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Immunology

Effect of ultraviolet radiation on production of epidermal cell thymocyte-activating factor/interleukin 1 *in vivo* and *in vitro*

(acute-phase reactant/desensitization)

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ABSTRACT **UV radiation was found to enhance the release by keratinocytes of epidermal cell thymocyte-activating factor (ETAF), a hormone-like molecule that is physiochemically identical to interleukin 1 (IL-1). This conclusion was supported by the following observations: (*i*) the keratinocyte cell line PAM 212 retained ETAF/IL-1-producing potential after exposure to UV radiation despite significant loss in cell viability; (*ii*) epidermal cells from normal and UV radiation-exposed mice were found to produce equivalent amounts of ETAF/IL-1 on a per cell basis with the density of epidermal cells in UV radiation-exposed skin being at least 5-fold above normal values; (*iii*) under the conditions used, ETAF/IL-1 could be detected in the serum of UV radiation-exposed, but not normal, animals; and (*iv*) many of the biologic consequences known to be mediated by elevations in ETAF/IL-1—i.e., neutrophilia, elevated levels of complement component 3, serum amyloid P, and plasma fibrinogen—were all observed in animals following a single UV radiation exposure. Animals subjected to chronic UV radiation showed an initial elevation in their levels of acute-phase reactants that returned to normal concentrations within 7 days. This correlates with observations made by others of a "desensitization" to ETAF/IL-1-mediated effects after chronic administration of known exogenous stimulators of inflammatory responses. Further, the UV radiation-induced desensitization took place in spite of demonstrable serum levels of ETAF/IL-1. These results suggest that the mechanism(s) responsible for desensitization is not an inhibition of ETAF/IL-1 synthesis but rather may result from inability of the target cells to perceive this endogenous mediator or to unavailability of serum-associated ETAF/IL-1 for the appropriate targets.**

Most products of the inflammatory response result from a systemic stimulation of some specific target organ and therefore require the transmission of information from the origin of tissue injury to distant sites including the brain, liver, lymphoid system, and bone marrow (1–5). In generating both local and systemic inflammatory responses, the perturbations induced by exogenous agents must be translated into endogenous signals (a language) that can be recognized and responded to by the various target organs throughout the body. Such a mechanism was first described by Beeson in 1948 (6), who determined that fever is caused by the generation of endogenous pyrogens in response to an exogenous insult. In recent years a number of biologic activities have been described that are induced by molecules that are indistinguishable from endogenous pyrogen. Names ascribed to these biologic activities include leukocyte endogenous mediator (7), lymphocyte-activating factor (8), serum amyloid A inducer (9), mononuclear cell factor (10, 11), and neutrophil-releasing factor (5). The term interleukin 1 (IL-1) has been adopted to describe this molecule or a closely related group of molecules that is derived from activated macrophages. The activities induced by this approximately 15,000 molecular weight protein include hepatocyte production of a number of molecules known collectively as acute-phase reactants (12), fibroblast secretion of collagenase and E-series prostaglandins (13, 14), the induction of fever (15), the peripherilization of neutrophils from bone marrow stores plus their stimulation to release specific lysosomal products (5, 16), and the activation of specific T-lymphocyte subsets (17).

Recent evidence suggests that keratinocytes, when stimulated, can produce a molecule that is physiochemically and biologically indistinguishable from macrophage-derived IL-1 (18–20). Since the epidermis serves as the major interface between the external environment and the internal organ systems, the induction of this protein, epidermal cell thymocyte-activating factor (ETAF), probably plays an important role in the local and systemic inflammatory responses that originate in the skin.

UV radiation is an environmental agent that can induce an inflammatory response, sunburn, subsequent to its action on the skin. Short-term (acute) UV radiation exposure induces the migration of inflammatory cells into the exposed area (21), while chronic exposure to fractionated doses of UV radiation results in gradual disappearance of the inflammatory cell infiltrate (22), the development of epidermal hyperplasia (22), and the many UV radiation-induced modifications of host immune responsiveness (23–25). Changes in immune responsiveness that occur following UV radiation exposure of animals include the functional inactivation of Langerhans cells (26), the inability to respond to contact sensitizing agents (26), the generation of a T-suppressor cell-mediated state of tumor susceptibility (24, 25), the alteration in lymphocyte trafficking patterns (27), and a modification of splenic antigen-presenting cell function (28), a condition caused by their migration from the spleen to peripheral sites (29).

Because of its ability to induce an inflammatory response *in situ*, we felt there was a high probability that UV radiation would also possess the capacity to enhance the release of ETAF/IL-1. The object of this investigation, therefore, was to analyze the effects of UV radiation on the elaboration of ETAF/IL-1 by a transformed keratinocyte cell line (PAM 212) and to determine whether epidermal cells from chronically UV radiation-exposed mice retain the capacity to secrete this hormone-like molecule. Preliminary results by us (30) and others (31, 32) suggest that sublethal doses of UV radiation actually enhance keratinocyte and macrophage ETAF/IL-1 production and release over normal values. Further, studies were carried out to establish the effects of UV radiation on products of the inflammatory response known to be stimulated in response to ETAF/IL-1. Finally, an as-

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Abbreviations: ETAF, epidermal cell thymocyte-activating factor; IL-1, interleukin 1; PMN, polymorphonuclear.

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say was developed to quantitate serum-associated ETAF/IL-1 and was used to demonstrate the presence of this mediator in the serum of UV radiation-exposed animals.

MATERIALS AND METHODS

Animals. C3H/HeN (MTV$^-$) were obtained from the National Cancer Institute animal production facility. All mice were housed at a maximum density of five animals per 7 × 11 inch cage and maintained on Wayne sterilizable Lab Blox and acidified water ad lib.

Generation of ETAF/IL-1 by Epidermal Cells. The transformed keratinocyte cell line PAM 212, derived originally from a newborn BALB/c mouse, was obtained from S. H. Yuspa (Laboratory of Experimental Pathology, National Institutes of Health, Bethesda, MD). Its characteristics and *in vitro* maintenance have been described (33). ETAF/IL-1 containing supernates from PAM 212 were generated by incubating 5×10^5 cells per ml in RPMI 1640 medium (M. A. Bioproducts, Walkersville, MD)/5% fetal calf serum (Sterile Systems, Logan, UT) for 72 hr.

Epidermal cells were obtained from normal and UV radiation-exposed animals and incubated for 24 hr at a density of 5×10^5 cells per ml in complete RPMI 1640 medium. All supernates were collected by centrifugation and exhaustively dialyzed at 4°C (Spectrapor Medical Industries; cut-off M_r 3,500) followed by membrane sterilization.

Thymocyte Proliferation Assay for ETAF/IL-1 Activity. Supernates were assayed for ETAF/IL-1 activity by their ability to enhance phytohemagglutinin (Sigma)-induced proliferation of murine thymocytes (enriched for peanut agglutinin-negative cells) using a procedure that has been described (19, 20). The amount of ETAF/IL-1 activity in a test supernate was quantitated by using a formula derived by Luger *et al.* (19).

UV Irradiation of Animals. The dorsal surfaces of all mice were shaved with electric clippers prior to exposure to UV radiation. Animals were exposed to wavelengths of radiation in the 280- to 320-nm range emitted by a bank of six Westinghouse FS40 fluorescent sun lamps. The energy emitted by these sun lamps was measured with a research radiometer (International Light) and found to average 2.8 (J/m^2)/sec. Animals were exposed to UV radiation for lengths of time giving the required energy in J/m^2.

Analysis of ETAF/IL-1 from Mouse Serum. Serum from individual mice was pooled and dialyzed. One-milliliter serum samples were placed in membrane tubing (Spectrapor) having a M_r cut-off of 50,000. This tubing was then placed in larger-diameter membrane tubing having a M_r cut-off of 3,500 and containing 4 ml of RPMI 1640 medium. Dialysis against two changes of 50 vol of phosphate-buffered saline at 4°C for a period of 24 hr was followed by dialysis in 50 vol of RPMI 1640 medium for 6 hr. The serum fraction containing molecules with a M_r greater than 3,500 and less than 50,000 was collected from the membrane tubing and filter sterilized prior to use in the thymocyte proliferation assay.

Assay for Acute-Phase Proteins. The concentration of plasma fibrinogen was determined by using techniques originally described by Ellis and Stransky (34) and Ratnoff and Menzie (35). Specific electroimmunoassays for serum amyloid P and serum complement component 3 have been described elsewhere in detail (36).

RESULTS

Effect of UV Radiation on ETAF/IL-1 Production: *In Vitro* Studies. In our initial experiments, we investigated the effects of UV radiation on the production of ETAF/IL-1 by keratinocytes, the major epidermal cell type. PAM 212, a transformed keratinocyte cell line, was used. This cell line has been useful in the biochemical characterization of ETAF/IL-1 because it constitutively produces this molecule (20). Prior to UV radiation exposure, PAM 212 was subcultured at 5×10^5 cells per ml in RPMI 1640 medium/5% fetal calf serum. Adherent cells were washed with phosphate-buffered saline and exposed to various doses of UV radiation. Cells were then incubated in RPMI 1640 medium/5% fetal calf serum at 37°C. Supernates collected 8 hr after irradiation contained equivalent amounts of ETAF/IL-1 activity (≈36 units/ml), and no reduction in cell viability from control values was detected. Fig. 1 presents the results obtained from the 8- to 24-hr time period. The highest UV radiation dose used (400 J/m^2) resulted in a dramatic decrease in secreted activity. This was paralleled by a large reduction in cell viability (remaining adherent cells). At lower doses of UV radiation, however, a different result was obtained. After exposure to 200 J/m^2, for example, the 24-hr cell viability was reduced to 28% of the control value, with no decrease in detectable ETAF/IL-1 activity. Whether the activity in UV radiation-exposed cultures is due to release of this mediator by UV radiation-damaged cells or is a result of dead cells releasing internal pools of ETAF/IL-1 could not be determined by this experiment.

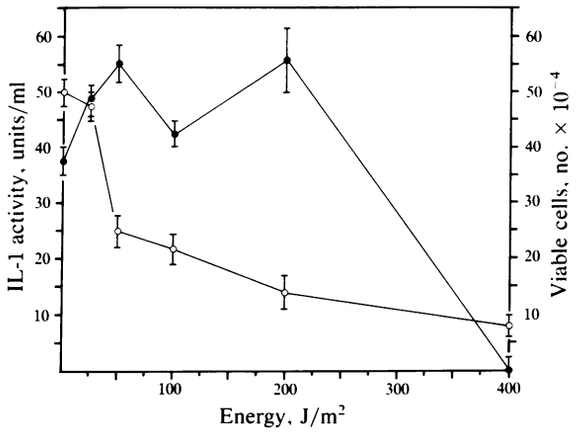

FIG. 1. ETAF/IL-1 secretion by PAM 212 keratinocytes after UV irradiation *in vitro*. Results represent ETAF/IL-1 activity (●) secreted by PAM 212 between 8 and 24 hr after UV irradiation. ETAF/IL-1 activity (mean ± SEM for triplicate cultures) was quantitated by comparing activity with a standard PAM 212 supernate given an arbitrary activity of 100 units/ml. Cell viability (○) was determined 24 hr after UV irradiation by trypan blue exclusion.

Overexposure to UV radiation results in an acute inflammatory response *in vivo*. With continued exposure to UV radiation a gradual decrease in the degree of inflammation is observed followed by a pronounced epidermal hyperplasia. We next analyzed the amount of ETAF/IL-1 produced by epidermal cells from chronically UV-irradiated and normal mice. When an equal number of epidermal cells from both normal and chronic UV-irradiated mice were incubated *in vitro* for 24 hr (Table 1), the supernates contained equivalent amounts of ETAF/IL-1 activity. Exposure to UV radiation *in vivo* results in a marked increase in the number of epidermal cells per unit area of skin rather than in the decrease in total cell number observed after UV radiation exposure of cells *in vitro* (12.7×10^3 cells per mm^2 in the epidermis of chronically UV-irradiated mice versus 1.23×10^3 cells per mm^2 in normal mice). Since UV radiation-exposed animals have a far greater number of epidermal cells at the exposed sites, their potential for ETAF/IL-1 secretion must be significantly enhanced.

UV Radiation Induction of Acute-Phase Reactants *in Vivo*. ETAF/IL-1 is known to stimulate a number of distinct cell types throughout the body (liver, brain, bone marrow, lymphocytes). Enhancement of the release of this mediator *in vivo* can therefore be analyzed by quantitating known sec-

Table 1. Detection of ETAF/IL-1 in supernates from cultured epidermal cells

	ETAF/IL-1 activity, units/ml		
	Exp. 1	Exp. 2	Exp. 3
Normal epidermal cells	81 ± 6	93 ± 10	83 ± 4
UV-exposed epidermal cells*	83 ± 5	129 ± 14	79 ± 2

ETAF/IL-1 activity in test supernates was quantitated by comparing activity with a standard PAM 212 supernate given an activity of 100 units/ml. Results represent mean ± SEM for triplicate cultures. Epidermal sheets from 117-mm^2 biopsy samples, taken from normal and chronically UV-irradiated mice, were exposed to a 1% DNase solution. Single cell suspensions of 5×10^5 cells per ml were incubated for 24 hr.

*Chronically UV-irradiated C3H/HeN mice were subjected to 30 min/day (5 days/week) of UV radiation from Westinghouse FS40 fluorescent sun lamps [2.8 (J/m^2)/sec] for 6 weeks.

ondary effects. We investigated whether UV radiation enhanced ETAF/IL-1 release by measuring changes in plasma fibrinogen, complement component 3, and serum amyloid P levels. The observed elevations in serum amyloid P, plasma fibrinogen, and serum complement component 3 after a single 3-hr UV radiation exposure are shown in Fig. 2. Minimal increases in plasma acute-phase reactant levels were found as little as 6 hr after UV radiation with maximal levels being achieved by 48–72 hr after UV radiation exposure.

Another known target for ETAF/IL-1 is the bone marrow, which is induced to release its neutrophil stores into the circulation in response to this mediator (5). Twenty-four hours after a single 3-hr exposure (30,000 J/m^2) to UV radiation, we observed a 5-fold increase in polymorphonuclear (PMN) cells in the peripheral blood. Normal nonirradiated mice averaged 1.5×10^6 ($\pm 3 \times 10^5$) PMN cells per ml of peripheral blood while the 3-hr UV radiation-exposed animals had 6.9×10^6 ($\pm 2 \times 10^5$) PMN cells per ml. At 48 hr after UV radiation exposure, the elevations in PMN levels had returned to normal. These results are in agreement with studies showing elevations in peripheral blood neutrophils within 8 hr of an inflammatory stimulus or following intravenous injection of IL-1 (5).

Observed elevations in acute-phase reactants and peripheral blood neutrophils are believed to result from the production of IL-1 at local sites of inflammation, which subsequently enters the circulation followed by the stimulation of appropriate targets. Therefore, we analyzed the serum from normal and 3-hr UV radiation-exposed mice for circulating levels of ETAF/IL-1. Serum from animals was obtained at various times after UV irradiation. After the dialysis procedure described in *Materials and Methods*, we tested the fraction of serum containing those molecules of M_r 3,500–50,000 for ETAF/IL-1 activity. Twenty-four hours after UV radiation exposure (Fig. 3), detectable levels of ETAF/IL-1 activity could be found in the serum. The highest concentration of ETAF/IL-1 activity was found 48 hr after UV irradiation and this value decreased thereafter. This direct correlation between UV radiation exposure, serum-associated ETAF/IL-1 levels, neutrophilia, and increases in acute-phase reactants was further substantiated by injecting the low molecular weight fraction (M_r 3,500–50,000) from 3-hr UV radiation-exposed animals into normal recipients (0.2 ml intravenously). An increase of 0.5 mg/ml of plasma fibrinogen over normal levels was observed after 24 hr (data not shown).

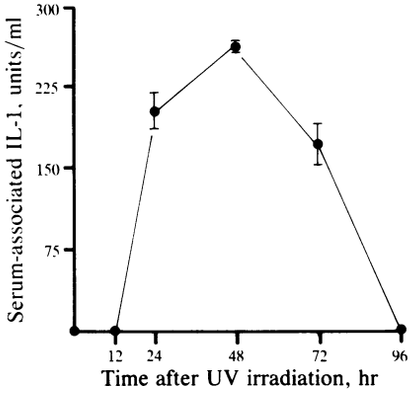

FIG. 3. Detection of serum-associated ETAF/IL-1 in animals after a single 3-hr exposure to UV radiation. The low molecular weight fraction of serum (M_r >3,500 but <50,000) from 3-hr UV radiation-exposed mice was tested for ETAF/IL-1 activity in a thymocyte proliferation assay. This fraction of serum had no mitogenic effect on thymocyte proliferation in the absence of phytohemagglutinin.

The majority of the research concerning the effects of UV radiation exposure on host immune responses has used chronically UV-irradiated animals. Groups of animals were exposed to a daily dose of 6,000 J/m^2 of UV radiation for 6 weeks. Plasma samples were taken at various times and analyzed for fibrinogen, complement component 3, and serum amyloid P. As shown in Fig. 4, acute-phase reactant levels peak by day 3 and then return to basal levels despite the maintenance of UV radiation exposure. These results were especially intriguing because we had previously shown that epidermal cells from chronically UV-irradiated animals remain fully capable of ETAF/IL-1 secretion. Quantitation of serum-associated ETAF/IL-1 in chronically UV radiation-exposed animals substantiated this finding. ETAF/IL-1 activity was detectable in the serum of UV radiation-exposed mice by day 3 (51 units/ml) and persisted throughout the assay period (63 units/ml at day 42). Therefore, the acute-phase reactant levels in UV radiation-exposed animals return to normal levels in spite of the presence of detectable levels of serum-associated ETAF/IL-1. Possible explanations for this observation are presented below.

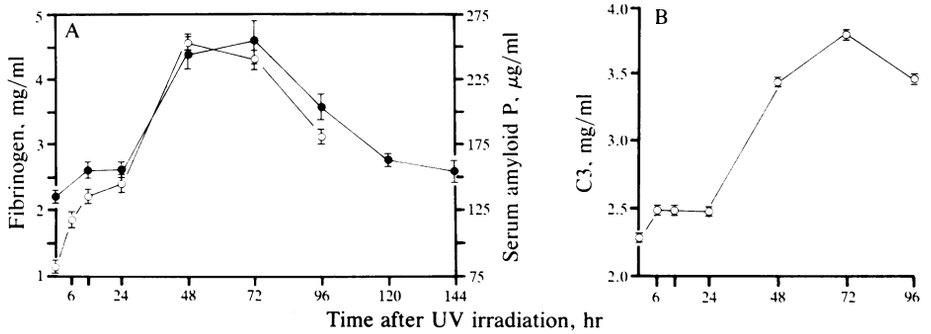

FIG. 2. Elevation in acute-phase reactants after a single 3-hr exposure to UV radiation. (*A*) Plasma fibrinogen (●) and serum amyloid P (○). (*B*) Serum complement component 3 (C3) (○). The levels of these acute-phase proteins in different groups of five animals were measured at various times after UV irradiation.

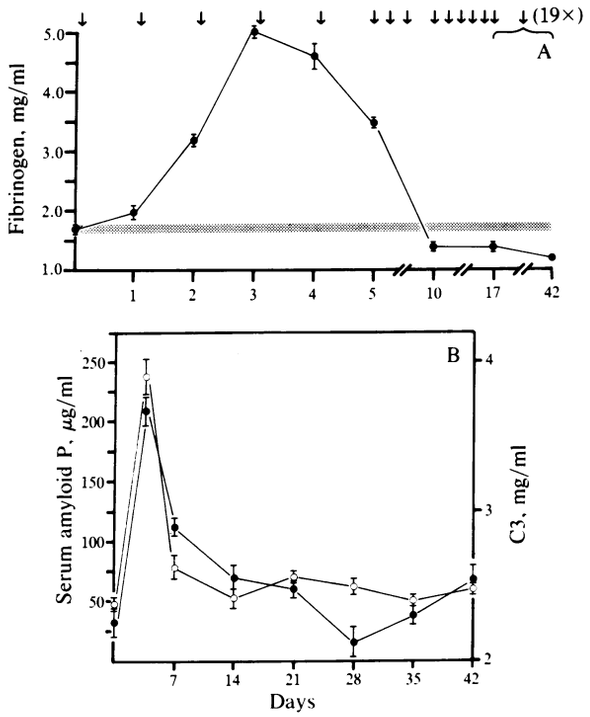

FIG. 4. Elevation in acute-phase reactants after multiple exposures of UV radiation. Animals were exposed to 30 min of UV radiation per day for 6 weeks. (*A*) Plasma fibrinogen levels were measured. The 30-min UV radiation exposures are represented as arrows while fibrinogen levels in normal animals are shown as a bar (▒). (*B*) Serum amyloid P (○) and serum complement component 3 (C3; ●) concentrations in animals receiving 30 min of UV radiation per day over a period of 6 weeks. The serum-associated ETAF/IL-1 activity (in units/ml) was 51 ± 11 at day 3, 100 ± 20 at day 28, and 63 ± 7 at day 42.

DISCUSSION

Our results support the hypothesis that UV radiation exposure of animals is capable of altering a number of physiological processes through its ability to enhance the production of the endogenous mediator ETAF/IL-1. This is contrary to reports in which conclusions are based on UV radiation-induced reductions in ETAF/IL-1 levels from *in vitro* experiments (37, 38). Exposure of the keratinocyte cell line (PAM 212) to a range of doses of UV radiation *in vitro* showed a marked effect on cell viability, with depression in ETAF/IL-1 activity being observed only at the highest and most cytotoxic doses used. These results are in agreement with data recently reported by Stingl *et al.* (39), which showed that exposure to low doses of broad-spectrum UV radiation does not impair the ability of normal epidermal cells to secrete ETAF/IL-1 over the first 48 hr after exposure. Sauder *et al.* (40) have shown that narrow-band UV radiation exposure induced a decrease in ETAF/IL-1 production by normal epidermal cells. Differences in the light sources used for irradiation may explain the discrepancy with our results. Our comparative analysis of ETAF/IL-1 activity derived from epidermal cell cultures of normal and chronically UV radiation-exposed skin showed that, on a per cell basis, both possessed equivalent potentials to secrete ETAF/IL-1 *in vitro*. Taken in the context of a significant epidermal hyperplasia present in skin from UV radiation-exposed mice, we conclude that an enhanced production of this mediator may be due to induced increases in the number of ETAF/IL-1-producing cells plus involvement of infiltrating cell types. Our data do not rule out, however, the possibility that cellular perturbations by UV radiation itself may enhance production of ETAF/IL-1 by individual cells in a dose-dependent manner.

Whether keratinocytes, or other resident cell types within the skin, are stimulated to enhanced ETAF/IL-1 production after UV radiation exposure is academic in the context of the whole organism. The essential issue relates to whether ETAF/IL-1 levels are increased in UV radiation-exposed animals and what biological implications these changes might imply. We were intrigued by the observation by Noonan *et al.* (28) that animals exposed to single, high doses of UV radiation develop a systemic depression in their response to contact sensitization through nonirradiated sites over a 3- to 5-day period. With no formal proof, those authors conclude their phenomenon to be due to an "antigen presenting cell (APC) defect." This is consistent with similar conclusions made by others using a different system (41). It has recently been reported that unirradiated skin from high-dose UV radiation-exposed animals has normal Langerhans cell density and function (42) and, further, that the antigen-presenting cell defect described to be present in the spleens of UV radiation-exposed mice is due to the migration of such a cell from this central lymphoid compartment to peripheral sites (29). Collectively these findings leave the issue unresolved as to why high-dose UV radiation-exposed animals exhibit a systemic unresponsiveness to contact-sensitizing agents.

Our results provide possible insight into the mechanisms that underlie this change in immunologic potential. A quantitative kinetic analysis of plasma fibrinogen, serum amyloid P, and serum complement component 3 levels shows that high-dose UV radiation exposure causes an elevation in the liver production of acute-phase reactants. Observed elevations in peripheral blood neutrophils suggests that one other ETAF/IL-1-sensitive target organ is being similarly affected by this treatment. Elevations in acute-phase reactants in response to inflammatory insults have been hypothesized to impart an immunosuppressed environment, a condition that may be beneficial after extensive tissue injury to inhibit the development of autoimmune reactions (43, 44). While the cause–effect relationships between ETAF/IL-1 and its diverse range of *in vivo* and *in vitro* biologic activities have been studied by many investigators, our finding of serum-associated ETAF/IL-1 after UV radiation exposure provides additional support for our hypothesis.

The majority of reports that describe the immunologic changes taking place subsequent to UV radiation exposure have been based on studies using fractionated and chronic UV radiation treatment protocols (22, 24). The induction of a tumor-susceptible state, and UV radiation-induced tumors themselves (23, 24), the generation of suppressor T-cell populations (25), and the observed changes in lymphocyte recirculation patterns (27) all use a near-physiological exposure regimen to induce the effects. Our data indicate that mice exposed to UV radiation on a chronic basis exhibit an increase in circulating acute-phase reactant levels that relaxes with time, in spite of continued stimulation. This is in full agreement with the work of other investigators who describe a "desensitization" in animals undergoing a chronic exposure to exogenous chemical inducers of ETAF/IL-1 (44). Of importance, however, is our finding that circulating levels of serum-associated ETAF/IL-1 are present in these chronically UV radiation-exposed animals. This suggests that "desensitization" may be taking place at the level of the target cell (liver, bone marrow, or brain) and results from an impaired or modified capacity to perceive the endogenous signal. Whether this is due to a down regulation of target cell surface receptor density, a depression of receptor affinity for ligand, or an elevation in specific inhibitors of ETAF/IL-1 activity awaits further study.

The coupling of our understanding as to how polypeptide

hormones are regulated *in situ* (45–47) to our knowledge of the mechanisms that underly the action of ETAF/IL-1 allows us to make predictions concerning the immunoregulatory effects mediated by chronic exposure to UV radiation. Under conditions in which the sensitivity of a target cell (helper T-lymphocyte) to stimulation by ETAF/IL-1 has been depressed or the essential interaction between target and ligand is physically suppressed, necessary second signals for effective immune function would be unavailable. This would be especially evident in situations involving low-grade antigenic stimulation, reflected by minimal elevations in ETAF/IL-1 levels. Tumors induced by UV radiation, where the majority are immunologically rejected by normal syngeneic hosts (23), may be given an immunologic advantage when placed in such an environment. The experimental evidence reported here, without detracting from the suppressor T-cell-mediated events known to exist in UV radiation-exposed animals (48), is quite supportive of such a concept.

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1. Dinarello, C. A. & Wolff, S. M. (1982) *Am. J. Med.* **72,** 799–819.
2. Bornstein, D. L. (1982) *Ann. N.Y. Acad. Sci.* **389,** 323–337.
3. Bornstein, D. L. & Walsh, E. C. (1978) *J. Lab. Clin. Med.* **91,** 236–245.
4. Maizel, A. L., Mehta, R. J., Ford, R. J. & Lachman, L. B. (1981) *J. Exp. Med.* **153,** 470–475.
5. Kampschmidt, R. F., Long, R. D. & Upchurch, H. F. (1972) *Proc. Soc. Exp. Biol. Med.* **139,** 1224–1226.
6. Beeson, P. B. (1948) *J. Clin. Invest.* **27,** 524 (abstr.).
7. Kampschmidt, R. F., Pullman, L. A. & Upchurch, H. F. (1980) *J. Lab. Clin. Med.* **95,** 616–623.
8. Sztein, M. B., Vogel, S. N., Sipe, J. D., Oppenheim, J. J. & Rosenstreich, D. L. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40,** 1161 (abstr.).
9. Sztein, M. B., Vogel, S. N., Sipe, J. D., Murphy, P. A., Mizel, S. B., Oppenheim, J. J. & Rosenstreich, D. (1981) *Cell. Immunol.* **63,** 164–176.
10. Dayer, J., Passwell, J. H., Schneeberger, E. E. & Krane, S. M. (1980) *J. Immunol.* **124,** 1712–1720.
11. Dayer, J., Krane, S. M., Russell, R. G. G. & Robinson, D. R. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 945–949.
12. Sipe, J. D., Vogel, S. N., Ryan, J. L., McAdam, K. P. W. J. & Rosenstreich, D. L. (1979) *J. Exp. Med.* **150,** 597–606.
13. Mizel, S. B., Dayer, J., Krane, S. M. & Mergenhagen, S. E. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2474–2477.
14. Postlewaite, A. E., Lachman, L. B., Mainardi, C. L. & Kang, A. H. (1983) *J. Exp. Med.* **157,** 801–806.
15. Kaiser, H. K. & Wood, W. B. (1962) *J. Exp. Med.* **115,** 27–47.
16. Klempner, M. S., Dinarello, C. A. & Gallin, J. I. (1978) *J. Clin. Invest.* **61,** 1330–1336.
17. Mizel, S. B. (1982) *Immunol. Rev.* **63,** 51–72.
18. Sauder, D. N., Carter, C. S., Katz, S. I. & Oppenheim, J. J. (1982) *J. Invest. Dermatol.* **79,** 34–39.
19. Luger, T. A., Stadler, B. M., Katz, S. I. & Oppenheim, J. J. (1981) *J. Immunol.* **127,** 1493–1498.
20. Luger, T. A., Stadler, B. M., Luger, B. M., Mathieson, B. J., Mage, M., Schmidt, J. A. & Oppenheim, J. J. (1982) *J. Immunol.* **128,** 2147–2152.
21. Hawk, J. L. M. & Parrish, J. A. (1982) in *The Science of Photomedicine*, eds. Regan, J. D. & Parrish, J. A. (Plenum, New York), pp. 219–260.
22. Gurish, M. F., Roberts, L. K., Krueger, G. G. & Daynes, R. A. (1981) *J. Invest. Dermatol.* **76,** 246–251.
23. Roberts, L. K., Spellman, C. W. & Daynes, R. A. (1980) *J. Immunol.* **125,** 663–672.
24. Daynes, R. A. & Spellman, C. W. (1977) *Cell. Immunol.* **31,** 182–187.
25. Spellman, C. W. & Daynes, R. A. (1978) *Cell. Immunol.* **36,** 383–387.
26. Lynch, D. H., Gurish, M. F. & Daynes, R. A. (1981) *J. Immunol.* **126,** 1892–1897.
27. Spangrude, G. J., Bernhard, E. J., Ajioka, R. S. & Daynes, R. A. (1983) *J. Immunol.* **130,** 2974–2981.
28. Noonan, R. P., Kripke, M. L., Pedersen, G. M. & Greene, M. I. (1981) *Immunology* **43,** 527–533.
29. Gurish, M. F., Lynch, D. H. & Daynes, R. A. (1982) *Transplantation* **33,** 280–284.
30. Daynes, R. A. & Gahring, L. C. (1983) *J. Invest. Dermatol.* **80,** 329 (abstr.).
31. Ansel, J. C., Luger, T. A. & Green, I. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42,** 961 (abstr.).
32. Ansel, J. C., Luger, T. A. & Green, I. (1983) *J. Invest. Dermatol.* **81,** 519–523.
33. Yuspa, S. H., Hawley-Nelson, P., Koehler, B. & Stanley, J. R. (1980) *Cancer Res.* **40,** 4694–4703.
34. Ellis, B. C. & Stransky, A. (1961) *J. Lab. Clin. Med.* **58,** 477–488.
35. Ratnoff, O. D. & Menzie, C. (1950) *J. Lab. Clin. Med.* **37,** 316–320.
36. Pepys, M. B., Baltz, M. L., Mussallam, R. & Doenhoff, M. J. (1980) *Immunology* **39,** 249–254.
37. Sauder, D. N., Noonan, F. B., DeFabo, E. C. & Katz, S. I. (1981) *Clin. Res.* **30,** 607.
38. Jakway, J. P., Sauder, D. N. & Shevach, E. M. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41,** 369 (abstr.).
39. Stingl, L. A., Sauder, D. N., Iijima, M., Wolff, K., Pehamberger, H. & Stingl, G. (1983) *J. Immunol.* **130,** 1586–1591.
40. Sauder, D. N., Noonan, F. P., DeFabo, E. C. & Katz, S. I. (1983) *J. Invest. Dermatol.* **80,** 485–489.
41. DeFabo, E. C. & Kripke, M. L. (1979) *Photochem. Photobiol.* **30,** 385–390.
42. Lynch, D. H., Gurish, M. F. & Daynes, R. A. (1983) *J. Invest. Dermatol.* **81,** 336–341.
43. Sipe, J. D. & Rosenstreich, D. L. (1981) *Cellular Functions in Immunity and Inflammation* (Elsevier/North-Holland, New York), pp. 411–429.
44. Macintyre, S. S., Schultz, D. & Kushner, I. (1982) *Ann. N.Y. Acad. Sci.* **389,** 76–87.
45. Knauer, D. J. & Cunningham, D. D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2310–2314.
46. Knauer, D. J., Wagner, F. W. & Smith, G. L. (1981) *J. Supramol. Struct. Cell. Biochem.* **15,** 177–191.
47. Kaplan, J. (1981) *Science* **212,** 14–20.
48. Daynes, R. A., Schmitt, M. K., Roberts, L. K. & Spellman, C. W. (1979) *J. Immunol.* **122,** 2458–2464.