Ultraviolet Radiation and Immunology: Something New under the Sun—Presidential Address

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Abstract

The carcinogenic activity of solar radiation has been known for nearly a century. However, within the past few years, we have realized that exposing the skin to sunlight also has profound immunological effects on the host and that these immunological changes can contribute to the development of skin cancer and alter host resistance to infectious diseases. These findings have led to the development of a new field of research, termed photoimmunology, which is concerned with the effects of UV radiation on immunological processes.

Our interest in this field arose from studies on the antigenic properties of skin cancers induced in mice by chronic exposure to UV-B (280—320 nm) radiation. These cancers are highly antigenic and many are immunologically rejected upon transplantation to normal syngeneic recipients. In studying how these cancers were able to survive and grow in the primary host, we discovered that exposing the skin to UV radiation altered some types of immune responses, including the immune response against skin cancers.

Studies on the nature and mechanism of the immunological alterations brought about by exposure to UV radiation suggested that UV-induced DNA damage triggers a cascade of events, leading ultimately to a state of antigen-specific, systemic T lymphocyte-mediated immunosuppression. Key components of this cascade are epidermal cytokines, which modulate the immune response to antigens introduced into the UV-irradiated host and divert the response toward a state of specific immunosuppression.

The finding that UV radiation can redirect the immune response from an effector to a suppressor pathway has raised the possibility that immune responses to infectious diseases might also be influenced by exposure of the host to UV radiation. Interest in the health consequences of stratospheric ozone depletion, with its attendant increase in solar UV-B radiation, has stimulated recent investigations on the effects of UV radiation on the pathogenesis of infections in animal models and on immune responses in humans. In addition, attempts are being made to use information about UV-induced specific immunosuppression to eliminate unwanted immune responses, such as transplant rejection and graft-versus-host reactions.

Thus, studies on the immunological effects of UV radiation are providing new information on how immune responses are regulated as well as improving our understanding of the role of the immune system in skin cancer induction. This information should facilitate the development of more effective measures for preventing the deleterious effects of overexposure to UV radiation.

Introduction

The fact that UV radiation has any effects on the immune system at all is both unexpected and remarkable. It is unexpected, because UV radiation has little power of penetration through living tissues, and most is absorbed by the outer few mm of the skin. It is remarkable because life on earth has evolved in an environment containing UV radiation, and there is no obvious reason why the immune system should be accessible to it. Twenty years ago, no one would have predicted that shining UV light on the skin would have immunological consequences, much less that this would turn into an entire field of investigation. Nonetheless, the field of photoimmunology, which is the study of the interactions between photons and the immune system, has become an important facet of both photobiology and dermatology research.

The photons of most significance for photoimmunology are those in the UV-B (280—320 nm) region of the solar spectrum. These are the wavelengths that benefit human health by catalyzing the production of vitamin D; they are also the primary wavelengths responsible for sunburn and skin cancer (1). Furthermore, it is this region of the solar spectrum that is affected by ozone depletion. Most UV-B radiation from the sun is filtered out by ozone in the stratosphere, and a reduction in ozone concentration will permit an increase in the amount of UV-B radiation in sunlight (2). The threat of ozone depletion in recent years has focused considerable attention on the potential immunological effects of UV-B radiation.

In my presentation I will first describe how our studies of UV-induced skin cancer led us into the realm of photoimmunology; second, I will summarize our current state of knowledge regarding the mechanisms by which UV radiation modifies immune responses; finally, I will address the significance of these findings for human health.

Historical Background

Many years ago, we set out to study the antigenic properties of skin cancers induced in mice by UV radiation, using the approach of classical tumor immunology, namely tumor transplantation. Unexpectedly, while trying to propagate the tumors by transplantation, we found that most failed to grow in normal, syngeneic hosts, but they grew progressively in immunoedeficient mice (reviewed in Refs. 3—6). This finding suggested that the tumors were highly antigenic, and it raised the question of why they were able to grow in the primary host without succumbing to immunological rejection. The answer to this question was equally unexpected; we found that exposing mice to UV radiation rendered them unable to reject these highly antigenic tumors. The alteration was systemic because tumors implanted anywhere in the UV-irradiated hosts grew progressively.

The systemic alteration induced by UV radiation was immunological in nature because it could be transferred with T lymphocytes. Somehow, UV irradiation induced suppressor T cells that inhibited the rejection of these highly antigenic tumors. The suppressor cells were specific in that the recipients were still able to reject other types of tumors. We also demonstrated that the suppressor cells were important in the induction of primary skin cancers by showing that injection of suppressor cells into mice early in carcinogenesis shortened the latent period for tumor induction (7).

From these early studies, we learned that UV-induced tumors were highly antigenic, that UV irradiation caused systemic immunosuppression, and that this immunosuppression contributed to the development of the primary skin cancers. Therefore, UV radiation seemed to play a multifaceted role in carcinogenesis: It transformed normal
cells into cancer cells; it caused stable, heritable antigenic changes in these cells; and it depressed the immune response to these antigenic cancers, thereby permitting their outgrowth. These findings raised two important questions: (a) How does shining a light on the skin alter the immune response? (b) Why does UV alter the immune response?

**Mechanisms of UV-induced Immunosuppression**

Most of what we know about how UV alters the immune response comes from studying other cell-mediated immune responses, rather than tumor rejection. Information on this topic has been contributed by many laboratories, as well as my own. Early on, we and others looked at the effects of UV irradiation on many immune responses (3–6). We found that early in the course of UV irradiation, some other immune responses were also modified, particularly delayed hypersensitivity and CHS2 responses; other immune responses, such as graft rejection and antibody production, seemed to be unaffected.

The human prototype of the CHS response is poison ivy. In this reaction, a chemical is applied to the skin, and antigen-presenting cells (mainly epidermal Langerhans cells) take up the antigen and migrate to the regional lymph nodes, where they interact with T helper cells to initiate the response. After expansion and differentiation, these T cells enter the circulation. When they contact the antigen in the skin, they release cytokines (chemical mediators) and cause the local symptoms of redness, itching, and swelling. The DTH response is similar, except that the antigen is introduced by the i.d. or s.c. route, and a different population of antigen-presenting cells is involved.

**Local Immunosuppression.** There are two models of UV-induced immunosuppression, termed local and systemic suppression (reviewed in Refs. 6, 8, and 9). In local suppression, the antigen is applied topically or i.d. in the site of UV irradiation. In the mouse, application of a contact sensitizing chemical to UV-irradiated skin induces a decreased CHS response, and one can find in the spleen of these mice antigen-specific T cells that inhibit the induction of CHS when transferred to another animal. Examination of the UV-irradiated skin reveals that epidermal Langerhans cells are morphologically altered and reduced in number. These findings led to the hypothesis that UV irradiation was altering the function of cutaneous antigen-presenting cells and that this somehow changed the type of immune response elicited.

To test this hypothesis, we used an in vivo approach in which the fluorescent antigen FITC is used as a contact sensitizer. This enabled us to follow the fate of the antigen-presenting cells in vivo. Eighteen h after painting FITC onto the skin of a normal mouse, the DLN contain a small number of FITC+ dendritic cells. Characterization of these cells by sorting FITC+ cells demonstrated that they are Ia+ and F4/80+ and contain Birbeck granules, which are ultrastructural markers of epidermal Langerhans cells (10). Injection of the DLN cells into a secondary host induces CHS because of the presence of these FITC-bearing antigen-presenting cells (11). We used this model to ask what happens to the activity of the DLN cells in mice that are first exposed to UV radiation and then painted epicutaneously with FITC. DLN cells from unirradiated mice induce CHS when injected into the footpads of normal syngeneic recipients. In contrast, DLN cells from UV-irradiated mice fail to induce CHS; instead, they induce or transfer suppressor T cell activity (12).

One possible explanation for the decreased antigen-presenting activity is that the FITC+ dendritic cells never leave the skin after UV irradiation and thus are not present in the DLN. This is not the case, however; FACS analysis demonstrated that equivalent numbers of FITC+ cells are present in the DLN of normal and UV-irradiated mice (12). At least some of the FITC+ cells come from the skin of the UV-irradiated mice. This was demonstrated by sorting the FITC+ cells and staining them with a monoclonal antibody specific for pyrimidine dimers; the dimers are present only in the DNA of cells exposed directly to UV radiation. In the DLN of UV-irradiated mice, all of the dimer-containing cells were present in the FITC+ antigen-presenting cell population, indicating that they had been exposed to UV radiation in the skin and migrated into the DLN after sensitization. Recent experiments demonstrated that FACS-purified, FITC+, Ia+ DLN cells have reduced antigen-presenting activity.

Thus, our studies support the following model: UV irradiation alters antigen-presenting cells in the skin; these cells reach the DLN after sensitization with FITC, and their activity is altered. There is also evidence suggesting that inflammatory cells that have entered the skin in response to UV radiation may also function as antigen-presenting cells and thereby contribute to the altered immune response (13). Recent work from Verneer and Streilein suggests, in addition, that cytokine mediators may also play a role in this form of UV-induced immunosuppression. Treating UV-irradiated mice with antibody against TNFα can partially inhibit immunosuppression (14). There are probably other contributors to the local suppressive milieu, such as prostaglandin E2, IL-1 and IL-10, and cis-urocacid, all of which are present in UV-irradiated skin. The net result is inhibition of the CHS pathway and activation of the suppressor pathway. Recent studies demonstrated that UV radiation has a similar effect in humans (15, 16).

**Systemic Immunosuppression.** UV irradiation can also suppress immune responses initiated outside UV-irradiated skin. In this case, slightly more UV radiation is required, and the antigen can be painted topically or injected s.c. The result is the same as in the local suppression model, however; instead of the DTH or CHS response being induced, antigen-specific suppressor T cells are induced (6).

Clearly, this form of suppression is not due to a direct effect of the UV on antigen-presenting cells in the skin, and it must involve soluble mediators. Evidence for the participation of soluble mediators was provided by experiments demonstrating that exposing murine keratinocytes to UV radiation in vitro caused the production of soluble factors that mimicked whole-body UV irradiation: Injection of these factors into normal mice, followed by immunization, induced active suppression, instead of DTH or CHS (17, 18). Recent studies demonstrated that IL-10 is a mediator of suppression of the DTH response (19), whereas systemic suppression of CHS involves TNFα (20).

**Molecular Mechanism of UV-Induced Immunosuppression.** To initiate a biological response, UV radiation must first be absorbed by molecules in the skin (photoreceptor) and transform its energy into a biochemical event. Several pathways for the effect of UV radiation on the immune response are possible. UV radiation can activate genes directly by causing structural alterations in DNA; it can also alter DNA indirectly by causing the formation of oxygen radicals, which secondarily damage DNA. UV radiation can cause lipid peroxidation in cell membranes, thereby activating genes by means of intracellular signaling pathways, and it can isomerize a molecule in the stratum corneum, urocacid, which has immunosuppressive properties.

Recently, we used an approach developed by Dr. Daniel Yarosh (Applied Genetics, Inc., Freeport, NY) to test the hypothesis that direct DNA damage, in the form of pyrimidine dimers, is involved in UV-induced immunosuppression. The approach involves the use of

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2 The abbreviations used are: CHS, contact hypersensitivity; DLN, draining lymph nodes; i.d., intradermal; DTH, delayed-type hypersensitivity; TNFα, tumor necrosis factor α; IL, interleukin.


4 A. Vink, F. Strickland, L. Roza, and M. Kripke, unpublished data.
liposomes to deliver an excision repair enzyme to cells in the skin in vivo. Bacteriophage T4 endonuclease V, an excision repair enzyme specific for pyrimidine dimers in DNA, is encapsulated in acid-labile liposomes and applied topically to murine skin. There, it is taken up by keratinocytes and Langerhans cells and reaches the nucleus of these cells (21). Previous studies demonstrated that these T4N5 liposomes increased the repair of pyrimidine dimers in mouse skin in vivo and human keratinocytes in vitro (22, 23).

In these studies, we wished to determine whether reducing the amount of DNA damage by application of the T4N5 liposomes would also reduce the amount of immunosuppression induced by UV irradiation. C3H mice were exposed to FS40 sunlamp irradiation and immunized 3 days later at an unexposed site with a contact sensitizing chemical to induce CHS or with *Candida albicans* to induce DTH. Both responses were inhibited in UV-irradiated mice. Application of T4N5 liposomes immediately after UV irradiation abrogated the immunosuppressive effects of the irradiation, whereas application of liposomes containing heat-denatured enzyme had no effect. The T4N5 liposomes were effective only when applied to the UV-irradiated skin, not to distant, unexposed skin, and only when applied immediately after the irradiation (24).

In subsequent studies, we demonstrated that T4N5 liposomes also reduce UV-induced damage to epidermal Langerhans cells (25). These studies and others (26) strongly suggest that DNA damage is the primary mechanism by which UV radiation triggers its immunosuppressive effects and that the formation of pyrimidine dimers is likely to be an essential first step in the pathway leading to immunosuppression. Since TNFα and IL-10 have been demonstrated to play a role as soluble mediators of UV-induced immunosuppression (14, 19, 20), our current studies are directed toward determining whether application of T4N5 liposomes reduces the amount of these cytokines in UV-irradiated skin.

Our working model for systemic immunosuppression is that UV-induced DNA damage activates transcription of cytokine genes, thereby initiating the release of immunosuppressive cytokines. These cytokines shift the balance of the immune response away from a DTH (T helper 1) type of response toward a suppressive (T helper 2) type of response, so that antigens introduced during a critical period after UV irradiation induce active suppression instead of immunity. There is growing sentiment that the suppressor T cells associated with UV irradiation may simply be T helper 2 cells, which produce cytokines, such as IL-10, that down-regulate T helper 1 cells (27, 28).

To put together the models for both local and systemic suppression, we propose that with low doses of UV radiation, antigen-presenting cells in the irradiated skin are modified, either directly by the action of UV or indirectly by the influx of inflammatory cells or by the local release of cytokines that modify their activity. Larger doses of UV radiation may bring about the release of these cytokines systemically, causing an alteration in the activity of antigen-presenting cells in distant lymphoid organs and activation of the T helper 2 pathway. Of course, we speculate that this is the mechanism by which UV irradiation suppresses the immune response to skin cancers, although there is as yet no direct evidence for this.

**Significance**

These studies may have significance for DNA repair deficiency diseases. If DNA damage is responsible for triggering immunosuppression through alterations in epidermal cytokine production, then persons who are unable to repair UV-induced DNA damage may have cytokine imbalances, in addition to a high incidence of skin cancer. This may help to explain some of the secondary manifestations of xeroderma pigmentosum and other sun sensitivity syndromes which could arise from cytokine dysregulation.

These studies clearly have significance for preventing the deleterious effects of sunlight exposure. Understanding the mechanisms of immunosuppression is suggesting new approaches for preventing UV-mediated damage. In addition to preventing DNA damage, the approach used by all current sun-blocking agents, it may be possible to reverse DNA damage by increasing DNA repair; to inhibit the activity of specific cytokines, such as TNFα; and to prevent the release of cytokines from epidermal cells. All of these approaches may serve to reduce the immunological effects of UV irradiation. On the other hand, we may be able to use this information to induce specific immunosuppression to prevent undesirable immune responses, such as transplant rejection or graft-versus-host disease (29).

If immunology is important in the induction of human skin cancer, then one might expect that sensitivity to UV-induced immunosuppression should correlate with skin cancer risk. Evidence supporting this idea was provided by Yoshikawa et al. (15), who demonstrated that under certain experimental conditions, about 40% of the normal human population is highly susceptible to local suppression of the CHS response by UV radiation; however, nearly 100% of persons who have had one or more skin cancers are highly sensitive to UV-induced immunosuppression. These results are consistent with the hypothesis that sensitivity to immunosuppression by UV is an additional risk factor for skin cancer development in susceptible individuals.

With regard to immunology, these studies illustrate that the immune system is important in skin cancer development and that immunological mechanisms may play a determining role in the outcome of the host-tumor interaction. They also make another point. Because the immune system is so intimately connected to the skin, it is accessible to external influences, and such seemingly superficial events as shining a light on the skin may have profound systemic immunological consequences. Therefore, what we put on our skin or encounter in our environment may influence our systemic immune response.

These studies also have environmental significance. Because the types of immune responses affected by UV radiation are important in defense against infectious diseases, this has raised the question of whether UV irradiation can impair immunity to infectious agents. Studies from our laboratory and others have demonstrated that not only can UV irradiation of mice interfere with the induction of immunity to certain infectious agents (reviewed in Ref. 30), but in some instances, UV irradiation can also increase the duration and severity of the disease process. This is illustrated in experiments in which mice were exposed once to UV radiation and then infected with *Mycobacterium leprae* (31). This bacterium causes a slow, progressive infection that ultimately results in death after about 1 year. A single dose of UV radiation decreased the DTH response, decreased the rate of clearance from lymphoid tissues, and accelerated the rate of death from this organism. These studies illustrate that the condition of the immune system during the first encounter with an infectious agent may be critical to the outcome of disease.

Such findings have raised a concern that increased solar UV radiation, resulting from ozone depletion, may alter the balance of the host-disease relationship in favor of the pathogen for some diseases. For example, infection of some mouse strains with *Leishmania* activates a T helper 2 response and results in a progressive, lethal infection; in others, a T helper 1 response is activated, the infection is self-limiting, and the animals develop immunity (32). Therefore, a shift from the T helper 1 to the T helper 2 type of response by UV irradiation could have serious consequences for infectious diseases. Thus, ozone depletion may result not only in more cases of skin cancer but also in an increase in the incidence, severity, or duration of
some infectious diseases, particularly in countries where infectious diseases constitute a major public health problem.

What, then, is the evolutionary significance of UV-induced immunosuppression? Why would UV exposure, which is necessary for life, cause immunosuppression? The answer is, of course, unknown, but the popular immunological answer, suggested first by Dr. George Klein (Karolinska Institute, Stockholm, Sweden), is that because UV radiation is mutagenic and causes antigenic changes in the skin, immunosuppression is a protective response designed to keep us from rejecting our skin every time we develop a sunburn. However, the recent findings on DNA damage and epidermal cytokines raise another possibility: we now know that keratinocytes can produce many cytokines; some of these, like IL-10, are involved in immunosuppression, but others, like granulocyte-macrophage colony-stimulating factor, IL-1, IL-4, and IL-6, are involved in hematopoiesis and lymphoid differentiation. Perhaps epidermal cytokine production represents an “SOS” type of response to DNA damage in general and is designed to restore hematopoietic balance following exposure to DNA-damaging agents. Because of coordinate regulation of cytokines or pleiotropic activity of cytokines, this response is accompanied by a brief period of immunosuppression. Thus, UV irradiation may produce a small gap in immune surveillance in exchange for rapid mobilization of hematopoietic cells in response to DNA-damaging agents. Regardless of the evolutionary function of UV-induced immunosuppression, however, it seems fair to conclude that studies of UV radiation and immunology have indeed provided us with something new under the sun.

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