Ultraviolet Radiation-Induced Impairment of the Early Initiating and the Late Effector Phases of Contact Hypersensitivity to Picrylchloride: Regulation by Different Mechanisms

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Two types of antigen-specific T cells are needed for the elicitation of contact hypersensitivity reactions. They act in an obligate sequence to mediate the early initiating and late effector phases of contact hypersensitivity, which are accompanied by skin-swelling responses at 2 and 24 h after challenge, respectively. The magnitude of the late ear swelling depends on that of the early swelling.

We studied the influence of ultraviolet radiation on both phases of contact hypersensitivity to picrylchloride. Mice were exposed to subedemal doses of ultraviolet radiation on the shaved backs for four consecutive days. Four days later mice were sensitized on non-irradiated skin. Four days after sensitization mice were challenged on the ears, and swelling was measured 2, 4, and 24 h after challenge. The early and late phases of contact hypersensitivity were largely suppressed in ultraviolet-irradiated, actively sensitized mice. Transfer of immune lymphoid cells from donor mice that were sensitized 4 d earlier induced early and late components of contact hypersensitivity in naive recipients after challenge. Transfer of immune lymphoid cells from donors that were sensitized 1 d earlier only induced the early component of contact hypersensitivity. Ultraviolet irradiation of donor mice significantly reduced the capacity of the immune lymphoid cells to induce contact hypersensitivity. We show that lymphoid cells responsible for the early and late components of contact hypersensitivity are both affected. Key words: ultraviolet radiation/CHS/mice/photoimmunology. J Invest Dermatol 102:923–927, 1994

The immunosuppressive properties of ultraviolet radiation (UVR) have been known for several years. Studies of Fisher and Kripke [1,2] and of Spellman et al [3] showed in inbred mice that the UVR-induced suppression of rejection of highly antigenic, UVR-induced tumors is transferable with lymphoid cells. UVR also suppresses delayed-type hypersensitivity (DTH) to a variety of antigens such as haptens [4,5], allogeneic spleen cells [6,7], viruses [8], bacteria [9], fungi [10], and parasites [11]. DTH assays are convenient assays to study the mechanisms of UVR-induced suppression of cellular immunity because they are relatively easy to perform.

In the above-mentioned studies, the suppression of the “classical” DTH reaction, measurable as a skin-swelling reaction that peaks 24–48 h after antigen challenge, was investigated. However, Van Loveren and colleagues reported that elicitation of murine DTH reactions is dependent on the sequential activities of at least two different antigen-specific Thy-1+, CD5+ , CD8+ cells [12,13]. These two different T cells mediate separate early and late steps in the DTH reaction that are accompanied by maximum skin swellings at 2 and 24 h after challenge [12–15]. More recently the phenotype of these cells was more extensively characterized [14]. The early DTH-initiating cell, which has recently been cloned [16], is Thy-1+ , CD5+ , CD4+ , CD8+ , CD3+ , CD45RA+ , and expresses IL-3 receptors but no IL-2 receptors. On the other hand, the classical, late DTH effector T cell is Thy-1− , CD5+ , CD4+ , CD8+ , CD3+ , CD45RA− , and expresses IL-2 receptors but no IL-3 receptors. After sensitization, the DTH-initiating T cells in lymphoid organs produce an antigen-specific factor that can subsequently be found in the circulation as early as 1 d after sensitization [17]. This factor sensitizes the extravascular tissues such as the skin [18]. Elicitation of DTH with specific antigen leads initially to an immediate hypersensitivity-like early reaction that is due to local release of the vasoactive amine serotonin by mast cells [18] and possibly by other cells [19,20]. These cells are sensitized with this antigen-specific DTH-initiating factor that is analogous to IgE antibodies [13,21,22]. Serotonin renders the vascular endothelium locally permeable [23,24], allowing late-acting antigen/major histocompatibility complex–restricted T cells to enter the site. Histologic proof for this early swelling reaction was provided by Kops et al [24]. Serotonin also activates the recruited late-acting T cells through specific serotonin-2 receptors [25]. These recruited classical DTH T cells are activated by binding of α:β T-cell receptors to antigen/major histocompatibility complexes on local antigen presenting cells and produce lymphokines that attract the 24-h perivascular infiltrate of non-specific inflammatory cells. For humans similar mechanisms are suggested [26].

The purpose of the present investigation was firstly, to examine whether UVR, besides suppressing the late 24-h swelling reaction, also suppresses the early 2-h swelling reaction of the contact hypersensitivity reaction (CHS) to Picryl Chloride (PCI); and secondly,
because the early initiating phase of DTH facilitates the full expression of the late effector phase of DTH [12,27], to investigate whether impairment of the late effector phase was in fact due to an effect of UVR on the early initiating phase.

MATERIALS AND METHODS

Mice DBA/2 mice (8-12 weeks old, male) were obtained from Harlan, UK. Mice were rested at least 2 weeks before use and were housed in macronol cages with unlimited access to standard mouse chow and tap water.

General Reagents PCl (Chemotronix, Swannanoa, NC) was recrystallized three times from methanol/H2O before use, and protected from light.

UVR Irradiation The UVR source was a Kromayer UV lamp (Hanovia Ltd, Slough, UK), that was equipped with a Schott WG 305 filter. This lamp had an irradiance of 140 (J/m²)/second in the UVR range (wavelengths 305-400 nm), as measured by a Kipp E11 thermopile [28]. After dorsal hair was removed with electrical clippers, two circular areas (6.3 cm² in total) on the backs of the mice were irradiated, and the ears were protected from UVR. No anesthetics were used during irradiation to avoid interactions between UVR and anesthetics. Instead mice were immobilized in cylindrical containers (diameter 3 cm). Mice were irradiated for 16 seconds on the shaved backs on four consecutive days. The dose received by each mouse was 2.2 kJ/m² at each exposure, a dose that was subedemal. Control mice were sham-sensitized.

Measurement of Contact Hypersensitivity (CHS) Ear-Swelling Responses Four days after sensitization, both ears of the mice were challenged by topical application of one drop (27-gauge needle) of 5% PCl in ethanol/acetone (3:1) to the non-UVR-irradiated shaved abdomen, chest, and four feet. Control mice were sham-sensitized by topical application of 0.8% PCl in olive oil.

Adoptive Transfer of Lymphoid Cells (Passive Sensitization) Suspensions of lymphoid cells pooled from the axilla and inguinal lymph nodes and the spleen were prepared from mice that were UV-irradiated and sensitized to PCl as described before. Cell suspensions were made by gently pressing the lymph nodes and spleens through a stainless steel screen. The cell suspensions were washed three times in phosphate-buffered saline. Subsequently, cell counts were performed and viability was checked by trypan blue exclusion. Generally, viability was higher than 90%. 5 X 10⁷ lymphoid cells in 0.3 ml PBS were transferred into the retroorbital plexus of normal recipient mice under light ether anesthesia. These recipients were challenged 1 d later, as indicated above.

Statistics Levels of significance were calculated using the Student t test; p < 0.05 was taken as a significant difference between groups.

RESULTS

Effect of UVR Irradiation on the Early and Late Swelling Reactions of the CHS Reaction to PCl To determine the effect of UVR on the early and late swelling reactions of the CHS response to PCl, we irradiated mice on four consecutive days and actively sensitized them to PCl 4 d after the last irradiation on the non-UVR-irradiated skin. Four days after sensitization, mice were challenged with PCl on the ears, and ear swelling was measured at certain intervals after challenge. In non-UVR-irradiated control animals, the early swelling response at 2 h was accompanied by a noticeable swelling and redness of the ears. In duplicate experiments, the 2-h ear-swelling response, which represents the early component of the CHS response, was significantly and almost completely inhibited in UV-irradiated sensitized animals (UV) as compared to the non-UV-irradiated sensitized controls (NR). At 4 h after challenge, the swelling reactions in both groups were not significant. The 24-h ear-swelling response, which represents the late component of the CHS response, was also greatly reduced in UV-irradiated, sensitized animals compared to the non-irradiated, sensitized controls (Fig 1).

Adoptive Transfer of Lymphoid Cells From UV-irradiated Sensitized Donor Mice Fails to confer the Early and Late Components of CHS in Naive Recipient Mice Sensitization for the early and late CHS responses can be adoptively transferred by lymphoid cells from a sensitized donor to a naive recipient. To check whether UVR would also impair this transfer of sensitization, we UV-irradiated donor mice for 4 consecutive d and sensitized them to PCl 4 d after the last exposure. Four days after sensitization the donors were sacrificed. Splen and lymph nodes were suspended and injected intravenously into syngeneic, naive recipients. The recipients were challenged on the ears with PCl the next day, and ear swelling was measured 2 and 24 h after challenge. In duplicate experiments, we were not able to induce a 2-h swelling response (Fig 2a, panel C) nor a 24-h swelling response (Fig 2b, panel C) after transfer of lymphoid cells from UV-irradiated and PCl-sensitized donor mice, whereas both swelling reactions were present when the donor mice were not irradiated. (Fig 2a, panel A and Fig 2b, panel A).

Figure 1. Time course of the ear-swelling response in mice contact sensitized with PCl, with (UV, dashed line) or without (NR, solid line) prior exposure to UVR, showing the UVR-induced impairment of the 2- and 24-h swelling responses. DBA/2 mice were sensitized by contact painting with PCl, and their ears were challenged by topical application of PCl 4 d later. Before and at various times after challenge ear thickness was measured and the increase in ear thickness was calculated. The background increase in similarly challenged nonsensitized controls was subtracted from responses of sensitized animals at each time point. There were five mice/group. Units, 10⁻³ cm. Mean values ± SEM are given. *p < 0.05 for UV versus NR.
obtained when lymphoid cells from PCI-sensitized, non-irradiated donors are transferred 4 d after sensitization (Fig 2a, panel A). However, the reduction of the 24-h ear swelling reaction by UVR irradiation of the PCI sensitized donor mice (Fig 2b, panel C) was not abolished by addition of day 1 immune lymphoid cells to the day 4 UV-immune lymphoid cells (Fig 2b, panel D). This shows that the UV-induced reduction of the 24-h ear-swelling response is not solely due to the reduction of the 2-h ear-swelling response. If impairment of the early initiating phase is the primary effect of UVR on CHS, then reconstitution of the 2-h response should have resulted in reconstitution of the 24-h response as well.

DISCUSSION
In these studies the systemic effects of low-dose UVR on the early initiating phase and the late effector phase of CHS reactions to PCI were investigated. The UV-irradiation protocol that was used suppressed both the early and the late components in actively sensitized mice as well as prevented the adoptive transfer of either component with lymphoid cells from the irradiated, sensitized donors. The early, initiating phase of CHS, which is mediated by an antigen-specific T-cell factor, facilitates the full expression of the late effector phase of CHS [12,17]. UVR-induced impairment of the early, initiating phase could therefore result in a reduced expression of the late effector phase. In other words, the UVR-induced impairment of the late effector phase could be an indirect effect of UVR. To test this hypothesis, we co-transferred lymphoid cells from UVR-exposed donor mice that were sensitized 4 d earlier together with lymphoid cells taken from non-irradiated animals 1 d after PCI sensitization. The latter cells are known to transfer only the early initiating phase of CHS. Transfer of immune lymphoid cells from UVR-exposed donor mice that were sensitized 4 d earlier resulted in the absence of the early swelling and a weak late swelling response after challenge of the recipients. Addition of day 1 immune lymphoid cells from PCI-sensitized, non-irradiated animals to day 4 immune lymphoid cells from UVR-irradiated, PCI-sensitized animals reconstituted the early ear-swelling response, but failed to re-establish the late swelling response. If the UVR-induced impairment of the late ear-swelling response was in fact due to a reduction of the early response, reconstitution of the early response (“preparing the way”) should have resulted in reconstitution of the late ear-swelling response. That this did not occur indicates that UVR-induced impairment of both phases is regulated by two different mechanisms.

From earlier studies it was already known that UVR could impair the late effector phase in DTH in both local and systemic ways. Work by several investigators has shown that the UVR-induced immunosuppression of CHS is associated with the appearance of UVR-induced antigen-specific suppressor T lymphocytes [29]. These UVR-induced suppressor T lymphocytes produce hapten-specific suppressor factor(s) in vivo that inhibit CHS response in vivo [30].

Several possible mechanisms have been suggested to explain UVR-induced activation of suppressor T cells. Direct action of UVR on photoreceptors in the skin such as DNA [31,32] or uracil-DNA glycosylase [33,34] has been proposed as the initiating step in the generation of suppressor T cells. Suppressor factors released from keratinocytes upon UVR appear to be involved in the generation of suppressor T cells. These include interleukin-1 [35] or interleukin-1, a-2 induces generation of prostaglandins [36] and tumor necrosis factor-α [37] as well as a 20-kD serum factor described by Schwartz et al. [38] that is possibly identical to factors described by Schwarz [39] and Molendijk. Tumor necrosis factor-α is probably one of the most important products released by keratinocytes after UVR exposure and may be responsible for UVR-induced suppression of CHS by affecting the activity of Langerhans cells [40]. The susceptibility for UVR-induced immunosuppression appears to be genetically determined. The lipopolysaccharide locus in mice correlates very well with UVR susceptibility trait. Moreover tumor necrosis factor-α, a cytokine produced extensively by mice that possess the lipopolysaccharide allele, appears to mediate UVR-induced suppression of the cellular immune system. Intradermal injection of tumor necrosis factor-α at the sites of hapten application mimics the effects of UVR by impairing the induction of CHS. Another possible mechanism leading to UVR-induced suppression of CHS may be the altered expression of co-stimulatory molecules such as intercellular adhesion molecule-1 and CD80/BB1. It has recently been demonstrated that UVR can diminish intercellular adhesion molecule-1 expression, leading to impaired migration of lymphocytes and dendritic cells to the epidermis [41]. This will result in less inflammatory infiltrate at 24–48 h after ear challenge, thereby resulting in a reduced late swelling reaction. The B7/BB1 expression is inhibited by UVR in a dose-dependent way [42]. Because the interaction of B7/BB1 on dendritic cells and CD28 on T cells is known to stimulate T-cell proliferation by a direct effect on interleukin-2 gene transcription and production [43,44], inhibition of B7/BB1 by UVR could result in T-cell anergy. This is in agreement

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with the findings of Simont et al [45], who showed that UV induces a long-lasting anergy of Th1 cells, but not of Th2 cells, which results from a lack in their ability to produce interleukin-2.

The different mechanisms of UVR-induced immunosuppression are recently reviewed by Goettsc h et al [46]. The precise modes of action of the skin-derived UVR-induced substances are still unclear, as is the mechanism by which the UVR-induced suppressor T cells are activated.

The mechanisms described only account for the suppression of the late effector phase of DTH reactions. In the present study, evidence is provided that the early initiating phase of CHS is also impaired by UVR. Moreover, this impairment of the early initiating phase seems to be regulated by another mechanism than the impairment of the late effector phase of CHS.

From earlier studies it is known that the expression of the early initiating phase of CHS is dependent on the production of an antigen-specific T-cell factor [17]. The production of this factor is regulated by a feedback mechanism. The factor induces T cells with a suppressor function to produce non-antigen-specific suppressor factors that suppress the T cells that are responsible for production of the DTH-initiating, antigen-specific T-cell factors [47,48]. Therefore, the factor suppresses indirectly the initiation of DTH responses to contact sensitizers, sheep erythrocytes, and allogeneic tumor cells [47-49] in a non-antigen-specific manner. It might well be possible that UVR induces the production of an UVR-specific factor that impairs the initiation of the immune response to PCL. However, there is no evidence (yet) for the presence of an UVR-specific factor. Whether tumor necrosis factor-α or other cytokines that can inhibit CHS are also involved in the suppression of the early phase reaction is not known. Adhesion molecules such as intercellular adhesion molecule-1, which are necessary for the migration of cells into the epidermis, are thought to be involved in UVR-induced immunosuppression [50]. The early swelling reaction is not due to cellular infiltrate [24] but due to an increased vascular permeability, i.e., an edematous swelling reaction. For this reason a role for adhesion molecules alterations due to suppression of the early swelling reaction is not likely.

A possible mechanism by which UVR could impair both early and late phases of the CHS response to PCL lies in the observation that both early and late phases of DTH can be reduced by systemic administration of transforming growth factor-β at the time of antigen challenge [51]. UV irradiation of murine skin might result in a systemic increase in the release of transforming growth factor-β, thus mimicking the effect of systemic administration. However, although it is demonstrated that the levels of various epidermal cytokines are increased after UV irradiation (reviewed in [52]), it has not (yet) been reported that transforming growth factor-β release is increased by UVR exposure.

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