077 | 43 | 3,669 |
| 16 | 63 | 1,856 | 37 | 2,502 |
| 32 | 219 | 2,020 | 131 | 2,195 |
| 64 | 1,721 | 1,750 | 1,421 | 1,671 |

[a] Plasma from one healthy volunteer prior to or following UV exposure was tested in the B9 hybridoma assay. Plasma samples were heat treated 30 min at 56°C or kept at room temperature. Results are expressed as mean cpm of triplicates of B9 cell $^3$H-thymidine incorporation. SE were within 10%. $^3$H-thymidine uptake of B9 cells alone was 1,525 cpm.

J/m² due to pigmentation type [12]. After informed consent was obtained, test persons received a single total-body UV exposure equivalent to four MED. Genitalia and buttocks which had been used for MED determination were covered. UV irradiation of all volunteers was performed at noon (12:00 A.M.). Blood was drawn from the antecubital vein into sterile, heparinized tubes immediately before UV exposure, and 1, 3, 6, 12, 24, 48, 72, and 96 h after UV treatment.

Venipuncture was also performed the day before UV exposure at 12:00 A.M., 6:00 P.M., 9:00 P.M., and 12:00 P.M. in order to exclude changes in IL-6 levels due to circadian rhythm. Plasma samples were centrifuged and immediately frozen in aliquots at −20°C and kept frozen until used. Axillary body temperature was evaluated every 3 h within the first 24 h after UV exposure, on the following days temperature was measured once per day (12:00 A.M.).

Test subjects were not allowed to take any drugs two weeks before and during the entire study. In order to mitigate severe sunburn reaction, topical application of indifferent oil in water emulsions not containing corticosteroids or other specific ingredients was allowed.

**IL-6 Assay** For the detection of IL-6, the murine hybridoma cell line B9 was used [10]. Briefly, $5 \times 10^4$ ml B9 cells were cultured in RPMI 1640 supplemented with $5 \times 10^{-5}$ M 2-mercaptoethanol, 5% FCS, penicillin and streptomycin. The assay was carried out in 96 well flat bottom microtiter plates in 200 μl volume. Cells were pulsed at 68–72 h with 1 μCi ($^3$H)-thymidine and radioactivity was measured. Unless otherwise stated, plasma samples to be tested were thawed immediately before use, heat inactivated at 56°C for 30 min, titrated at various dilutions in triplicates and compared to a standard IL-6 preparation containing 10 U/ml IL-6 activity. Results are expressed as counts per minute ± SEM of triplicate cultures or as ng/ml.

In order to test the specifity of IL-6 activity in plasma samples, a rabbit antiserum directed against highly purified *E. coli*-derived IL-6 was used (starting dilution, 1 : 100) kindly provided by L. May (The Rockefeller University, New York).

**CRP Assay** Quantitation of circulating CRP levels was performed by standard nephelometry using commercially available kits (Behring Diagnostics, Marburg, FRG).

**High-Performance Liquid Chromatography** Serum samples (100 μl) were subjected to a size exclusion column (Bio-Sil TSK 125, 300 × 7.5 mm, Bio-Rad, Richmond) and elution was carried out with PBS (pH 7.2) at a flow rate of 1.0 ml/min.

## RESULTS

When plasma IL-6 activity was investigated in human volunteers prior and post UV exposure, usually no or little IL-6 activity could be detected in untreated samples. After heat inactivation, however, samples of unexposed volunteers exhibited very low or undetectable B9 cell promoting activity, which appeared significantly enhanced following UV exposure (Table I). The effect of heat inactivation may be due to the removal of unspecific inhibitors, sometimes present in serum samples. Therefore in all further experiments heat-inactivated plasma was used. Elevation of IL-6 levels was first observed between 1 and 3 h after therapy, peaking 12 h post UV when clinical sunburn reaction started to become most severe. Still, enhanced IL-6 amounts were found within 72 h, returning to base line within 96 h (Table II). In order to exclude the possibility that the increase of IL-6 reflected only variations due to the circadian rhythm, venipuncture had been performed 1 d before UV treatment, at distinct time points. Pre-UV samples, however, contained low or undetectable IL-6 activity (Table II).

In order to evaluate the specifity of the B9 cell inducing activity in plasma of UV-treated human subjects, a rabbit antiserum directed against human recombinant IL-6 was used. Addition of this antiserum resulted in a dose-dependent inhibition of the B9 cell promoting capacity demonstrating that this is in fact due to IL-6 (Fig 1). Co-incubation of plasma samples with a normal rabbit serum as a control did not affect the stimulating activity (Fig 1). When plasma obtained 12 h after UV treatment was subjected to size-exclusion HPLC, IL-6 activity eluted at around 20 kD (Fig 2).

In all volunteers, emergence of clinical symptoms (freezing, fatigue, burning sensations) was observed 6 h after UV treatment. In addition, body temperature was significantly increased when measured 12-h post-UV exposure (Table III). When the plasma samples were evaluated for CRP, significantly increased levels were detected 48 h after UV treatment (Table III). The complete pre- and post-UV IL-6 kinetic and fever course of one volunteer is shown in Fig 3.

**Table II.** IL-6 in Plasma of UV-Treated Volunteers

| Volunteer[a] | IL-6 (ng/ml)[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | −24 h | −12 h | 0 h | 3 h | 6 h | 12 h | 24 h | 48 h | 72 h | 96 h |
| I | 1.5 | 1.0 | 1.3 | 4.8 | 5.0 | 5.7 | 3.6 | 3.3 | 2.7 | 1.9 |
| II | n.d.[c] | n.t.[d] | 1.8 | n.t. | 4.9 | 7.7 | 8.4 | 6.0 | 5.5 | 2.7 |
| III | n.t. | n.d. | 2.6 | 3.6 | 3.7 | 5.1 | 3.5 | n.d. | n.t. | n.t. |
| IV | 1.7 | n.t. | 2.0 | 5.4 | 5.9 | 11.6 | 10.6 | 6.4 | 6.1 | 5.1 |
| V | 3.1 | 3.0 | 3.6 | 4.9 | 6.6 | 27.8 | 13.3 | 7.2 | 5.2 | 5.2 |
| VI | n.d. | 3.2 | 3.7 | 4.8 | n.t. | 7.3 | 5.2 | 3.2 | 2.5 | n.t. |
| Mean | 1.26 | 1.8 | 2.5 | 4.7 | 5.22 | 10.8 | 7.43 | 4.35 | 4.4 | 3.7 |
| SE | 1.08 | 1.16 | 0.94 | 0.77 | 0.9 | 2.8 | 1.91 | 1.57 | 1.22 | 1.21 |

[a] Human volunteers were total-body exposed to UV light equivalent to 4 MED.
[b] IL-6 activity was measured by using the B9 hybridoma assay.
[c] n.d., not detectable.
[d] n.t., not tested.

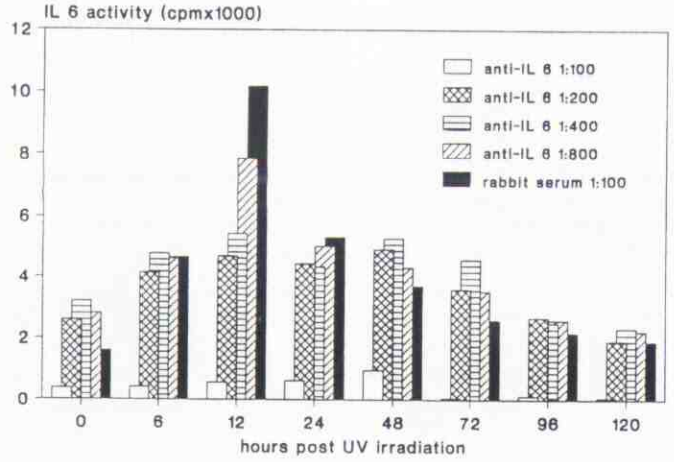


**Figure 1.** Effect of anti-IL-6 antiserum on plasma IL-6 activity. Plasma samples obtained at different time points immediately before and after UV exposure were incubated with various dilutions of rabbit anti-IL-6 antiserum or normal rabbit serum and tested in the B9 assay. Results are expressed as cpm of one representative experiment.

## DISCUSSION

The present study demonstrates that a single total-body UV exposure causing severe sunburn reaction leads to an increase of circulating IL-6 in the serum of UV-treated human subjects. The specifity of these findings is supported by the observation that an antiserum directed against recombinant human IL-6 blocked plasma IL-6 activity. Similar to previous studies [3], low IL-6 levels were usually detected in blood samples obtained before UV exposure, indicating that spontaneous release of IL-6 into the circulation is only minimal in healthy human subjects. Whether these low circulating IL-6 levels which were detected in healthy normal volunteers using different assays are of biologic relevance at present is unclear [3]. However, they may reflect a host response to frequent minor bacterial or viral infections without clinical relevance. The failure to detect increased levels at different time points before phototreatment excludes the possibility that enhanced IL-6 activity found after UV exposure is just a reflectance of physiologic variations due to circadian rhythm.

Interleukin 6 exhibits a variety of different biologic activities on different target cells [4]. It is a major mediator of the acute-phase response, induces fever, is an important mediator of B-cell growth

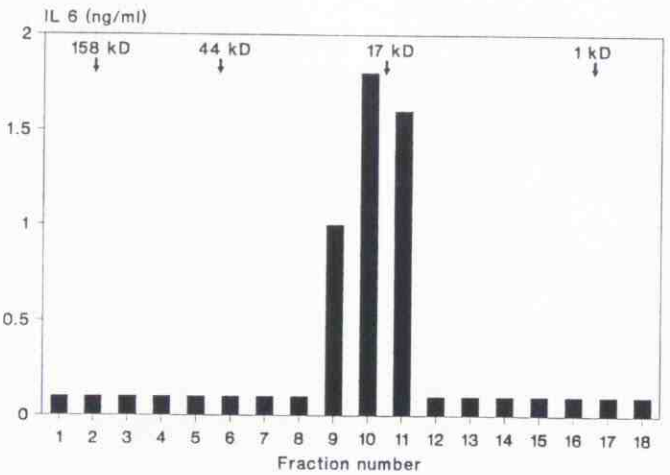


**Figure 2.** HPLC gel filtration (TSK 125) of plasma obtained 12 h after UV exposure. Fractions were tested in the B9 assay.

**Table III.** Plasma IL-6, Circulating CRP, and Body Temperature Following UV-Exposure

| | Pre-UV | 12 h | 48 h |
|---|---|---|---|
| IL-6 (ng/ml)[a] | 2.5 ± 0.9 | 10.9 ± 2.9 | 4.2 ± 1.7 |
| Fever (Δ°C)[b] | | 1.75 ± 0.37 | 0.4 ± 0.17 |
| CRP (mg/dl) | <0.27 | 0.3 ± 0.004 | 2.3 ± 1.3 |

[a] Results are expressed as mean ± SE of data obtained from six volunteers.
[b] Results are expressed as increase (Δ°C) in body temperature compared to values measured before UV exposure.

and differentiation, enhances immunoglobulin production, functions as a second signal in T-cell activation, and synergizes with colony-stimulating factors. Through these multiple effects, IL-6 has been addressed as a major mediator of immunologic and inflammatory reactions in response to various injurious stimuli, such as infection or severe burns [3,5,7].

In the present study, all six human subjects received the same relative amount of UV light, equivalent to four MED. Nevertheless, there was a broad individual variation in the induction of IL-6 levels. However, there seemed to be a correlation between IL-6 levels and clinical symptoms, and along with the elevation of IL-6 there were a consistent rise in body temperature peaking 12 h after UV treatment and a delayed increase in circulating CRP. Serum CRP was maximally enhanced after 48 h, when IL-6 and fever were already decreasing. Similar results have also been observed in other studies [5] and may be explained by the fact that acute-phase proteins start to be synthesized in the liver after induction by mediators.

Therefore, the present study suggests that IL-6 may be involved in the induction of fever and acute-phase protein production following extensive UV-exposure; however, the study does not permit an evaluation whether IL-6 is the only cytokine involved and

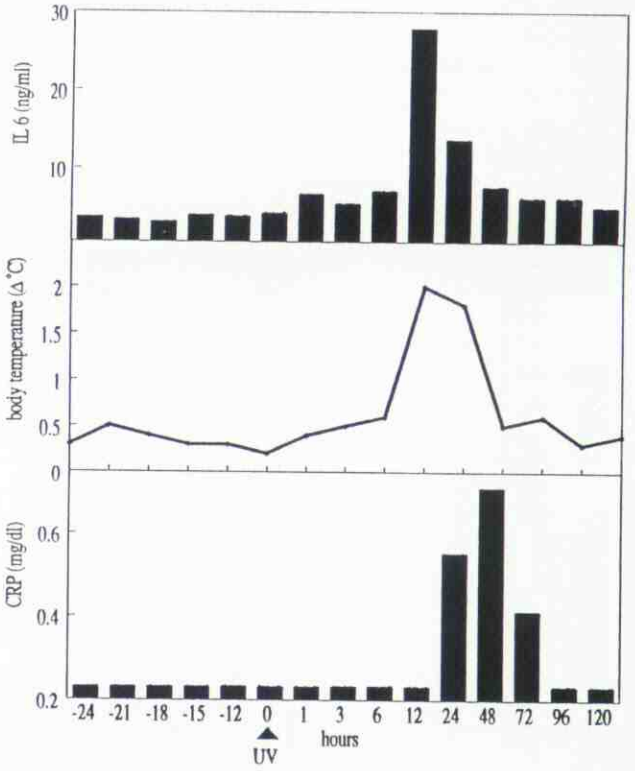


**Figure 3.** Kinetics of plasma IL-6, circulating CRP, and fever course of one UV-exposed volunteer. Plasma samples were obtained at different time points before and after UV exposure and tested in the B9 assay, increase of body temperature was evaluated at identical time points, and CRP levels were measured by nephelometry.

whether it causes its effects directly or via induction of other factors. It recently has been demonstrated the UV light in vitro and in vivo induces the release of IL-1 [2,14,15], which shares some similar biologic effects with IL-6, such as induction of fever. Accordingly, IL-1 plasma levels have been found enhanced in psoriatics undergoing phototherapy [16]. In this study, IL-1 activity was measured by the LAF-assay and interestingly IL-1 activity in plasma samples could not be completely blocked by IL-1 antibodies [16]. According to the present data, it appears to be very likely that the activity not neutralized by IL-1 antibodies is due to IL-6, which also exhibits some LAF-activity [17]. As IL-1 is a potent inducer of IL-6 [18], it remains to be determined whether increase of IL-6 is mediated directly by UV light and/or via IL-1 or other mediators such as tumor necrosis factor-$\alpha$ [19].

The cellular source of circulating IL-6 in response to UV light is not yet completely clear. Currently, it has been demonstrated that besides monocytes, endothelial cells, and fibroblasts, human keratinocytes in vivo and in vitro produce IL-6, and that synthesis and release can be dramatically enhanced by UV light [10,11]. As keratinocytes represent the main target for UV light, they appear to be a likely source for the increased IL-6 found in the circulation.

UV light probably is one of the most important physical impacts affecting human beings. Acute sun exposure leads to local changes and systemic effects; chronic UV irradiation induces cancer formation [20], causes immunosuppression [21], and exacerbates autoimmune diseases such as lupus erythematosus [22]. There is strong evidence that soluble mediators released by various cells upon UV exposure are involved in the pathogenesis of these reactions [2]. Prostaglandins secreted by keratinocytes mediate UV-induced erythema [23], and there is recent evidence that immunosuppressive factors produced by epidermal cells contribute to UV-induced immunosuppression [24–26]. Because of its B-cell stimulatory capacity, increased IL-6 production following UV irradiation may be of importance in autoimmune diseases such as lupus erythematosus. Accordingly, it has to be investigated in the future whether IL-6 as a B-cell stimulatory factor through the induction of autoantibodies may be responsible for UV-induced exacerbation of lupus erythematosus.

In summary, the present study demonstrates that in addition to IL-1, IL-6 is found increased in the circulation after total-body UV exposure and, according to their biologic activities, there is strong evidence that both cytokines play an essential role in the pathogenesis of systemic sunburn reaction. Thus specific inhibitors of IL-1 and IL-6 emerge as potential candidates to treat severe sunburn.

*The authors gratefully thank M. Bednar for the excellent secretarial assistance and R. Frost for the expert technical assistance in preparing the graphs.*

## REFERENCES


1. Ansel JC, Luger TA, Green I: Fever and increased serum IL-1 activity as a systemic manifestation of acute phototoxicity in New Zealand white rabbits. J Invest Dermatol 89:32–37, 1987
2. Schwarz T, Luger TA: Effect of UV irradiation on epidermal cell cytokine production. J Photochem Photobiol B4:1–13, 1989
3. Fong A, Moldawer LL, Marano M, Wei H, Tatter SB, Clarick RH, Santhanam U, Sherris D, May LT, Sehgal PB, Lowry SF: Endotoxemia elicits increased circulating $\beta$2-interferon/interleukin-6 in man. J Immunol 142:2321–2323, 1989
4. Wong GC, Clark SC: Multiple actions of interleukin 6 within a cytokine network. Immunol Today 9:137–139, 1988
5. Nijsten MWN, DeGroot ER, TenDuis HJ, Klasen HJ, Hack CE, Aarden LA: Serum levels of interleukin-6 and acute phase response. Lancet II:921, 1987
6. Neuner P, Kapp A, Kirnbauer R, Schwarz T, Krutmann J, Luger TA: Monocytes derived from patients with psoriasis synthesize and release increased levels of interleukin 6. J Invest Dermatol 92:490A, 1989
7. Helfgott DC, Tatter SB, Santhanam U, Clarick RH, Bhardway N, May LT, Sehgal PB: Interferon $\beta$2/interleukin 6 in plasma and body fluids during acute bacterial infection. J Immunol 142:948–953, 1989
8. Shenkin A, Fraser WD, Series J, Winstanley FP, McCartney AC, Burns HJG, Van Damme J: The serum interleukin 6 response to elective surgery. Lymphokine Res 8:123–127, 1989
9. Luger TA, Schwarz T: Epidermal cell derived cytokines. In: Bos J (ed). Skin Immune System. CRC Press, Boca Rotan (in press)
10. Kirnbauer R, Köck A, Schwarz T, Urbanski A, Krutmann J, Borth W, Damm D, Shipley G, Ansel JC, Luger TA: IFN-$\beta$2, B cell differentiation factor 2, or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. J Immunol 142:1922–1928, 1989
11. Kirnbauer R, Köck A, Krutmann J, Schwarz T, Urbanski A, Luger TA: Different effects of UVA and UVB irradiation of epidermal cell-IL 6 expression and release. J Invest Dermatol 92:459A, 1989
12. Pathak MA, Fitzpatrick TB, Greiter F, Kraus EW: Preventive treatment of sunburn dermatoheliosis and skin cancer with sunprotective agents. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF (eds.). Dermatology in General Medicine. McGraw Hill, New York, 1987, pp 1507–1522
13. Wolff K, Gschnait F, Hönigsmann H, Konrad K, Parrish JA, Fitzpatrick TB: Phototesting and dosimetry for photochemotherapy. Br J Dermatol 96:1–10, 1977
14. Ansel JC, Luger TA, Green I: The effect of in vitro and in vivo irradiation on the production of ETAF activity by human and murine keratinocytes. J Invest Dermatol 81:619–624, 1983
15. Granstein RD, Sauder DN: Whole-body exposure to ultraviolet radiation results in increased serum interleukin-1 activity in humans. Lymphokine Res 3:187–193, 1987
16. Konnikov J, Pincus SH, Dinarello C: Elevated plasma interleukin-1 levels in humans following ultraviolet light therapy for psoriasis. J Invest Dermatol 92:235–239, 1989
17. Lotz M, Jirik F, Kabouridis P, Tsoukas C, Hirano T, Kishimoto T, Carson D: B cell stimulatory factor 2/interleukin 6 is a costimulant for human thymocytes and T lymphocytes. J Exp Med 167:1253–1258, 1988
18. Santhanam U, Tatter SB, Helfgott DC: Structure, genetics and function of human "$\beta$2-interferon/B-cell stimulatory factor-2/hepatocyte stimulating factor" (interleukin-6). In: Powanda MC, Oppenheim JJ, Kluger MH, Dinarello CA (eds.). Monokines and Other Non-Lymphocytic Cytokines. Alan R. Liss Inc., New York, 1988, pp 29–34
19. Jablons DM, Mule JJ, McIntosh JK, Sehgal PB, May LT, Huang CM, Rosenberg SA, Lotze MT: IL-6/IFN-$\beta$2 as a circulating hormone. J Immunol 142:1542–1547, 1989
20. Kripke ML: Immunology and photocarcinogenesis. J Am Acad Dermatol 14:149–155, 1986
21. Roberts LK, Samlovski WE, Daynes RA: Immunological consequence of UV radiation exposure. Photodermatol 3:284–298, 1986
22. Ansel JC, Mountz J, Steinberg AD, DeFabo E, Green I: Effects of UV radiation on autoimmune strains of mice: Increased mortality and accelerated autoimmunity in BXSB male mice. J Invest Dermatol 85:181–186, 1985
23. Snyder DK, Eaglstein W: Intradermal anti-prostaglandin agents and sunburn. J Invest Dermatol 62:47–50, 1974
24. Schwarz T, Urbanska A, Gschnait F, Luger TA: Inhibition of the induction of contact hypersensitivity by a UV-mediated epidermal cytokine. J Invest Dermatol 87:289–291, 1986
25. Schwarz T, Urbanska A, Gschnait F, Luger TA: UV-irradiated epidermal cells produce a specific inhibitor of interleukin 1 activity. J Immunol 138:1457–1463, 1987
26. Schwarz T, Urbanski A, Kirnbauer R, Köck A, Gschnait F, Luger TA: Detection of a specific inhibitor of interleukin 1 in sera of UVB-treated mice. J Invest Dermatol 91:536–540, 1988