Ultraviolet B–Irradiated Antigen-Presenting Cells Display Altered Accessory Signaling for T-Cell Activation: Relevance to Immune Responses Initiated in Skin

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A principal mechanism by which ultraviolet (UV) B radiation exerts its selective and antigen-specific suppressive influence on immune responses is through its effects on the capacity of antigen-presenting cells (APC) in skin, primarily Langerhans cells (LC), to differentially activate T-cell subsets. Recent evidence has indicated that LC, following UVB radiation, lose the capacity to stimulate proliferation of CD4+ Th1, but not of Th2, clones. Additional work has shown this acquired unresponsiveness of Th1 cells to represent a long-lasting form of clonal anergy that results from a block in their ability to produce IL-2. Although not completely delineated, these defects appear to be the result of preserved delivery of the primary signal transduced by interaction of the MHC/antigen complex on APC with the T-cell receptor complex, in the absence of a viable second signal normally delivered by interaction of a co-stimulatory factor from APC with its appropriate ligand on the T cells. These findings support the notion that the outcome of certain immune responses depends greatly upon conditions that are brought to bear on APC and T cells during the time of antigen presentation. J Invest Dermatol 98:66S–69S, 1992

Cutaneous exposure to ultraviolet B (UVB) radiation reverses, from effector to suppressor, the outcome of certain immune responses that are initiated in skin. Induction of these suppressor responses has been demonstrated at an in vivo level for contact allergy in mice and humans, for delayed-type hypersensitivity (DTH) in mice, and for immunosurveillance against UV-induced skin cancer, also in mice [1,2]. Several mechanisms have been implicated to explain these immunosuppressive effects [3,4]. In our review, a consolidation of evidence derived principally from work conducted at laboratories in three cities (Dallas, Cleveland, and Freiburg) is presented to advance the view that these suppressive effects of UVB radiation on cutaneous immune responses are attributable to alterations in accessory signal transmission during antigen presentation.

RELATIVELY LOW DOSES OF UVB RADIATION INHIBIT THE INDUCTION OF CONTACT ALLERGIC RESPONSES IN VIVO

The idea that suppression of immune responses by ultraviolet B (UVB) radiation might be related to UVB effects on antigen-presenting cells (APC) was first suggested by experiments with C57BL/6 mice reported in 1980 by Toews et al [5]. They observed that exposure of abdominal wall skin to relatively low doses of UVB (<200 J/m²/d on four consecutive days) had at least two effects. The first was morphologic, a diminution in the density of Langerhans cells (LC) within the epidermis of irradiated skin, as judged by a loss of cell dendricity and a loss of membrane ATPase activity. The second change was functional, a profound reduction in the capacity of mice sensitized with the reactive hapten dinitrofluorobenzene (DNFB) through irradiated skin to acquire ear-swelling responsiveness. These two parallel changes suggested that LC were crucial APC for the induction of contact allergy to hapten such as DNFB, and that the unresponsiveness to sensitization to DNFB produced by local exposure to low doses of UVB radiation was attributable to a loss in this APC function.

UVB RADIATION INHIBITS THE ANTIGEN-PRESENTING CAPACITY OF LC IN VITRO

The notion that UVB radiation had the capacity to inhibit antigen presentation by LC was fortified by work performed in other laboratories, which demonstrated in vitro irradiation (single doses of 25–600 J/m² UVB) to cause human or mouse epidermal cells (contain-
UVB RADIATION CONVERTS LC FROM IMMUNOGENIC TO TOLERGENIC APC IN CONTACT ALLERGY IN VIVO

To this point, the evidence that UVB exerted its immunosuppressive effect on LC was largely circumstantial, awaiting the development of techniques by which epidermal cells could be sorted into relatively pure populations. Once this had been achieved, it was shown by Cruz et al [8] that a single dose of 200 J/m² UVB in vitro would abrogate the capacity of hapten-derivatized, FACS-purified LC to induce allergic responses, when infused intravenously into syngeneic mice (Fig 1). More importantly, it was also demonstrated in these studies that mice infused with UVB-irradiated and hapten-derivatized LC became incapable of mounting normal ear-swelling responses, even when the same hapten was painted on unirradiated normal skin at a later date [8]. These studies indicated that UVB radiation did not prevent antigen presentation; rather, it converted the function of LC from one that induces an immunogenic signal to one that is tolerogenic.

CD4⁺ T CELLS MEDIATE THE SUPPRESSION OF CONTACT ALLERGY RESPONSES INDUCED BY UVB RADIATION IN VIVO

One mechanism by which UVB radiation has been shown to induce antigen-specific tolerance is through the generation of suppressor T cells. In adoptive transfer studies in which the identities of T-cell populations were determined via in vitro depletion with antibody and complement lysis, Elmets et al [9] demonstrated that the suppression of contact allergy by low doses of UVB radiation in vivo was mediated by CD8⁺ T cells. This observation was recently corroborated and extended by Cruz et al [10]. Using in vivo T-cell depletion via monoclonal antibodies against either CD4 or CD8, they showed that the down-regulation of contact allergy induced by intravenous infusion of UVB-irradiated, hapten-derivatized LC required the presence in vivo of CD4⁺ (but not CD8⁺) T cells. Taken together, these studies suggested that T cells of the CD4 phenotype mediate the suppression of contact allergy that is induced by low-dose UVB radiation in vivo.

Low-dose UVB Radiation Reverses the Preferential Capacity of LC to Activate Th1 vis-a-vis Th2

Ultimately, the identification of mechanisms by which UVB distorts the activation of T cells must examine biologic events at the level of single cells, because this is the level at which activation takes place. In fact, work with LC had progressed to the extent that they could be added to experimental systems at relatively high purity, and it therefore became necessary to develop experiments in which lines or clones of antigen-specific T cells were employed.

Within this context, two subsets of CD4⁺ T cells in mice are currently under intense investigation (Table I). Cells of the Th1 subset are responsible for mediating DTH, a function that has been attributed to their ability to secrete interferon γ (IFNγ) [11]. Th1 cells have been shown to mediate tumor cytolysis and to secrete other cytokines including IL-2, IL-3, GM-CSF, and lymphokine [11]. By contrast, cells of the Th2 subset produce IL-4 and IL-5, cytokines that enable B cells to efficiently secrete antigen-specific antibodies, including IgE [11]. Importantly, Th1 and Th2 cells appear to counter-regulate each other [12]: INFγ (from Th1) can inhibit the proliferation of Th2, whereas the newly identified IL-10 (from Th2) can block activation of Th1 [13].

The availability of Th1 and Th2 clones specific for the protein, keyhole limpet hemocyanin (KLH), allowed Simon et al [14] to test the possibility that the UVB-induced alteration in APC function of LC might be reflected in differential activation of these contrasting CD4⁺ T cell subsets. As demonstrated in Table II, unirradiated LC, when pulsed with KLH, were observed to induce vigorous proliferation of Th1 and, to a lesser extent, of Th2. On the other hand, LC irradiated with a single dose of 200 J/m² UVB lost their ability to stimulate Th1, while fully retaining their capacity to stimulate Th2. These experiments demonstrated UVB to be capable of reversing the normal capacity of LC to activate Th1 preferentially over Th2 cells in vitro.

UVB-Irradiated LC Can Induce Clonal Anergy in CD4⁺ Cells of the Th1 Subset

As cited previously, UVB-irradiated, hapten-derivatized LC were observed to induce initial unresponsiveness and subsequent down-regulation (i.e., tolerance) when infused intravenously into syngeneic mice [8]. This finding prompted Simon et al [15] to test whether the proliferative unresponsiveness that had been observed when UVB-irradiated LC were employed as APC for the KLH-specific Th1 clones represented simply a null event. Note in Table III that this was not the case. Th1 clones were subjected to two sequential stimulations, initially to KLH in the presence of unirradiated or UVB-irradiated LC, and then (after 3 or 16 d) to KLH and unirradiated LC, or to exogenous IL-2 alone. These experiments indicated that exposure to the UVB-irradiated

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**Table I. Two Functionally Disparate Subsets of CD4⁺ T Cells in Mice**

<table>
<thead>
<tr>
<th></th>
<th>Th1</th>
<th>Th2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent function</td>
<td>Mediation of &quot;cellular&quot; immunity, such as DTH and tumor cytotoxicity</td>
<td>Mediation of &quot;humoral&quot; immunity by helping B cells to produce Ab, including IgE</td>
</tr>
<tr>
<td>Characteristic lymphokines</td>
<td>IL-2, IFNγ</td>
<td>IL-4, IL-5, IL-10</td>
</tr>
<tr>
<td>Optimal APC</td>
<td>Macrophages and dendritic cells, including LC</td>
<td>B cells</td>
</tr>
<tr>
<td>Costimulatory factor</td>
<td>Not yet identified</td>
<td>IL-1</td>
</tr>
</tbody>
</table>

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**Table II. Differential Effects of UVB Radiation on the APC Function of LC for CD4⁺ Th1 and Th2 Clones**

<table>
<thead>
<tr>
<th>APC</th>
<th>Antigen</th>
<th>T-Cell Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unirradiated LC</td>
<td>KLH</td>
<td>++++</td>
</tr>
<tr>
<td>UVB-irradiated LC</td>
<td>KLH</td>
<td>+++</td>
</tr>
</tbody>
</table>

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**Figure 1.** In vitro UVB radiation converts LC from immunogenic to tolerogenic APC in contact allergy in vivo.
Table III. Induction of Anergy in Th1 Clones by UVB-Irradiated LC

<table>
<thead>
<tr>
<th>Initial Incubation of Th1 with</th>
<th>Subsequent Incubation of Th1 with</th>
<th>Proliferation of Th1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unirradiated LC and KLH</td>
<td>Unirradiated LC and KLH</td>
<td>+++++</td>
</tr>
<tr>
<td>UVB-irradiated LC and KLH</td>
<td>Unirradiated LC and IL-2 alone</td>
<td>++/−</td>
</tr>
</tbody>
</table>

(but not to unirradiated) LC rendered these Th1 cells tolerant to subsequent stimulation with KLH and LC. Furthermore, the finding that such tolerant Th1 retained the capacity to proliferate in response to exogenous IL-2 indicated that they were not physically deleted, but rather made functionally anergic.

**Distortion or Deletion of a Costimulatory Activity by UVB Converts LC from Immunogenic to Tolerogenic APC**

Optimal antigen presentation requires the engagement of several different membrane-bound or soluble ligands from APC to appropriate receptors on the surface of responder T cells. These ligand-receptor interactions deliver signals to T cells that eventuate in their differentiation, cytokine production, and proliferation (Fig 2) [16]. The antigen-specific signal (signal 1) is transduced when the MHC molecule in association with processed antigen (on the APC) becomes engaged to the T-cell receptor (TCR) complex on the selected T cell. A second signal (signal 2) is transduced by the interaction of a costimulatory activity from the APC to its corresponding receptor on the T cell. Simultaneous transduction of both signals enables T cells to secrete IL-2, which in turn fuels their proliferation [17]. In the absence of one signal or the other, T cells do not proliferate. In fact, transduction of signal 1 alone renders T cells anergic, a state that is associated with the failure to secrete IL-2 [17].

Two features of the Th1 tolerance induced by UVB-irradiated LC suggest that it results from stimulation by MHC/antigen in the absence of the required costimulatory activity. First, Th1 cells that were rendered tolerant by this method also failed to produce IL-2, whereas they could proliferate in response to exogenous IL-2 [15]. Second, addition of allogeneic and unirradiated APC to the original combination of UVB-irradiated LC, antigen, and Th1 cells prevented the induction of tolerance [15]. Because MHC-disparate APC could not by themselves cause Th1 proliferation, UVB could not have affected signal 1; rather, it distorted or deleted signal 2. The most direct interpretation of this finding is that the allogeneic APC provided the costimulatory activity that is missing from the irradiated LC.

**ICAM-1 as a Model of a Costimulatory Factor Distorted or Deleted by UVB on LC**

The identity of the UVB-sensitive costimulatory activity for Th1 cells is not known. Studies conducted by Simon et al [15] suggest that this factor is membrane associated, but not encoded by MHC genes, with one attractive candidate being ICAM-1. Insight into this possibility is derived from the studies of Krustmann et al [18–20], which have shown UVB to abrogate the capacity of human peripheral blood monocytes (PBM) or LC to induce OKT3-driven T-cell proliferation. This occurred in parallel with down-regulated ICAM expression by these APC. Moreover, T cells stimulated by UVB-irradiated PBM displayed increased intracellular free calcium and expressed IL-2 receptors (albeit, at a reduced level), but failed to produce IL-2 [18–20]. Each of these findings corresponds in parallel with changes in mouse T cells rendered tolerant in the experiments reported by Simon et al using UVB-irradiated APC [15] and by Mueller et al using chemically fixed APC [21].

Recently, macrophages and spleen dendritic cells were reported to serve as optimal APC for Th1, whereas B cells were optimal APC for Th2 [22]. These observations link two additional findings: 1) Hertl et al [23] noted that UVB had no effect on the accessory function of human B cells and did not down-regulate ICAM-1 expression by these cells, and 2) Simon et al [24] showed that mouse Th1, but not Th2, clones require an ICAM-1–mediated activation signal from LC for optimal proliferation. Taking into consideration the fact that Th1 clones were sensitive, whereas Th2 clones were resistant, to the tolerogenic effects of UVB-irradiated LC, these findings constitute further circumstantial evidence to support the possibility that ICAM-1 serves as a UVB-sensitive accessory factor for Th1 cells alone. To date, however, no studies have yet demonstrated that deletion of the signal transduced by ICAM-1 from APC to T cells leads to tolerance.

**Relevance to Immune Responses Initiated in Skin**

Most immune responses that are initiated in skin appear to be T-cell rather than B-cell mediated. In addition, most, if not all, reported immunosuppressive effects of UVB radiation affect T-cell rather than B-cell responses. We postulate this peculiar cutaneous predilection for T-cell–driven reactions to reflect an inherent capacity of the principal APC in skin, namely, LC, to preferentially activate T cells that mediate “cellular” immunity (i.e., CD4+ Th1 cells) over those T cells that promote “humoral” immunity (i.e., CD4+ Th2 cells).

Almost all of the reproducible forms of suppression evoked by UVB are antigen specific. In addition, unlike other immunosuppressive agents, UVB radiation does not cause pan-immunosuppression; rather, it produces a highly selective immunosuppression. For example, UVB suppresses the induction, but not the elicitation, of contact allergy [25]. These two characteristics, antigen specificity and affection of primary but not secondary immune responses, are consistent with the notion that antigen presentation, specifically the APC interaction with naïve T cells during antigen–driven activation, is the perturbed locus for UVB-induced immunosuppression.

The finding that UVB-irradiated LC exert disparate effects on activation of CD4+ Th1 and Th2 cells in vitro provides a useful framework for rationalizing several in vivo observations. Thus, the long-lasting unresponsiveness induced by UVB in contact allergy and DTH assays may be explained by the tolerogenic effects of irradiated LC on the T cells deemed to be responsible for mediating

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**Figure 2.** Simultaneous transduction of (antigen-specific) signal 1 and (costimulatory) signal 2 enables Th1 cells to secrete IL-2, which fuels their proliferation. By contrast, transduction of signal 1 alone renders Th1 cells incapable of producing IL-2, leading to a state of anergy.
these reactions, i.e., Th1 cells. In the absence of direct evidence, it is tempting to speculate that Th2 cells, whose proliferative capacity remains intact despite contact with irradiated LC, are the cells responsible for mediating suppression of Th1-mediated responses in vivo. Recall that Th2 cells can secrete IL-10, which has been shown to inhibit activation of Th1. The notion that the antigen-specific suppressor T cells that had been adoptively transferred in the early studies of Elmets et al. [9] were Th2 cells awaits experimental verification.

Our final suggestion concerns the possibility that UVB-irradiated LC play a double-edged role through their tolerogenic function. On the one hand, such cells may serve a beneficial role in preventing the induction of autoimmune cutaneous disease that ordinarily would be triggered by undeleted peripheral T cells, which recognize autoantigens or neoantigens (in keratinocytes for instance) produced by the UVB radiation. On the other hand, the existence of such cells also implies detrimental consequences, particularly with respect to the loss of immunosurveillance against tumor antigens or infectious agents.

REFERENCES


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