EFFECTS OF CRUDE OIL AND ULTRAVIOLET RADIATION ON IMMUNITY WITHIN MOUSE SKIN

Kim Burnham
and
Marcie Bey
Department of Botany and Microbiology
Oklahoma State University

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ABSTRACT

Previous studies indicate that crude oil leads to increased pigmentation and erythema (sunburn) in response to sunlight in exposed individuals. However, no information is currently available concerning whether crude oil exposure might enhance the immunosuppressive effects of solar ultraviolet radiation (UVR) on the skin. In order to address this question, the back skin of shaved, female C3H/HeN mice was exposed to crude oil with or without subsequent treatment with medium wave (UVB) (200 J/M^2) or long wave (UVA) (20,000 J/M^2) UVR. Immune function was assessed in treated mice by measuring their ability to mount contact hypersensitivity responses to a hapten (2,4,-dinitro-1-fluorobenzene) (DNFB) applied to the site of crude oil, and UVR treatment as determined by ear swelling upon subsequent challenge. Since Langerhans cells represent an important component of immunity within the skin, and because suppression of contact hypersensitivity following UVR treatment is often accompanied by the disappearance of Langerhans cells from the epidermis, the impact of these agents on epidermal Langerhans cell density was also analyzed. This was accomplished by enumerating IA positive cells within the epidermis of treated skin. In these studies, crude oil alone induced a mild inhibition of contact hypersensitivity but had no effect on epidermal Langerhans cells. In contrast, combined treatment with crude oil and UVA led to depletion of epidermal Langerhans cells accompanied by marked suppression of contact hypersensitivity responses.

INTRODUCTION

Mammalian skin contains all of the components necessary for the elicitation, performance, and regulation of immune responses. Collectively these components have been named skin associated lymphoid tissue (SALT) (Streilein, 1978). Specialized antigen presenting cells required to assist in the recognition of antigen by lymphocytes constitute an important part of the SALT system (Stingl et al., 1978). These specialized cells, termed Langerhans cells, are found only in the skin.

Recent studies indicate that Langerhans cells are quite sensitive to wavelengths of ultraviolet radiation (UVR) contained within normal sunlight (Toews et al., 1980). Virtually all of the biological effects of sunlight on the skin of higher vertebrates are mediated by radiation that falls within the ultraviolet region of the solar spectrum. For the sake of convenience, ultraviolet radiation has been divided into three sub-regions (Coblentz, 1932). These include longwave (UVA) (greater than 320 nm), mediumwave (UVB) (280-320 nm) and shortwave (UVC) (less than 280 nm). Of these regions, only UVB and UVC have significant effects on skin under physiological conditions. However, UVC is of little natural importance at this time since the majority of UVR within this region is absorbed from sunlight by the ozone layer. Physiological doses of UVA are capable of causing alterations in the skin only in the presence of photosensitizing chemicals (discussed below) (Santamaria et al., 1966, 1980). In contrast, exposure of skin to UVB results in an array of effects including enhanced vitamin D synthesis, inflammation (sunburn), increased pigmentation, and epidermal cell division (hyperplasia) associated with aging of the skin. UVB treatment also induces skin cancer in rodents and appears to play an important role in the development of non-melanoma skin cancers in humans (Blum, 1959, Urbach et al., 1974).

One of the most interesting set of results to come out of studies involving UVB treatment of rodents is that which pertains to the effect of this physical agent on immune responses in the skin. Relatively low doses of UVB ($400J/M^2$) are capable of suppressing contact hypersensitivity to haptens applied to the UVB exposed site (Toews et al., 1980), whereas larger doses ($30,000J/M^2$) suppress contact hypersensitivity to haptens applied at distant as well as UVB treated sites (Lynch et al., 1983). Results from previous studies indicate1 that the loss of Langerhans cells from the epidermis as well as the local release of prostaglandins are involved in initiating the local suppression observed following exposure of mice to low doses of UVB (Toews et al., 1980, Chung et al., 1986). Interesting corollaries to these studies are those which indicate that UVB plays at least

two separate roles in the induction of skin cancer in rodents. In addition to its role as a mutagen, UVB also appears to allow the growth and survival of arising tumors within hosts due to down regulation by UVB of potential tumor directed immune responses (Kripke et al., 1984, Daynes et al., 1985).

A variety of industrial and pharmaceutical chemicals are able to enhance the effect of UVR on human or animal skin (reviewed by Magnus, 1976). This type of photosensitization has been observed following both ingestion as well as dermal exposure. Classically, photosensitization is demonstrated as a sunburn type of reaction following combined treatment of subjects with potential photosensitizers and longwave UVR (UVA). Petroleum compounds have been observed to photosensitize humans to sunlight as detected by enhanced sunburn (erythema) and pigmentation (WHO, 1982). The chemicals responsible for this phenomenon have not been elucidated yet, nor have the wavelengths of light involved. Furthermore, no information is available concerning whether photosensitization with petrochemicals impacts on local immunity within the skin.

Recently, a greater appreciation has been generated for the impact of complex mixtures on human and animal health. This has come about due to a realization that such a mixture is "more than the sum of its parts" in terms of its biological effects (Politzer et al., 1985). This notion is supported by synergisms and antagonisms observed between specific petrochemicals in the induction of cancer in rodents (reviewed by Politzer et al., 1985). Assessment of the biological impact of petroleum is further complicated by potential interactions of petrochemicals with other aspects of the environment, such as sunlight. As stated above, such interaction does, in fact, occur. In addition to photosensitization, which requires UVR exposure of animals that have ingested or absorbed chemicals, evidence exists that light can also directly enhance the toxicity of petrochemicals in the environment by inducing chemical changes within these substances (Ross and Crosby, 1985).

Since petrochemicals are capable of enhancing the inflammatory and pigmentation inducing effects of sunlight on the skin of exposed individuals, it is important to know whether these substances also enhance the capacity of UVR to inhibit local immune responses. This is important since impaired immunity in the skin could result in increased susceptibility of exposed individuals to infections or cancer at exposed sites.

In this study, the effects of crude oil and UVR on contact hypersensitivity and epidermal Langerhans cells were analyzed. The results of these studies indicate that crude oil induces low levels of suppression independent of noticeable effects on Langerhans cell density, while combined treatment of mice with crude oil and UVA leads to depletion of Langerhans cells accompanied by enhanced suppression of contact hypersensitivity.

METHODS

Animals
Female C3H/HeN mice were obtained from Charles River (Wilmington, MA) and maintained in a licensed facility. Mice were 6 to 8 weeks old at the initiation of experiments.

Reagents
Crude oil was obtained from a well on the Covington lease in Jack County, Texas, in June of 1989 and stored at 4°C. The hapten, 2,4-dinitro-1-fluorobenzene (DNFB), was obtained from Sigma (St. Louis, MO). IAk specific monoclonal antibody consisted of supernatant from cultured 26-7-11S hybridoma cells obtained from the American Type Culture Collection (Rockville, MD). This antibody is specific for the private specificity IA2 and exhibits no known cross-reactivity with other MHC determinants.

UVR Treatment
Mice were shaved and the hair removed with a commercial depilatory reagent (Neet) prior to UVR exposure. Mice treated with UVB were immobilized in a holder wrapped with black electrician's tape to prevent exposure of the ears. (Neither application nor removal of tape effects the responsiveness of ear skin to contact hypersensitivity (results not shown)). Mice treated with UVA each received an I.P. injection containing 2 mg of Ketamine hydrochloride (Bristol Laboratories, Syracuse, NY) prior to treatment, and their ears were covered with electrician's tape. For UVB treatment, a bank of two 15-watt lamps, which emit monochromatic UVR at 302 nm, was used. Mice received 6.5 $J/M^2/sec$ of UVB at a tube-to-target distance of 63 cm. The total dose of UVB received was 200 $J/M^2/sec$. For UVA treatment, a bank of two lamps, which emit monochromatic UVR at 302nm, was used. Mice received 11 $J/M^2/sec$ of UVA at a tube to target distance of 35 cm. The total dose of UVA received was 20,000 J/M^2.

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Sensitization and Challenge

Mice were sensitized on the shaved back with 25 υl of 0.5% DNFB in a vehicle of 4:1 acetone/olive oil immediately following crude oil and/or UVR treatment and again 24 hours later. Subjects were challenged 5 days following the second sensitization by application of 10 υl of 0.2% DNFB in 4:1 acetone/olive oil to the right ear. Controls consisted of mice that received sensitization and challenge (but no other treatment) and those that received only a challenge (as a measure of non-specific swelling). Ear swelling was measured with the aid of an engineer's micrometer (MSC, Plainview, NY) at daily intervals following challenge. Ear thickness measurements were made under ether anesthesia, and each ear was measured at three different locations. Ear swelling was determined for each individual mouse by subtracting the thickness of the left ear from that of the right ear. Ear swelling for each group was reported as the mean difference in swelling plus or minus the standard error of the mean.

## Evaluation of IAk Positive Epidermal Cells

Mice were sacrificed with ether and the back skin removed. Subcutaneous tissue was scraped away from the dermis with the aid of a scalpel. Resected skin was subsequently cut into pieces of approximately 1 cm^2 in area and incubated, dermal side down, in an isotonic solution containing 26 mM EDTA (Sigma) in phosphate buffered saline (PBS). After 3 hours at 37°C, epidermal sheets were removed from the dermis with forceps, fixed in acetone, and rehydrated with PBS. The epidermal sheets were subsequently incubated in supernatant containing IAk specific monoclonal antibody or tissue culture medium (as a negative control). Tissue bound antibody was assessed by an indirect immunoperoxidase method employing a universal mouse specific peroxidase-anti-peroxidase kit (Accurate Chemical and Scientific Corp., Westbury, NY) with 3-amino-9-ethylcarbazole as the developing substrate. Stained sections were mounted on slides in glycerol and examined by light microscopy. The number of IA positive Langerhans cells was determined by randomly counting 20 fields at 400 X with an ocular grid of known area. From these values the mean number of Langerhans cells/mm^2 of tissue was calculated.

RESULTS

Effects of Crude Oil and UVB

The class of chemicals in crude oil that is most likely to exhibit photosensitizing capacity consists of the polynuclear aromatic hydrocarbons (PAHs). These substances all absorb in the UVB range *in vitro*. Therefore, we were interested in whether crude oil would alter the effect of UVB on immunity within the skin. Before addressing this question, it was important to know if crude oil alone had an effect on immunity within the skin and to determine whether the dose of UVB selected would induce a mild suppression as expected. (The dose of UVB used (200 J/M^2) was one half that employed to generate optimal local suppression in previous studies (Chung et al., 1986).) In order to accomplish these goals, female C3H/HeN mice were shaved and the back hair removed with a commercial depilatory reagent (Neet). Groups of mice received either UVB or crude oil treatments on four consecutive days. On the fourth day, mice were sensitized following their final treatment with crude oil or UVB by application of 2,4,-dinitro-1-fluorobenzene (DNFB). Mice were re-sensitized on the following day and then challenged on the right ear with DNFB five days after the second sensitization. Control groups included mice that were sensitized and challenged but otherwise untreated (Positive C) and mice that were only ear challenged (Negative C). Ear swelling was determined by measurement with an engineer's micrometer at 24 and 48 hour intervals. The bars in Figure 1 represent mean ear swelling for groups of 5 mice plus or minus the standard error of the mean. As shown in Figure 1, the dose of UVB employed resulted in a partial suppression of contact hypersensitivity as predicted. Interestingly, crude oil application also resulted in suppression, although to a lesser extent.

Figure 1
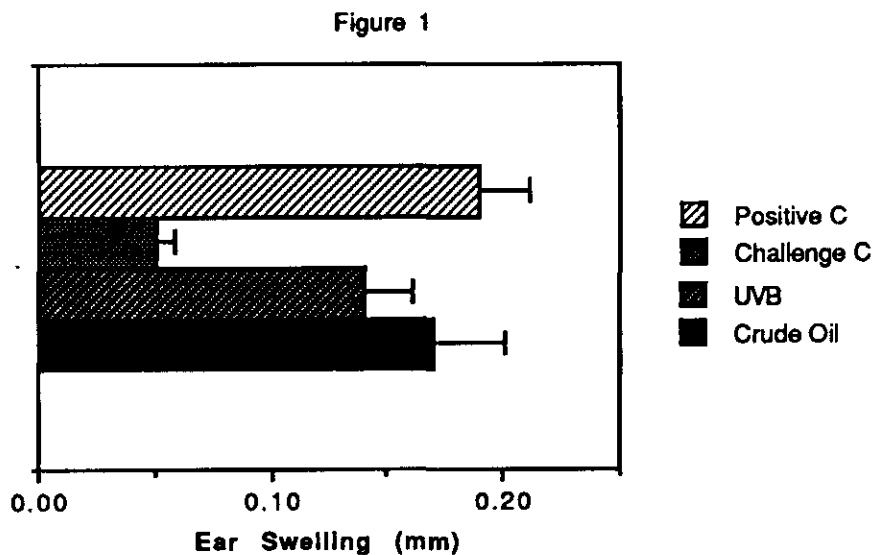
A similar experiment was performed to determine whether crude oil and UVB might exhibit synergy in their effects on contact hypersensitivity. In this experiment, an additional group of mice received both crude oil and UVB on four consecutive days. In this case, UVB was always administered 2 hours following crude oil application. Ear swelling was assessed at 24, 48, and 72 hours following challenge. The results of 24 hour measurements are depicted in Figure 2. As shown, UVB and crude oil suppressed contact hypersensitivity as observed previously. However, no synergy between these two agents (or even an additive effect) was observed. Forty-eight and 72 hour measurements were consistent with those obtained at 24 hours (Figure 3).
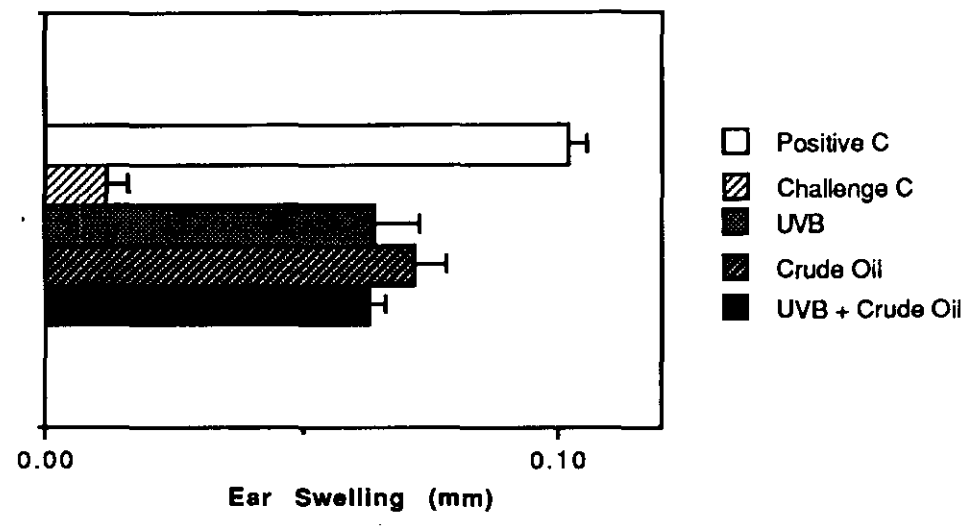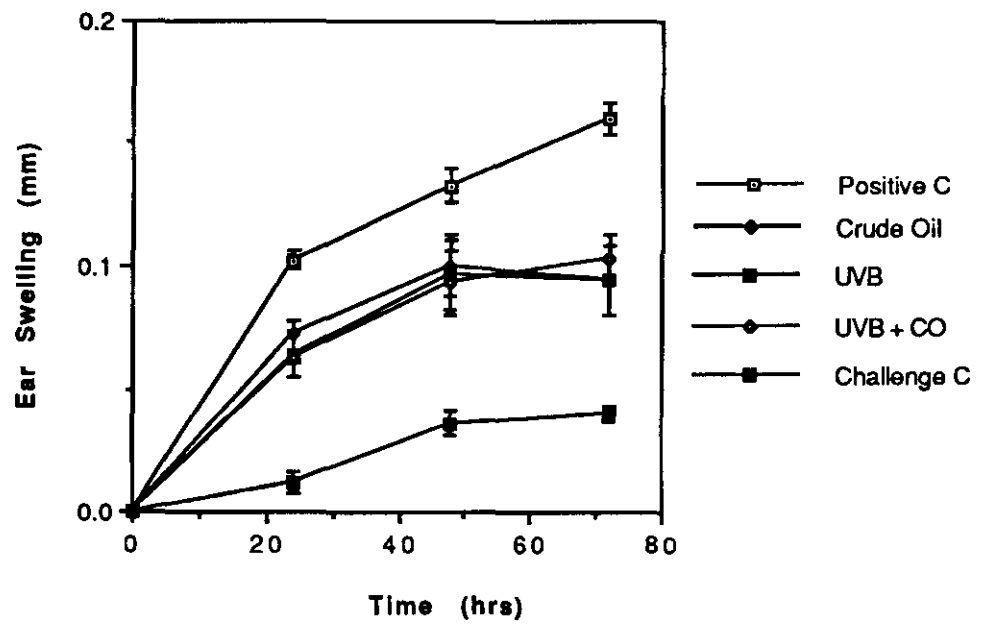
Figure 2

Figure 3

Effects of Crude Oil and UVA

It has been reported that methoxypsoralen absorbs maximally in the UVB range *in vitro*. However, this substance photosensitizes mice to the effects of UVA *in vivo* (Santamaria, 1980). Therefore, the combined effect of crude oil and UVA on contact hypersensitivity was examined. These experiments were performed as previously described, except that UVA was employed (20,000 J/M^2) in the place of UVB, and the dose of crude oil used was increased from 10 to 25 ʋl per mouse per treatment. In addition, three daily treatments were used rather than four. The effects of crude oil and UVA on contact hypersensitivity are depicted in Figure 4. As expected, crude oil induced a mild, but statistically significant, suppression of contact hypersensitivity. However, combined treatment with UVA yielded a greater level of suppression, which was equivalent to that optimally observed in previous studies employing 400 J/M^2 of UVB (Chung et al., 1986).

Since suppression of contact hypersensitivity by UVR often correlates with depletion of Langerhans cells from the epidermis, we examined the effect of crude oil and UVA on the density of these cells in skin from exposed mice. Mice were treated in a fashion identical to the way those in the previous experiment were treated prior to sensitization. Twenty four hours following the last treatment with crude oil and/or UVA, mice were sacrificed and the exposed skin removed. Epidermal sheets were obtained from these skin samples and stained specifically for IAk by an indirect immunoperoxidase
method. The number of Langerhans cells per unit area was subsequently determined with the aid of a microscope. As shown in Table 1, no depletion of Langerhans cells resulted from treatment with crude oil or UVA alone. However, combined treatment with these two agents led to a marked decrease in IAk positive cells. From these results, it appears that the mild suppression of contact hypersensitivity mediated by crude oil is independent of Langerhans cell density, while the increased level of suppression induced by crude oil and UVA correlates with epidermal Langerhans cell depletion.
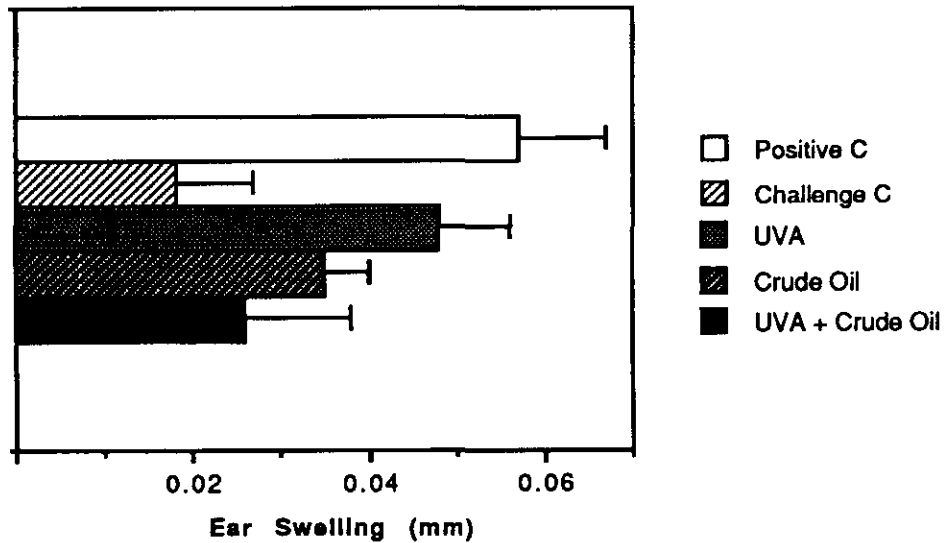
Figure 4

Ear Swelling (mm)

Legend:
- □ Positive C
- ▨ Challenge C
- ▩ UVA
- ▨ Crude Oil
- ■ UVA + Crude Oil

TABLE 1. Effect of UVA and Crude Oil on Langerhans Cell Density

Group	LC/mm^2 + SEM
Control	950 + 210
UVA	1300 + 260
Crude Oil	1000 + 220
UVA + Crude Oil	150 + 80

Discussion

Previous studies have demonstrated photosensitization of individuals by petroleum to the inflammatory (sunburning) and pigment inducing (tanning) effects of sunlight (WHO, 1982). However, to our knowledge, no previous studies have examined the potential effects of combined exposure to petroleum and solar UVR on immunity within mammalian skin. In contrast, much is known about the effects of UVB and UVA on immunity within cutaneous tissues. Low doses of UVB (in the physiological range) are known to induce suppression of contact hypersensitivity responses in rodents that is restricted to the site of exposure (Toews, 1980), while high doses of this physical agent lead to systemic suppression (Lynch et al., 1983). In contrast, UVA alone (in physiological doses) has no inhibitory effect on contact hypersensitivity unless given in combination with a photosensitizing chemical such as methoxypsoralen (Kripke et al., 1983). The local suppression of contact hypersensitivity responses by UVR correlates with the disappearance of Langerhans cells from exposed epidermis (Toews et al., 1980). In addition to the role of Langerhans cell depletion, prostaglandins also appear to play a role in the local suppression of contact hypersensitivity by UVB (Chung et al., 1986), and increased release of archidonic acid (a metabolic precursor of prostaglandins) is observed among mammalian cells exposed to UVB in culture (DeLeo et al., 1985).

Although the induction of suppression following UVR treatment of skin appears to involve effects on Langerhans cells and prostaglandin production, the maintenance of suppression appears to involve suppressor T cells (Bergstresser et al., 1985). In addition to the effects on lymphoid tissues listed above, UVR has also been observed to cause changes in the membrane fluidity, Fc receptor expression, and migration of mononuclear phagocytes (Krutmann et al., 1988, Gurish et al., 1982).

In this study, crude oil treatment led to a mild, yet consistent, inhibition of contact hypersensitivity in the absence of effects on epidermal Langerhans cell density. It is possible that crude oil inhibits contact hypersensitivity responses by inducing local prostaglandin release within the skin similar to that observed following UVB exposure. We are presently examining the role of prostaglandins in crude oil induced inhibition of contact hypersensitivity. Although Langerhans cell depletion correlated with the suppression induced following combined treatment of mice with crude oil and UVA, it is possible that prostaglandin production could also contribute to the suppression observed in response to these agents. Due to this fact, we are also investigating the potential role of prostaglandins in mice treated with crude oil and UVA.

Although crude oil presumably enhanced the effect of UVA on Langerhans cell density and contact hypersensitivity in this study, the reverse is also possible. That is, UVA could have potentiated the suppressive action of crude oil. Visually detectable crude oil disappeared from treated mice fairly rapidly, such that noticeable discoloration was gone within one hour. This disappearance was not due to licking by mice of their own treated skin nor by their companions. (No such activity was detected upon prolonged observation, nor did individual housing of mice reduce the rate of disappearance of crude oil on the skin). In contrast to mice that received crude oil alone, discoloration persisted for longer periods of time on mice that received both crude oil and UVA. Therefore, it is possible that UVA treatment could have potentiated the effects of crude oil on contact hypersensitivity by inhibiting clearance from the skin. Nevertheless, Langerhans cell depletion following combined treatment with crude oil and UVA was most likely due to photosensitization of UVA mediated effects by crude oil, since this agent had no discernable inhibitory effects on epidermal Langerhans cells in the absence of UVA.

Petroleum contains many potentially toxic chemicals. The majority of these, however, are contained in the polynuclear hydrocarbon (PAH) class of compounds. This group of chemicals contains numerous carcinogens and tumor promoters (reviewed by Politzer et al., 1985). Interestingly, topical application of one such carcinogen (dimethylbenzanthracene) resulted in epidermal Langerhans cell depletion (Odling et al., 1987). Rodent studies support the concept that immunosuppression mediated by UVB and UVA plus methoxypsoralen (a photosensitizer) contributes to the development of skin cancer in chronically exposed subjects (Kripke et al., 1984, Daynes et al., 1985). It is highly possible that the immunosuppressive effects of combined exposure to petrochemicals and solar UVA might prevent the destruction of cells transformed by carcinogenic PAHs, and thereby increase the risk of cancer.

Carcinogenesis, as well as other adverse effects of environmental contaminants, often involve multiple steps mediated by a combination of environmental insults. The results of this study indicate that combined exposure to crude oil and sunlight can lead to profound inhibition of cell mediated immunity within the skin. Consequently, the combined action of these two agents could significantly increase the risk of microbial infections and cancer within the skin of exposed individuals.

BIBLIOGRAPHY

Bergstresser, P.R, C.A. Elmets, and J.W. Streilein. Local effects of ultraviolet radiation on immune function in mice. In *The Effects of Ultraviolet Radiation on the Immune System.* pp. 73-86, Brunswick, N.J.: Johnson and Johnson Baby Products Co., 1983.

Blum, H.F. *Carcinogenesis by Ultraviolet Light.* Princeton, N.J.: Princeton University Press, 1959.

Chung, H.T., D.K. Burnham, B. Robertson, L.K. Roberts, and R.A. Daynes. Involvement of prostaglandins in the immune alterations caused by the exposure of mice to ultraviolet radiation. *J. Immunol.* 137:2478-2483, 1986.

Coblentz, W.W. 1932. The biologically active component of ultraviolet in sunlight and daylight. *Transactions of the Illuminating Engineering Society* 26:572-577, 1932.

Daynes, R.A., D.K. Burnham, C.W Dewitt, L.K. Roberts, and G.G. Krueger. The immunobiology of ultraviolet-radiation carcinogenesis. *Cancer Surveys* 4:51-99, 1985.

DeLeo, V., D. Hanson, I.B. Weinstein, and L.C. Harber. Ultraviolet radiation stimulates the release of arachidonic acid from mammalian cells in culture. *Photochem. Photobiol.* 41:51-56, 1985.

Gurish, M.F., D.H. Lynch, and R.A. Daynes. Changes in antigen-presenting cell function in the spleen and lymph nodes of ultraviolet irradiated mice. *Transplantation* 33:280-284,1982.

Kripke, M.L. Immunological unresponsiveness induced by ultraviolet radiation. *Immunol. Rev.* 80:87-102, 1984.

Kripke, M.L., W.L. Morrison, and J.A. Parish. Systemic suppression of contact hypersensitivity in mice by psoralen plus UVA radiation (PUVA). *J. Invest. Dermatol.* 81:87-92, 1983.

Krutmann, J., M. Athar, D. Mendel, I.U. Khan, P.W. Gyre, H. Mukhtar, and C.A. Elmets. Inactivation of the ligand binding site of the human monocyte Fc receptor following porphyrin photosensitization. *Clin. Res.* 36: 377A, 1988.

Lynch, D., M. Gurish, and R. Daynes. The effects of high-dose UV exposure on murine Langerhans cell function at exposed and unexposed sites as assessed using *in vivo* and *in vitro* assays. *J. Invest. Dermatol.* 81:336-341, 1983.

Magnus, I.A. *Dermatological Photobiology,* pp. 211-235, Osney Mead, Oxford: Blackwell Scientific Publications, 1976.

Odling, K.A., G.M. Halliday, and H.K. Muller. Enhanced survival of skin grafts depleted of Langerhans' cells by treatment with dimethylbenzanthracene. *Immunol.* 62:379-385, 1987.

Politzer, I.R., R.D. Ildefonso, and J.L Laseter. *Impact on Human Health of Petroleum in the Marine Environment,* pp. 43-83, Washington, D.C.: American Petroleum Institute, 1985.

Ross, R.D. and D.G. Crosby. Photooxidant activity in natural waters. *Environ. Toxicol. Chem.* 4: 773-778, 1985.

Santamaria, L., A. Arnaboldi, P. Daffara, and A. Bianchi. Photocarcinogenesis by methoxypsoralen, neutral red and proflavine. *Bolletino Chimico Farmaceutico* 118:356- 362, 1980.

Santamaria, L., G.G. Giordano, M. Alfisi, and F. Cascione. Effects of light on 3,4 benzpyrene carcinogenesis. *Nature* 210:8214-825, 1966.

Stingl, G., S. Katz, L. Clements, I. Green, and E.M. Shevach. Immunologic functions of Iabearing epidermal Langerhans cells. *J. Immunol.* 121:2005-2013, 1978.

Streilein, J. Lymphocyte traffic, T-cell malignancies and the skin. *J. Invest. Dermatol.* 71:167171, 1978.

Toews, G., P. Bergstresser, and J. Streilein. Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J. Immunol.* 124:445-453, 1980.

Urbach, F., P.D. Forbes, R.E. Davies, and F. Berger. Cutaneous photobiology: past, present and future. *J. Invest. Dermatol.* 67:209-224,1976.

World Health Organization. *Environmental Health Criteria 20. Selected Petroleum Products.* Geneva, Switzerland: WHO, 1982.