Interleukin-12 Prevents Ultraviolet B-Induced Local Immunosuppression and Overcomes UVB-Induced Tolerance

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Ultraviolet (UV) light abrogates contact hypersensitivity (CHS) responses and induces hapten-specific tolerance. Because Th-1 cells are critically involved in CHS and are induced to develop by the cytokine interleukin (IL)-12, we asked whether IL-12 might overcome UV-induced local immunosuppression. C3H/HeN mice exposed to low doses of UV light over 4 d and hapten sensitized through the irradiated skin area with dinitrofluorobenzene showed profound inhibition of the CHS response, which was completely prevented upon intraperitoneal injection of murine recombinant IL-12 (rIL-12) after the last UV exposure. UV-treated mice resensitized 14 d after the first challenge displayed hapten-specific tolerance, whereas UV-exposed mice injected with rIL-12 before the first sensitization exhibited a vigorous CHS response. Furthermore, mice that were initially sensitized through UV-exposed skin also produced a significant CHS reaction when they received rIL-12 before resensitization. Adoptive transfer of spleen and lymph node cells from UV-irradiated mice treated with rIL-12 had no effect on the CHS response in recipient mice, whereas transfer of cells from UV-treated mice inhibited the immune response. These findings demonstrate that rIL-12 can prevent UV-induced local immunosuppression and overcome UV-induced hapten-specific tolerance. ***Key words: ultraviolet light/contact hypersensitivity. J Invest Dermatol 106:1187–1191, 1996***

Ultraviolet (UV) radiation, in particular UVB (290–320 nm), has suppressive effects on the immune system (Kripke, 1990). UV light can induce a systemic form of immunosuppression, as suggested by the disruption of delayed-type hypersensitivity and contact hypersensitivity (CHS) to haptens that are injected or applied to distant, unirradiated skin areas in mice chronically exposed to high-dose UV light (Jessup *et al*, 1978; Noonan *et al*, 1981). Because UVB light is almost completely absorbed within the epidermis and cannot directly affect cells outside the irradiated layer, one potential mechanism involves the release of immunomodulatory cytokines by epidermal cells (Schwarz *et al*, 1994b). Findings by Rivas and Ullrich (1992) suggest a primary role for the cytokine interleukin (IL)-10 in mediating UV-induced systemic immunosuppression.

Toews *et al* (1980) showed that acute low-dose UV exposure of skin drastically reduces the number of epidermal Langerhans cells and that application of reactive haptens on the cutaneous areas depleted of Langerhans cells by UV light results in the absence of CHS. Unlike the observations after chronic exposure to high-dose UV light (Jessup *et al*, 1978; Noonan *et al*, 1981), CHS was induced when hapten was applied to cutaneous sites at a distance from the UV-treated site, indicating that the UV effect was local rather than systemic. Like the systemic alterations by high-dose UV light, local immunosuppression induced by low-dose UV leads to hapten-specific tolerance as well. Despite similar biologic consequences, however, different mechanisms appear to be involved in systemic and local immunosuppression: (i) Local immunosuppression can be induced only in certain genetically defined mouse strains (C3H/HeN, C57BL/6), whereas other strains (BALB/c, C3H/HeJ) are resistant to low-dose UV light (Streilein and Bergstresser, 1988); and (ii) in systemic immunosuppression, the release of IL-10 appears to be important (Ullrich, 1994), whereas local immunosuppression seems to act primarily on antigen-presenting cells, probably via the release of tumor necrosis factor-α (Toews *et al*, 1980; Aberer *et al*, 1981; Vermeer and Streilein, 1990; Yoshikawa and Streilein, 1990).

IL-12 is a recently described heterodimeric cytokine composed of covalently linked 35-kDa and 40-kDa subunits (Chehimi and Trinchieri, 1994). IL-12 is expressed by phagocytic cells, B lymphocytes, and, as shown recently, by keratinocytes and dendritic cells (Aragane *et al*, 1994; Müller *et al*, 1994; Macatonia *et al*, 1995). Besides its stimulatory effects on both natural killer cells (Kobayashi *et al*, 1989) and cytotoxic T lymphocytes (Stern *et al*, 1990), IL-12 has co-stimulatory and regulatory effects on T-helper cells. Recently, IL-12 was found to be critically involved in the development of Th1 cells (Hsie *et al*, 1993; Manetti *et al*, 1993, 1994; Trinchieri, 1993; Wu *et al*, 1993). Because Th1 cells appear to play an essential role in mediating CHS (Hauser, 1990), Schmitt *et al* (1995) recently investigated whether IL-12 could prevent systemic UV-induced immunosuppression. Administration of recombinant IL-12 (rIL-12) blocked systemic suppression of delayed-type hy-

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Abbreviations: CHS, contact hypersensitivity; r, recombinant.

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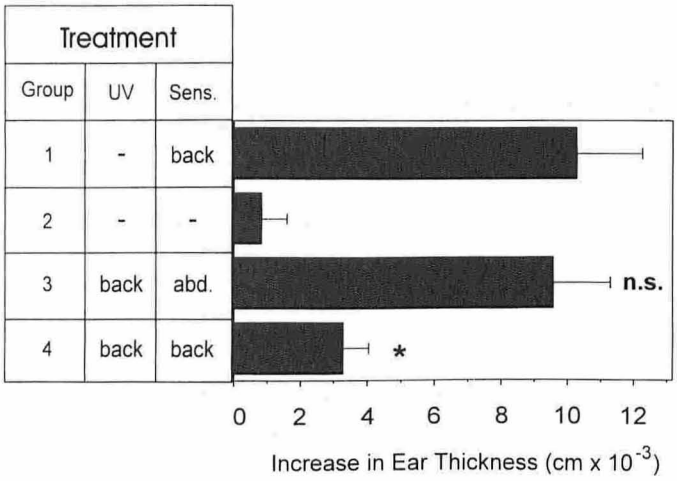

Figure 1. UV light at 4 × 1,000 J/m^2 does not induce systemic immunosuppression. Mice were treated daily with UV light (1,000 J/m^2) on 4 consecutive days on the shaved back and sensitized (sens.) 24 h after the last exposure through UV-exposed back skin (group 4) or through unirradiated abdominal (abd.) skin (group 3). Five days later, mice were challenged on the left ear, and ear swelling was measured 24 h later. Positive control mice were sensitized and challenged (group 1), whereas negative control animals were only ear challenged (group 2). Ear-swelling response is expressed as the difference (cm × 10^{-3}, mean ± SD) between the thickness of the challenged ear and that of the vehicle-treated ear. *p < 0.001 *vs* positive control.

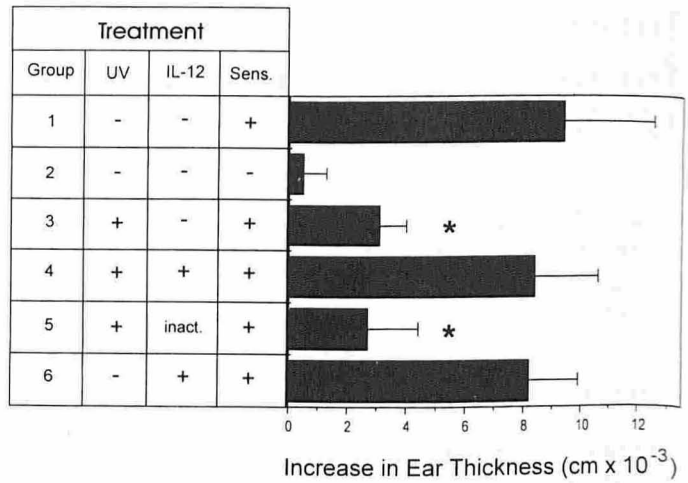

Figure 2. Recombinant IL-12 prevents UV-induced local immunosuppression. Mice were treated daily with UV light (1,000 J/m^2) on 4 consecutive days on the shaved back and sensitized (sens.) 24 h after the last exposure through UV-exposed back skin. Five days later, mice were challenged on the left ear, and ear swelling was measured 24 h later (group 3). Group 4 received 100 ng of rIL-12 i.p. at 21 h after the last UV exposure; group 5 was treated with heat-inactivated (inact.) IL-12; group 6 was treated as in group 4 but was not UV irradiated. Positive control mice were sensitized and challenged (group 1), whereas negative control animals were only ear challenged (group 2). Ear-swelling response is expressed as the difference (cm × 10^{-3}, mean ± SD) between the thickness of the challenged ear and that of the vehicle-treated ear. *p < 0.001 *vs* positive control.

persensitivity and CHS in mice given a single high-dose UV exposure. Those data clearly demonstrated that IL-12 can overcome UV-induced systemic immunosuppression, most likely by antagonizing IL-10. Because local and systemic immunosuppression appear to be mediated via different pathways, we investigated whether IL-12 can prevent local UV-induced immunosuppression and overcome hapten-specific tolerance induced by low-dose UV exposure.

MATERIALS AND METHODS

Mice C3H/HeN mice (8–12 weeks old) were purchased from Charles River Deutschland (Sulzfeld, Germany).

Contact Hypersensitivity Mice were sensitized by painting 25 μl of 2,4-dinitrofluorobenzene (DNFB; Sigma Corp., St. Louis, MO) solution (0.5% in acetone:olive oil, 4:1) on the shaved back on day 0, as described (Schwarz *et al*, 1994a). On day 5, 20 μl 0.3% DNFB was applied to the left ear, and acetone/olive oil was applied to the right ear. Ear swelling was quantified with a spring-loaded micrometer (Mitutoyo, Japan) 24 h after challenge. CHS was determined as the amount of swelling of the hapten-challenged ear compared with the thickness of the vehicle-treated ear in sensitized animals and was expressed in cm × 10^{-3} (mean ± SD). Mice that were ear-challenged without prior sensitization served as negative controls. Resensitization was performed as described above through nonirradiated abdominal skin 14 d after the first challenge. The second challenge was performed on the right ear 5 d after the second sensitization.

UV Irradiation The shaved back was exposed to UV light from a bank of four FS-20 fluorescent lamps (Westinghouse Electric Corp., Pittsburgh, PA), which emit most of their energy within the UVB range (290–320 nm) with an emission peak at 313 nm. The UV output measured at 310 nm using an IL1700 research radiometer (International Light, Newport, MA) was 8.0 W/m^2 at a tube-to-target distance of 28 cm. Mice were exposed to UVB daily for 4 consecutive days (1,000 J/m^2 per exposure). Twenty-four hours after the last UV exposure, DNFB was applied carefully to the surface of the irradiated area, as described above. The UV regimen applied did not cause systemic immunosuppression **(Fig 1)**.

Injection of rIL-12 Recombinant IL-12 was kindly provided by S. Wolf (Genetics Institute, Cambridge, MA). For *in vivo* injection, rIL-12 was diluted in sterile endotoxin-free saline and injected intraperitoneally (i.p.) in doses ranging from 10 to 500 ng. Control mice were treated i.p. with equal volumes of saline, which had no effect on the outcome of the sensitization procedure or on the suppressive effect of UVB exposure. Heat-inactivated rIL-12 (95°C for 30 min) was used as a negative control.

Adoptive Transfer of Immune Response Donor mice were exposed to UV light and sensitized with DNFB through UV-exposed skin, and were ear-challenged 5 d later. Ten days thereafter, spleens and regional lymph nodes were removed, and single-cell suspensions were prepared. Cell number was adjusted to 2.5 × 10^8 cells/ml, and 200 μl was injected intravenously into each recipient mouse. Recipients were sensitized 24 h later by epicutaneous application of DNFB on the shaved abdomen. After 5 d, mice were challenged on the left ear, and ear swelling was evaluated 24 h later. One group of donor mice was treated with rIL-12 after the last UV exposure.

Statistical Analysis Data were analyzed by Student's t test, and differences were considered significant at $p < 0.05$. Each experiment was performed at least three times.

RESULTS

rIL-12 Prevents UV-Induced Local Immunosuppression Mice were exposed on the shaved back skin to a low-dose UV regimen, i.e., four consecutive daily exposures to UV light (1,000 J/m^2/d), and were sensitized 24 h after the last exposure by topical application of DNFB onto the UV-exposed skin. Positive control mice received the same dose of DNFB but were not UV irradiated. To determine the degree of sensitization, we challenged the ears of these mice 5 d later and determined the ear-swelling response 24 h later. As shown in **Fig 2**, application of DNFB to UV-exposed skin resulted in failure to induce sensitization. In contrast, mice injected with 100 ng of rIL-12 i.p. at 21 h after the last UV exposure and sensitized through UV-exposed skin 3 h later were fully sensitized and responded with a vigorous CHS response. Injection of rIL-12 into mice that had not been UV exposed, but were sensitized and challenged identically to positive control mice, did not significantly affect the ear-swelling response. As a control for the possibility that the effect on UV-induced local immunosuppression was due to contaminants such as lipopolysaccharide, rIL-12 was heat-inactivated by boiling. The ear-swelling response in mice that were UV exposed and treated with inactivated rIL-12 was as weak as that

Table I. Dose Response[a]

UV Light[b]	IL-12[c] (ng)	Sensitization[d]	Increase in Ear Thickness[e]
			0.5 ± 0.5
		DNFB (0.5%)	7.7 ± 2.8
+		DNFB (0.5%)	2.5 ± 0.5[f]
+	10	DNFB (0.5%)	2.8 ± 1.3[f]
+	50	DNFB (0.5%)	3.8 ± 1.3[f]
+	100	DNFB (0.5%)	6.1 ± 1.0
	100	DNFB (0.5%)	7.5 ± 3.2

[a] IL-12 prevents UVB-induced suppression of CHS in a dose-dependent manner.
[b] Mice were exposed to four UV exposures (1,000 J/m^2) on 4 d on the shaved back.
[c] rIL-12 was injected i.p. 21 h after the last UV exposure.
[d] Sensitization was performed on back skin 24 h after the last UV exposure. All groups were challenged 5 d thereafter with 0.3% DNFB applied to the left ear.
[e] Increase in ear thickness was measured 24 h after challenge and expressed in cm × 10^{-3} (mean ± SD).
[f] $p < 0.005$ *vs* positive control (sensitized and challenged).

observed in mice that were only UV exposed before sensitization (**Fig 2**). The effect of rIL-12 on UV-induced suppression of CHS was dose dependent, and the CHS response in mice injected with 100 ng was almost as pronounced as that in positive control mice (**Table I**).

rIL-12 Prevents UV-Induced Tolerance Hapten application on UV-irradiated skin not only fails to induce CHS, but also generates hapten-specific tolerance (Toews *et al*, 1980; Elmets *et al*, 1983). Because injection of rIL-12 after UV exposure prevented the UV-induced suppression of sensitization, mice were rechallenged after a resting period of 14 d. Mice exposed to UV light and subsequently sensitized through the UV-exposed skin area did not respond to the rechallenge with ear swelling, suggesting that the suppression of CHS by UV is long-lasting (**Fig 3**). In contrast, mice treated with 100 ng of rIL-12 after the last UV exposure and before sensitization revealed moderate ear swelling, which was significantly enhanced compared with that of mice that were only UV

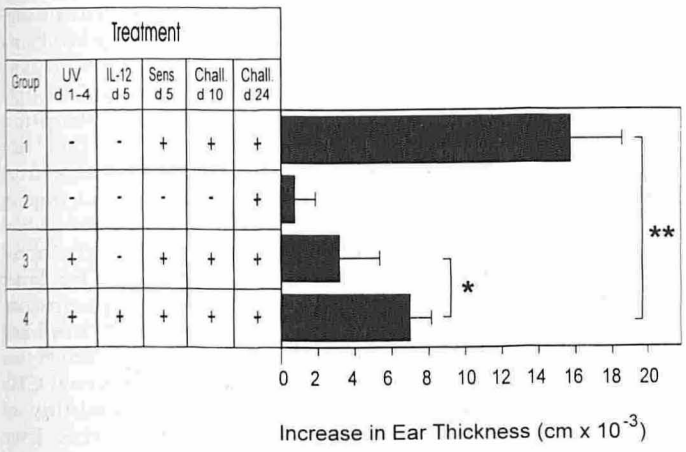

Figure 3. The preventive effect of 100 ng rIL-12 is not long lasting. Mice were treated daily with UV light (1,000 J/m^2) on 4 consecutive days on the shaved back and sensitized (sens.) 24 h after the last exposure through UV-exposed back skin. Five days later (day 10), mice were challenged (chall.) on the left ear. Fourteen days after the first challenge (day 24), mice were rechallenged on the right ear, and ear-swelling response was measured 24 h thereafter. Group 4 received 100 ng of rIL-12 i.p. at 21 h after the last UV exposure. Positive control mice were sensitized, challenged, and rechallenged (group 1), whereas negative control animals were only ear challenged on day 24 (group 2). Ear-swelling response is expressed as the difference (cm × 10^{-3}, mean ± SD) between the thickness of the challenged ear and that of the vehicle-treated ear. $^*p < 0.05$; $^{**}p < 0.001$.

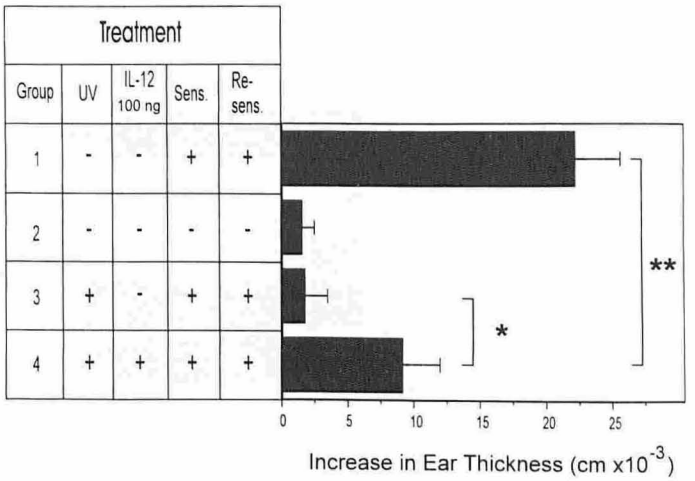

Figure 4. Recombinant IL-12 at 100 ng prevents the induction of UV-induced tolerance only partially. Mice were treated daily with UV light (1,000 J/m^2) on 4 consecutive days on the shaved back, sensitized (sens.) through UV-exposed back skin 24 h after the last UV exposure, and challenged on the left ear 5 d later. After 14 d, mice were resensitized (resens.) through the nonirradiated shaved abdominal skin and challenged on the right ear 5 d later (group 3). Group 4 received 100 ng of rIL-12 i.p. 21 h after the last UV exposure. Positive control mice were double-sensitized and challenged (group 1); negative control animals were ear challenged only (group 2). Ear-swelling response is expressed as the difference (cm × 10^{-3}, mean ± SD) between the thickness of the challenged left ear and that of the ear measured immediately before challenge. $^*p < 0.005$; $^{**}p < 0.001$.

treated and sensitized. Compared with positive control mice, which were only sensitized and challenged, however, the ear-swelling response was significantly lower.

To examine the effect of rIL-12 on the induction of hapten-specific tolerance by UV radiation, we performed resensitization experiments. Groups of mice were sensitized through UV-exposed skin with or without i.p. injection of 100 ng of rIL-12 at 21 h after the last UV exposure. Sensitization through UV-exposed skin was performed 3 h thereafter. Positive control mice were sensitized without prior UV exposure. After 14 d, all mice were sensitized a second time through nonirradiated abdominal skin. In positive control mice, this treatment resulted in a vigorous ear-swelling response upon challenge 5 d later (**Fig 4**). Mice that were initially sensitized through UV-exposed skin did not show an ear-swelling response after resensitization with the same hapten through non–UV-exposed skin, suggesting that hapten-specific tolerance was induced. In contrast, mice that received a single dose of 100 ng of rIL-12 before primary sensitization and were resensitized 14 d later in the absence of UV light or IL-12 injection showed a specific ear-swelling response. Although this response was significantly greater than that of UV-treated, non–IL-12-injected mice, the response was significantly less pronounced than that of positive control mice, which were not UV irradiated at all (**Fig 4**).

To determine whether this incomplete restoration induced by rIL-12 was due to an insufficient dose of rIL-12, we performed experiments in which mice were injected with 500 ng of rIL-12 immediately after UV exposure and again at 3 h before sensitization. This regimen allowed an almost complete immune response upon resensitization (**Fig 5**), suggesting that i.p. injected rIL-12 prevents induction of hapten-specific tolerance mediated by low-dose UV light. To determine whether rIL-12 is also able to overcome UV-induced tolerance, mice initially tolerized by hapten application through UV-exposed back skin received two 500-ng doses of rIL-12 before resensitization through nonirradiated skin (**Fig 5**, *group 5*). Whereas mice tolerized by hapten application through UV-treated skin remained tolerant after application of a

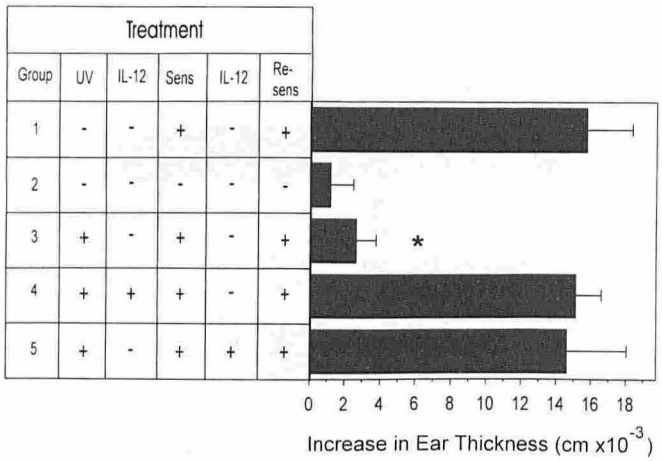

Figure 5. Recombinant IL-12 at 2 × 500 ng prevents UV-induced tolerance. Mice were treated daily with UV light (1,000 J/m^2) on 4 consecutive days on the shaved back, sensitized (sens.) through UV-exposed back skin 24 h after the last UV exposure, and challenged on the left ear 5 d later. After 14 d, mice were resensitized (resens.) through the nonirradiated shaved abdominal skin and challenged on the right ear 5 d later (group 3). Group 4 received 500 ng of rIL-12 i.p. immediately after the last UV exposure and 3 h before sensitization, whereas group 5 received 500 ng of rIL-12 24 and 3 h before resensitization. Positive control mice were double-sensitized and challenged (group 1); negative control animals were ear challenged only (group 2). Ear-swelling response is expressed as the difference (cm × 10^{-3}, mean ± SD) between the thickness of the challenged left ear and that of the ear measured immediately before challenge. *$p < 0.0001$ *vs* positive control.

second sensitizing dose of hapten to nonirradiated skin 14 d later, mice injected with rIL-12 before resensitization showed a specific CHS response comparable to that of double-sensitized positive control mice (**Fig 5**, *group 1*), suggesting that rIL-12 can overcome UV-induced tolerance.

Adoptive Transfer In light of previous findings demonstrating that the suppression induced by sensitization through UV-exposed skin is transferable by injection of lymphocytes from tolerized animals (Elmets *et al*, 1983), we tested whether rIL-12 might counteract such adoptive transfer. Spleen cells and regional lymph node cells were obtained from mice sensitized through UV-irradiated skin 10 d after challenge and transferred intravenously to normal recipients. Recipients were sensitized with DNFB 24 h after transfer and challenged 5 d later. The CHS response of mice injected with cells from UV-irradiated donor mice was remarkably suppressed, whereas recipients of cells from UV-irradiated and rIL-12–treated mice showed a still significant CHS response to DNFB **(Table II)**.

Table II. Effect of IL-12 on Adoptive Transfer of Immune Response[a]

Group	Cells Transferred[b]	Treatment of Recipients[c]	Increase in Ear Thickness[d]
1	None	None	0.8 ± 0.7
2	None	DNFB	9.7 ± 3.5
3	Normal	DNFB	9.1 ± 2.8
4	UV + DNFB	DNFB	3.0 ± 1.7[e]
5	UV + IL-12 + DNFB	DNFB	6.1 ± 1.2

[a] Treatment of donor mice with IL-12 prevents CHS suppression by adoptive splenocyte transfer.

[b] Spleen and lymph node cells were obtained from untreated mice (group 3), from mice sensitized through UV-exposed skin (group 4), or from mice that received IL-12 before sensitization through UV-exposed skin (group 5). At 10 d after sensitization, 5 × 10^7 cells were injected intravenously into normal recipients.

[c] Mice were sensitized with DNFB 24 h after cell transfer.

[d] Increase in ear thickness was measured 24 h after challenge and expressed in cm × 10^{-3} (mean ± SD).

[e] $p < 0.005$ *vs* positive controls (group 2 and 3, respectively).

DISCUSSION

Because the mechanisms involved in systemic and local immunosuppression induced by UV light differ, we analyzed whether IL-12 can overcome local immunosuppression. These data clearly demonstrate that a single i.p. injection of rIL-12 given after the last UV exposure and before hapten application to the irradiated skin area completely prevents suppression of CHS. Although rather low, and thus physiologically relevant, doses (1,000 J/m^2) were used, a pronounced inhibition of the immune response was achieved by this irradiation procedure in most experiments. On the other hand, the UV doses applied did not cause systemic effects. Interestingly, rather low concentrations of IL-12 (100 ng) sufficed to restore the immune response. In contrast, Schmitt *et al* (1995) had to inject at least 500 ng or 1 μg to antagonize systemic immunosuppression, whereas 100 ng had no effect. The preventing effect of IL-12 was specific and was not due to putative contaminants in the preparation, as heat-inactivated IL-12 was ineffective. We did not try to neutralize this *in vivo* effect of rIL-12 by concurrent injection with an anti–IL-12 antibody because we observed recently that such injection itself prevents the induction of CHS and induces hapten-specific tolerance (Riemann *et al*, 1996).

Because sensitization through UV-exposed skin not only results in the suppression of CHS, but also induces hapten-specific tolerance (Toews *et al*, 1980; Elmets *et al*, 1983), we analyzed whether the effect of IL-12 is long-lasting in mice rechallenged after a resting period of 14 d. Although the ear-swelling response in mice receiving 100 ng of rIL-12 was specific and statistically significant when compared with nonsensitized, but ear-challenged negative control mice, the response was remarkably less pronounced than that of positive control animals. In subsequent experiments, however, injection of two 500-ng doses between UV exposure and first sensitization, respectively, enabled a complete CHS response upon rechallenge. This dose was completely effective not only when IL-12 was injected before the first sensitization, but also when it was given exclusively before resensitization (**Fig 5**, *group 5*). These findings demonstrate that IL-12 prevents UV-induced immunosuppression upon early injection and also does so when injected into mice tolerized by application of haptens through UV-exposed skin. Unresponsiveness to DNFB produced by *in vivo* low-dose UV light can be adoptively transferred (Elmets *et al*, 1983), indicating that suppressor cells are generated by this regimen. The fact that immunosuppression was reduced in mice receiving cells from UV-irradiated and IL-12–treated donors suggests that IL-12 impairs the induction of suppressor cells. Alternatively, IL-12 might induce effector cells, presumably Th1 cells, that are concurrently transferred and overcome the activity of suppressor cells. The latter possibility appears to be likely because, in our transfer experiments, immunosuppression was only reduced and not completely inhibited by IL-12. Moreover, this is supported by the recent observation that suppressor T cells can be present in mice that display a normal CHS response (Glass *et al*, 1990). Because of the unavailability of appropriate antibodies to perform depletion studies, this issue remains unresolved.

The mechanism by which IL-12 prevents UV-induced local immunosuppression remains to be clarified. Because low-dose UV light results in depletion of Langerhans cells (Toews *et al*, 1980; Aberer *et al*, 1981), one possible mechanism by which IL-12 can prevent local suppression includes the rescue of Langerhans cells from UV damage. This seems unlikely, however, based on our preliminary experiments indicating that the number of Ia+ cells detected in epidermal cell suspensions by fluorescence-activated cell sorter analysis is equally reduced in skin from UV-exposed mice and in skin from mice treated with IL-12 after UV exposure. Moreover, in our system, IL-12 is not applied before UV irradiation, but after four UV light exposures at daily intervals. A variety

of cells within the skin have to be considered in that respect, e.g., macrophages, dermal dendritic cells, and endothelial cells. At present, we cannot exclude the involvement of macrophages, as preliminary experiments show that the restoring effect is not observed after *in vivo* depletion of macrophages by injection of dichloromethylene-diphosphonate-containing liposomes (van Roijen, 1989). Using this method, Kurimoto *et al* (1994) recently demonstrated that liposomes prevented the induction of CHS when hapten was painted on UV-exposed BALB/c mice, a strain that develops CHS when hapten is applied to UV-exposed skin. Those findings suggest that macrophages provide antigen-presenting cell function in the skin of UV-resistant mice after UV exposure. On the other hand, our preliminary experiments gave no indication that *in vitro* treatment of macrophages with IL-12 enables induction of the primary mixed lymphocyte reaction by these cells. The mechanism by which IL-12 prevents UV-induced local immunosuppression therefore remains to be determined.

NOTE IN PROOF

While this paper was in press, similar observations were reported by Müller *et al*, demonstrating that injection of IL-12 can prevent and reverse UV-induced tolerance (*J Immunol* 155:4661–4668, 1995).

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REFERENCES

Aberer W, Schuler G, Stingl G, Hönigsmann H, Wolff K: Ultraviolet light depletes surface markers of Langerhans cells. *J Invest Dermatol* 76:202–210, 1981

Aragane Y, Riemann H, Bhardwaj RS, Schwarz A, Sawada Y, Yamada H, Luger TA, Kubin M, Trinchieri G, Schwarz T: IL-12 is expressed and released by human keratinocytes and epidermoid carcinoma cell lines. *J Immunol* 153:5366–5372, 1994

Chehimi J, Trinchieri G: Interleukin-12: a bridge between innate resistance and adaptive immunity with a role in infection and acquired immunodeficiency. *J Clin Immunol* 14:149–161, 1994

Elmets CA, Bergstresser PR, Tigelaar RE, Wood PJ, Streilein JW: Analysis of the mechanism of unresponsiveness produced by haptens painted on skin exposed to low dose ultraviolet radiation. *J Exp Med* 158:781–794, 1983

Glass MJ, Bergstresser PR, Tigelaar RE, Streilein JW: UVB radiation and DNFB skin painting induce suppressor cells universally in mice. *J Invest Dermatol* 94:273–278, 1990

Hauser C: Cultured epidermal Langerhans cells activate effector T cells for contact sensitivity. *J Invest Dermatol* 95:436–440, 1990

Hsie CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM: Development of TH1 CD4+ cells through IL-12 produced by Listeria-induced macrophages. *Science* 260:547–549, 1993

Jessup JM, Hanna N, Palaszynski E, Kripke ML: Mechanisms of depressed reactivity to dinitrochlorobenzene and ultraviolet-induced tumors during ultraviolet carcinogenesis in BALB/c mice. *Cell Immunol* 38:105–115, 1978

Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, Loudon R, Sherman F, Perussia B, Trinchieri G: Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biological effects on human lymphocytes. *J Exp Med* 170:827–845, 1989

Kripke ML: Photoimmunology. *Photochem Photobiol* 52:919–924, 1990

Kurimoto I, van Rooijen N, Dijkstra CD, Streilein JW: Role of phagocytic macrophages in induction of contact hypersensitivity and tolerance by hapten applied to normal and ultraviolet B-irradiated skin. *Immunology* 82:281–287, 1994

Macatonia SE, Hosken NA, Litton M, Vieria P, Hsieh C, Culpepper JA, Wysocka M, Trinchieri G, Murphy KM, O'Garra A: Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. *J Immunol* 154:5071–5079, 1995

Manetti R, Gerosa F, Giudizi MG, Biagiotti R, Parronchi P, Piccinni MP, Sampognaro S, Maggi E, Romagnani S, Trinchieri G: Interleukin 12 induces stable priming for interferon-γ (IFN-γ) production during differentiation of human T helper (Th) cells and transient IFN-γ production in established Th2 cell clones. *J Exp Med* 179:1273–1278, 1994

Manetti R, Parronchi P, Giudizi MG, Piccinni MP, Maggi E, Trinchieri G, Romagnani S: Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 177:1199–1204, 1993

Müller G, Saloga J, Germann T, Bellinghausen I, Mohamadzadeh M, Knop J, Enk AH: Identification and induction of human keratinocyte-derived IL-12. *J Clin Invest* 94:1799–1805, 1994

Noonan FP, DeFabo EC, Kripke ML: Suppression of contact hypersensitivity by UV radiation and its relationship to UV-induced suppression of tumor immunity. *Photochem Photobiol* 34:683–689, 1981

Riemann H, Schwarz A, Grabbe S, Aragane Y, Luger TA, Wysocka M, Kubin M, Trinchieri G, Schwarz T: Neutralization of interleukin 12 *in vivo* prevents induction of contact hypersensitivity and induces hapten specific tolerance. *J Immunol* 156:1799–1803, 1996

Rivas JM, Ullrich SE:. Systemic suppression of DTH by supernatants from UV-irradiated keratinocytes: an essential role for IL-10. *J Immunol* 148:3133–3139, 1992

Schmitt DA, Owen-Schaub L, Ullrich SE: Effect of IL-12 on immune suppression and suppressor cell induction by ultraviolet radiation. *J Immunol* 154:5114–5120, 1995

Schwarz A, Grabbe S, Riemann H, Aragane Y, Simon M, Manon S, Andrade S, Luger TA, Zlotnik A, Schwarz T: *In vivo* effects of interleukin-10 on contact and delayed-type hypersensitivity reactions. *J Invest Dermatol* 103:211–216, 1994a

Schwarz T, Urbanski A, Luger TA: Ultraviolet light and epidermal cell derived cytokines. In: Luger TA, Schwarz T (eds.). *Epidermal Growth Factors and Cytokines.* Marcel Dekker, New York, 1994b, p 303

Stern AS, Podlaski FJ, Hulmes FD, Pan YC, Quinn PM, Wolitzky AG, Familetti PC, Stremlo DL, Truitt T, Chizzonte R, Gately MK: Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. *Proc Natl Acad Sci USA* 87:6808–6812, 1990

Streilein JW, Bergstresser PR: Genetic basis of ultraviolet-B effects on contact hypersensitivity. *Immunogenetics* 27:252–258, 1988

Toews GB, Bergstresser PR, Streilein JW: Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J Immunol* 124:445–453, 1980

Trinchieri G: Interleukin-12 and its role in the generation of Th1 cells. *Immunol Today* 14:335–338, 1993

Ullrich SE: Mechanisms involved in the systemic suppression of antigen-presenting cell function by UV irradiation. Keratinocyte-derived IL-10 modulates antigen-presenting cell function of splenic adherent cells. *J Immunol* 152:3410–3416, 1994

van Roijen N: The liposome-mediated macrophage 'suicide' technique. *J Immunol Methods* 124:1–6, 1989

Vermeer M, Streilein JW: UVB light induced alterations in epidermal Langerhans cells are mediated in part by tumor necrosis factor alpha. *Photodermatol Photoimmunol Photomed* 7:258–265, 1990

Wu CY, Demeure C, Kiniwa M, Gately M, Delespesse G: IL-12 induces the production of IFN-γ by neonatal human CD4 T cells. *J Immunol* 151:1938–1949, 1993

Yoshikawa T, Streilein JW: Tumor necrosis factor alpha and UVB light have similar effects on contact hypersensitivity in mice. *Reg Immunol* 3:139–144, 1990