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J Immunol 2010; 185:7207-7215; Prepublished online 15 November 2010; doi: 10.4049/jimmunol.1001693
http://www.jimmunol.org/content/185/12/7207
Ultraviolet Irradiation of Mice Reduces the Competency of Bone Marrow-Derived CD11c+ Cells via an Indomethacin-Inhibitable Pathway

Royce L. X. Ng,* Jacqueline L. Bisley,* Shelley Gorman,* Mary Norval,† and Prue H. Hart*

Direct UV irradiation of dendritic cells and Langerhans cells reduces their Ag presenting ability. However, the effects of UV on CD11c+ cells located distally to the point of irradiation are poorly understood. Three days after UV irradiation (8 kJ/m2) of BALB/c mice, bone marrow cells were isolated and cultured for 7 d with IL-4 and GM-CSF for the propagation of CD11c+ cells. Bone marrow-derived CD11c+ cells from UV-irradiated or nonirradiated mice were loaded with dinitrobenzene sulfonic acid and injected into the ear pinnas of naive BALB/c mice. After 7 d, the ears were painted with 2,4-dinitro-1-fluorobenzene and the ear swelling determined 24 h later. A reduced contact hypersensitivity response was found in mice injected with CD11c+ cells from the UV-irradiated animals compared with those injected with cells from the nonirradiated animals. Further, a long-lasting suppression of the memory response to 2,4-dinitro-1-fluorobenzene was created. This suppressed response corresponded to increased IL-10 in UV-irradiated animals compared with those injected with cells from the nonirradiated animals. Further, a long-lasting suppression of the memory response to 2,4-dinitro-1-fluorobenzene was created. This suppressed response corresponded to increased IL-10 and PGE2 secretion by freshly isolated bone marrow cells from UV-irradiated mice, and to increased myelopoiesis. The reduction in competence of bone marrow-derived CD11c+ cells from UV-irradiated mice was not due to delayed maturation, as it was maintained upon LPS exposure prior to CD11c+ cell purification. The UV-induced effect was reversed by the administration of indomethacin to mice prior to UV irradiation and could be reproduced by s.c. PGE2. These results show that UV irradiation of mice can affect the function of bone marrow-derived CD11c+ cells via a mechanism inhibitable by indomethacin; this pathway is likely to contribute to systemic UV-induced immunosuppression.


UC

Ultraviolet radiation is an important environmental factor with diverse biological roles, including induction of skin cancer, skin aging, skin pigmentation, and immunomodulation (1, 2). The immunomodulatory effects of UV can be classified as either local or systemic. Local immunosuppression describes the compromised immune response to Ags applied directly to sites previously UV irradiated. Systemic immunosuppression defines the reduced immunity that accompanies Ag exposure at locations distal to the point of UV irradiation.

Several mechanisms for UV-induced systemic immunosuppression have been proposed (for review see Refs. 1–4). These include the involvement of soluble mediators such as IL-10 (5), an anti-inflammatory cytokine that promotes a shift toward Th2 immunity (6), cis-urocacidic acid formed from trans-urocanic acid in the epidermis on UV exposure (7–10), and prostanoids (8, 9), with PGE2 being the major PG induced by UV (11). Prostanoids can regulate a number of dendritic cell (DC) functions, including their maturation, migration, cytokine production, receptor expression, and apoptosis. The effects of PGE2 vary depending on the location and the maturation status of the DCs (12). In the periphery, when proinflammatory cytokines such as TNF-α are present, PGE2 can act as a cofactor to promote DC activation (13). However, in lymphoid organs, PGE2 inhibits Ag presentation by DCs to T cells (14).

Direct UV irradiation of Langerhans cells (15) and bone marrow-derived DCs (16) in vitro impairs their ability to present Ags to T cells. Various mechanisms have been implicated, including downregulation of costimulatory molecule expression (15) and reduction of IL-12 production (17). IL-12 is a cytokine produced by APCs and results in the activation of Th1 cells (18, 19). In addition, it can reverse UV-induced immunosuppression (20) and enhance the Ag-presenting ability of DCs (21). It is not certain whether DCs at sites distal to the location of UV irradiation are altered. Gorman et al. (22) and Lappin et al. (23) found that the function and phenotype of CD11c+ cells in lymph nodes distal to UV exposure was unchanged at 1, 2, and 4 d post UV irradiation. In contrast, Noonan and colleagues (24) demonstrated that the Ag-presenting ability of splenic DCs was reduced at 7, but not 3, d after UV irradiation, suggesting that UV irradiation of skin may affect the function of DCs in the spleen in a time-dependent manner. Any effect of UV irradiation of skin on bone marrow-derived DCs and their subsequent role in UV-induced immunosuppression has not been examined previously.

There are a number of DC subsets, characterized according to cell marker expression. However, the expression of CD11c is widely accepted as the principal marker of DCs (25). DCs can be grouped into two general classifications: conventional DCs, which possess dendritic form and demonstrate DC functions in the steady...
state, and precursors of DCs, which require further development to acquire DC functions. Conventional DCs can be further divided into migratory DCs (Langerhans cells and dermal DCs) and lymphoid tissue-resident DCs (CD8α and CD8ε conventional DCs), whereas precursors of DCs include plasmacytoid DCs and monocytes. In response to inflammation or microbial stimuli, precursors of DCs can give rise to nonsteady state inflammatory DCs. Bone marrow-derived CD11c+ cells are most frequently generated by culture of bone marrow cells with the combination of IL-4 and GM-CSF. These cells exhibit properties similar to those of inflammatory DCs. There is, however, developmental flexibility in DC populations, which can be controlled by environmental influences.

In this study, UV irradiation of mice led to functional changes in bone marrow-derived CD11c+ cells by a pathway inhibitable by indomethacin. As bone marrow is a source of DCs to replenish the periphery, we propose that this is a novel mechanism of UV-induced systemic immunosuppression that, to our knowledge, has not been recognized previously.

Materials and Methods

Mice

Female BALB/c mice were obtained from the Animal Resources Centre (Murdoch, Western Australia) and were used when 6–10 wk old. All experiments were performed with the approval of the Teledton Institute for Child Health Research Animal Ethics Committee according to the guidelines of the National Health and Medical Research Council of Australia.

UV irradiation

A bank of TL40W/12RS lamps (Philips, Amsterdam, The Netherlands) emitting broadband UV with 65% UVB (280–320 nm) and peak emission at 313 nm was used. Prior (24 h) to irradiation, a uniform area of dorsal skin of mice was shaved (8 cm²). Mice were put in individual compartments of perspex cages, which were covered with 0.2-mm polyvinyl chloride plastic to eliminate wavelengths <290 nm. The cages were placed 20 cm below the sunlamps, and a dose of 8 kJ/m² UV, equivalent to three to four minimal erythema doses, was delivered. This dose of UV has been shown in this and other laboratories to suppress systemic contact hyper-sensitivity responses by >50% in BALB/c mice (27, 28). The output was measured using a UVX radiometer (UVX Products, Upland, CA). The mice were irradiated 3 d prior to euthanasia and isolation of bone marrow cells.

Isolation of bone marrow cells

Tibias and femurs of mice were removed and flushed using a solution of 11 mM Na-glucose, 5.5 mM KCl, 137 mM NaCl, 25 mM Na₂HPO₄, and 5.5 mM NaH₂PO₄·2H₂O (glucose-potassium-sodium buffer [GKN]) containing 10% FCS. Bone marrow cells (pooled from all mice within a group) were passed through cotton wool to remove bone debris and were cultured in RPMI 10 (Thermo Scientific, Waltham, MA) containing 10% FCS, 2% l-glutamine, 50 μM 2-ME, and 5 μg/ml gentamicin (RPMI 10) at a density of 8 × 10⁶ cells/ml in 24-well plates. Cells were cultured in the presence of 10 ng/ml IL-4 and 10 ng/ml GM-CSF (Peprotech, Rocky Hill, NJ) for 7 d to promote CD11c+ development. The medium was replaced after 48 and 96 h. After 7 d in culture, nonadherent cells were harvested and enriched to >95% CD11c+ cells (confirmed by flow cytometry), using anti-CD11c magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany).

Bone marrow cells (8 × 10⁵ cells/ml) were also incubated with 1 μg/ml LPS for 24 h at the first or final 24 h of culture and the supernatants collected (day 0 and day 7 supernatants, respectively). For the cells previously cultured for 6 d, the nonadherent cells were harvested and counted, but not enriched for CD11c+ expression, before further culture with LPS for 24 h. Freshly isolated bone marrow cells were also enriched to >85% CD11b+ and CD11b- cells (confirmed by flow cytometry), using magnetic microbeads (Miltenyi Biotec). As performed with unsorted cells, CD11b+ and CD11b- cells (8 × 10⁶ cells/ml) were incubated with 1 μg/ml LPS for 24 h. The concentrations of IL-10 and IL-12p70 in supernatants were determined using dissociation-enhanced time-resolved fluorescence immunoassays (IL-10 sensitivity, 50 pg/ml; IL-12 sensitivity, 20 pg/ml; BD Biosciences, Franklin Lakes, NJ), Europium (PerkinElmer, Waltham, MA) was used as the detection label. PGE₂ levels in bone marrow cell culture supernatants were determined using a PGE₂ EIA Kit according to the manufacturer’s instructions (Cayman Chemical, Ann Arbor, MI; sensitivity, 15 pg/ml).

Phenotyping of bone marrow and auricular lymph node cells

All cells were phenotyped using a washing solution of GKN containing 0.2% w/v BSA (Sigma-Aldrich, St. Louis, MO). Cells were preincubated with anti-CD16/CD32 Fc receptor Abs (BD Biosciences) for 5 min to prevent nonspecific Ab binding. Ab incubations and cell washes were performed at 4°C. Freshly isolated bone marrow cells were phenotyped, as previously described (29), by incubating cells for 30 min with Abs identifying erythroid, myeloid, and lymphoid precursors. Bone marrow cell cultures for 3 d were incubated for 30 min with biotinylated anti-CD44, biotinylated anti-CD80, or biotinylated anti-CD86. Freshly isolated auricular lymph node (ALN) cells were incubated for 30 min with FITC–anti-CD3, PE-Cy5–anti-CD4 and PE–anti-FoxP3, or FITC–anti-MHC class II (I-A/I-E), PE–anti-CD11c, biotinylated anti-B220, and allophycocyanin–anti-CD8. Cells were washed and incubated with streptavidin–PE-Cy5 (BD Biosciences) label as required. Appropriate isotype controls were included. All Abs were purchased from either eBio-science (San Diego, CA), BD Biosciences, or Biolegend (San Diego, CA). Data were acquired on a FACSCalibur flow cytometer or LSRII (BD Biosciences). Flow cytometry analysis was performed using FlowJo software (Treestar, version 8.7.3, Ashland, OR).

Adaptive transfer of bone marrow CD11c+ cells into naive recipients and contact hypersensitivity assay

After culturing the bone marrow cells for 7 d, purified CD11c+ cells at 10⁷ cells/ml in RPMI 10 were pulsed with 1 μm dinitrobenzene sulfonic acid-sodium salt (DNBS; Sigma-Aldrich) for 30 min at 37°C. CD11c+ cells were washed and resuspended to 5 × 10⁶ cells/ml in 0.9% saline (Baxter, Old Toongabbie, New South Wales, Australia). CD11c+ cells (10⁷) were injected into the ear pinnae of naive BALB/c mice (n = 8 ears per group). Controls included 1) mice that had their ears injected with CD11c+ cells without DNBS loading and 2) mice that had their ears injected with 20 μl 0.9% saline and mice that were UV irradiated (8 kJ/m²) 3 d prior to being injected with 20 μl 0.9% saline. At 7 d later, each side of the ears was painted with 10 μl 0.2% v/v 2,4-dinitro-1-fluorobenzene (DNFB, Sigma-Aldrich). Ear thicknesses were determined before and 24, 30, and 48 h after challenge with DNFB, using a spring-loaded micrometer (Mitutoyo, Aurora, IL). The contact hypersensitivity response was determined by subtracting the ear thicknesses before painting. When the ear swelling had subsided (at least 2 wk after the initial painting), the ears of mice were repainted with 0.2% DNFB and the ear swelling was measured after 24 h.

ALN culture, cytokine, and proliferation assays

The ALNs (four mice per group) were harvested 48 h after the first challenge of ears with 0.2% DNFB. The ALNs were physically disaggregated and passed through a FCS-washed nylon mesh filter. Cells were washed using GKN containing 5% FCS and resuspended to 10⁶ cells/ml in RPMI 10 containing 10 mM nonessential amino acids (Sigma-Aldrich) and 1 mM sodium pyruvate (Sigma-Aldrich). ALN cells were cultured in round-bottomed 96-well plates for 96 h, with or without 1 mM DNBS. Supernatants were collected, and concentrations of IL-10 (see above), IL-4, and IFN-γ were determined using a capture ELISA with a europium detection label. IL-10, IL-4, and IFN-γ capture and biotinylated Abs were obtained from Pharmingen (BD Biosciences, San Diego, CA). After culturing the bone marrow cells for 7 d, purified CD11c+ cells at 10⁷ cells/ml in RPMI 10 were pulsed with 1 μM DLNBS loading and 2) mice that had their ears injected with 20 μl 0.9% saline and mice that were UV irradiated (8 kJ/m²) 3 d prior to being injected with 20 μl 0.9% saline. At 7 d later, each side of the ears was painted with 10 μl 0.2% v/v 2,4-dinitro-1-fluorobenzene (DNFB, Sigma-Aldrich. Ear thicknesses were measured before and 24, 30, and 48 h after challenge with DNFB, using a spring-loaded micrometer (Mitutoyo, Aurora, IL). The contact hypersensitivity response was determined by subtracting the ear thicknesses before painting. When the ear swelling had subsided (at least 2 wk after the initial painting), the ears of mice were repainted with 0.2% DNFB and the ear swelling was measured after 24 h.

Indomethacin and PGE₂ administration

Indomethacin pellets containing 0.05 mg, with a constant release of 2.38 μg/day over 21 d (Innovative Research of America, Sarasota, FL), were s.c. implanted into the dorsal skin of mice 4 d prior to UV irradiation. Mice were also s.c. implanted with two PGE₂ pellets, each containing 0.1 mg, with a constant release of 4.76 μg/day over 21 d (total, 9.52 μg/day; Innovative Research of America). 3 d prior to isolation of bone marrow cells. As a control, mice were s.c. implanted with placebo pellets. Following isolation of bone marrow cells, 10 μM indomethacin (Sigma-Aldrich) was included during culture where indicated. Indomethacin is a nonselective inhibitor of cyclooxygenase (COX)-1 and COX-2 (30).
Statistical analysis

Statistical analysis was performed using the Student t test. Differences were considered statistically significant when p < 0.05.

Results

UV irradiation of mice increases myelopoiesis in the bone marrow

There was a small significant decrease in bone marrow cell yield from mice that had been UV irradiated (4.3 ± 0.2 × 10^7, mean ± SEM) 3 d previously, compared with nonirradiated mice (4.7 ± 0.2 × 10^7; n = 35 individual mice, p < 0.01). There were significantly more Gr1^+CD11b^+ myeloid cells in the bone marrow of UV-irradiated mice (28.6 ± 2.8%, mean ± SEM) compared with nonirradiated mice (24.1 ± 1.9%; n = 9 mice; p < 0.05; Fig. 1) (Table I). In addition, the percentage of Ter119^+CD11b^+ erythroid cells in bone marrow from UV-irradiated mice was significantly decreased (UV-irradiated, 42.4 ± 2.2%; nonirradiated, 49.3 ± 1.3%; n = 9; p < 0.05). UV irradiation did not change the proportion of B220^+CD11b^- lymphoid cells within the bone marrow.

UV irradiation of mice alters cytokine secretion by bone marrow cells

To determine if UV radiation modified cytokine production, bone marrow cells freshly isolated from UV-irradiated mice were incubated with 1 μg/ml LPS for 24 h. They secreted ~1.5-fold more IL-10 (Fig. 2A) and 2-fold greater amounts of PGE2 (Fig. 2B) than, but the same amount of IL-12 (Fig. 2C) as, bone marrow cells from nonirradiated mice. After 7 d culture with 10 μg/ml IL-4 and 10 μg/ml GM-CSF, aliquots of nonadherent cells (~50% CD11c^+) were stimulated with LPS (1 μg/ml) for 24 h. In the presence or absence of LPS, the levels of IL-10, IL-12, and PGE2 in the supernatants did not differ between UV-irradiated and nonirradiated mice (Fig. 2).

The CD11b^+ cells in the freshly isolated bone marrow population produce more IL-10, whereas following UV irradiation of mice, both the CD11b^+ and the CD11b^- cells produce more PGE2

To identify which cell populations were responsible for the up-regulation of IL-10 and PGE2 levels by UV radiation, production by the CD11b^+ and the CD11b^- cell subsets was determined. As shown in Fig. 1, the majority of bone marrow cells were CD11b^- (~70%). Of the purified CD11b^+ cells, >90% expressed Gr1 (data not shown). Upon exposure to LPS, CD11b^+ bone marrow cells secreted significantly greater IL-10 than did CD11b^- cells (Fig. 3A). Bone marrow CD11b^+ and CD11b^- cells from UV-irradiated mice secreted significantly higher quantities of LPS-induced PGE2 (Fig. 3B).

CD11c^+ cells derived from the bone marrow of UV irradiated mice have reduced priming capacity

CD11c^+ cells were recovered from bone marrow cell cultures after 7 d and enriched to ~95% by magnetic beads. CD11c^+ cells were incubated for 30 min at 37°C with DNBS (1 mM), and 10^6 cells

### Table I. Abs used for phenotyping freshly isolated bone marrow cells

<table>
<thead>
<tr>
<th>Cell Marker</th>
<th>Fluorescent Conjugate of Ab</th>
<th>Targeted Cell Population in Bone Marrow</th>
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<tr>
<td>Ter119</td>
<td>FITC</td>
<td>Erythroid precursors</td>
</tr>
<tr>
<td>CD11b</td>
<td>PE</td>
<td>Myeloid precursors</td>
</tr>
<tr>
<td>Gr1 (Ly-6G)</td>
<td>Biotin</td>
<td>Granulocyte and myeloid precursors</td>
</tr>
<tr>
<td>B220</td>
<td>Allophycocyanin</td>
<td>B cell (lymphoid) precursors</td>
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</tbody>
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FIGURE 2. Bone marrow cells freshly isolated from UV-irradiated mice secrete greater levels of IL-10 and PGE2. Bone marrow cells were isolated from mice that were either UV irradiated (closed bars) or not irradiated (open bars). Freshly isolated bone marrow cells (8 × 10^6 cells/ml) were stimulated ± 1 μg/ml LPS for 24 h (Day 0). Alternatively, bone marrow cells were cultured with IL-4 and GM-CSF for 7 d (Day 7). During the final day of culture, the nonadherent cells were replated at 8 × 10^6 cells/ml and incubated with and without LPS. Supernatants were harvested after 24 h and levels of IL-10 (A), PGE2 (B), and IL-12 (C) determined. Data represent mean ± SEM; n = 5 independent experiments. * p < 0.05.

FIGURE 1. UV irradiation of mice stimulates increased myelopoiesis in bone marrow at the expense of erythropoiesis. The shaved dorsal skin of female BALB/c mice was either UV irradiated (8 kJ/m^2, closed bars) or not irradiated (open bars), and bone marrow cells were isolated 3 d later. Cells were analyzed for their expression of the cell surface markers Gr1, CD11b, B220, and Ter119, using flow cytometry. Data represent mean ± SEM; n = 9 independent experiments. *p < 0.05.
were injected into the ears of naive mice. After a further 7 d, the ears were painted with DNFB (0.2% v/v) and ear swelling was measured 24 h later. The ears of naive mice that had been injected with CD11c+ cells cultured from bone marrow of UV-irradiated mice had a consistently reduced contact hypersensitivity response (Fig. 4A for ear swelling of a representative experiment; Fig. 4B for results from seven independent experiments). CD11c+ cells without DNBS loading did not prime an immunological response. In addition, there was no difference between the ear swelling in nonirradiated mice injected with saline and UV-irradiated mice injected with saline.

**CD11c+ cells from the bone marrow of UV-irradiated mice cause consistently reduced responses to DNFB**

To determine whether bone marrow-derived CD11c+ cells from UV-irradiated mice induced immunological tolerance, ear swelling resulting from the initial challenge with DNFB was allowed to subside (at least 2 wk) and the ears were repainted (challenge 2) with 0.2% DNFB. The ears of mice that had been injected with bone marrow-derived CD11c+ cells from bone marrow of UV-irradiated mice had a consistently reduced contact hypersensitivity response, compared with the ears of mice injected with bone marrow-derived CD11c+ cells from UV-irradiated mice and mice injected with saline (Fig. 4C). When the ears of mice were challenged for a third time (challenge 3, after a further 2-wk period), the ears of mice injected with bone marrow-derived CD11c+ cells from nonirradiated mice produced significantly greater contact hypersensitivity responses, compared with the ears of mice injected with bone marrow-derived CD11c+ cells from UV-irradiated mice, but not compared with mice injected with saline.

**FIGURE 3.** Bone marrow CD11b+ cells secrete greater levels of IL-10. Bone marrow cells were isolated from mice that were either UV irradiated (closed bars) or left not irradiated (open bars). Freshly isolated bone marrow cells (unsorted) were purified to >85% CD11b+ and CD11b− cells. Cell aliquots (8 × 10^6 cells/ml) were incubated with 1 µg/ml LPS for 24 h. Supernatants were harvested after 24 h and levels of IL-10 (A), and PGE2 (B) determined. A representative of two independent experiments is shown. Data represent mean ± SEM for supernatants from triplicate cultures. *p < 0.05.

**FIGURE 4.** Reduced contact hypersensitivity in mice that received CD11c+ cells from bone marrow of UV-irradiated mice. Bone marrow cells were isolated from mice that were either UV irradiated (closed bars) or left not irradiated (open bars). After 7 d of culture with IL-4 and GM-CSF, CD11c+ cells were purified and pulsed for 30 min with DNBS (1 mM) at 37°C. Ears of naive mice were injected with 10^6 CD11c+ cells (n = 8 ears per experiment). Controls included mice that had their ears injected with CD11c+ cells without DNBS loading, mice that had their ears injected with 20 µl saline (hatched bar), and recipient mice that were UV irradiated (8 kJ/m^2) 3 d prior to being injected with 20 µl saline (hatched bar, R). After 7 d, ears were painted with 0.2% DNFB, and ear swelling was measured after 24 h. A, Ear swelling for a representative of seven independent experiments, mean ± SEM. *p < 0.05. B, Results for seven independent experiments, mean ± SEM with saline measurements subtracted. *p < 0.05. C, Consistently reduced responses to DNFB. After the ear swelling due to challenge 1 had subsided (at least 2 wk after painting), the ears of mice were repainted with 0.2% DNFB (challenge 2), and the ear swelling was measured over 48 h. After an additional 2 wk, this procedure was repeated (challenge 3). Ear swelling for a representative of three independent experiments, mean ± SEM. *p < 0.05 for swelling between ears injected with CD11c+ cells from bone marrow of nonirradiated (−UV) and UV-irradiated mice (+UV). *p < 0.05 for swelling between ears injected with CD11c+ cells from bone marrow of nonirradiated mice and with saline.

Less competent CD11c+ cells derived from the bone marrow of UV-irradiated mice do not alter the phenotype of ALN cells or the cytokine environment

ALNs were harvested from mice 48 h after challenging the ears with DNFB and examined for cell phenotype and for cytokine secretion after culture for 96 h (cells pooled from four mice per
group per experiment). No significant difference was observed between the ALN cell numbers in mice injected with CD11c<sup>+</sup> cells cultured from UV-irradiated (3.8 ± 0.4 × 10<sup>5</sup>) and from non-irradiated mice (4.0 ± 0.5 × 10<sup>3</sup>; n = 7 independent experiments). However, the ALNs from mice injected with CD11c<sup>+</sup> cells had increased cell numbers in comparison with mice injected with saline (2.0 ± 0.2 × 10<sup>5</sup>, mean ± SEM; n = 7 independent experiments, p < 0.05). There was also no significant difference in the percentages of DCs (CD11c<sup>+</sup>, MHC class II<sup>+</sup>), B cells (MHC class II<sup>+</sup>, B220<sup>+</sup>), CD8<sup>+</sup> T cells, and CD3<sup>+</sup>CD4<sup>+</sup>FoxP3<sup>+</sup> T cells in ALNs of mice that were recipients of bone marrow-derived CD11c<sup>+</sup> cells cultured from UV-irradiated or nonirradiated mice (data not shown).

Single-cell suspensions from ALNs were cultured with and without DNBS (1 mM) for 96 h. Levels of IL-4, IL-10, and IFN-γ secreted by ALN cells from mice injected with bone marrow-derived CD11c<sup>+</sup> cells cultured from UV-irradiated and non-irradiated mice did not differ (data not shown). In addition, no differences in [³H]thymidine incorporation occurred over the last 24 h of culture (data not shown).

**Reduced priming ability of CD11c<sup>+</sup> cells derived from bone marrow of UV-irradiated mice is not a result of late CD11c<sup>+</sup> cell maturation**

To confirm that the reduced ear swelling (Fig. 4) was not due to differences in maturation of the injected CD11c<sup>+</sup> cells, bone marrow cells from UV-irradiated and nonirradiated mice were activated with LPS (1 μg/ml) during the final 24 h of the 7-d culture. With use of the in vivo priming assay described earlier, mice injected with CD11c<sup>+</sup> cells from UV-irradiated mice cultured both with and without the addition of LPS demonstrated reduced contact hypersensitivity responses (Fig. 5A shows a representative of two experiments).

Following 6 d in culture, stimulation of bone marrow cells with LPS for 24 h upregulated expression of the costimulatory molecules CD40, CD80, and CD86 on CD11c<sup>+</sup> cells (Fig. 5B). However, there was no difference in the expression of these costimulatory molecules between CD11c<sup>+</sup> cells cultured from the bone marrow of UV-irradiated mice and those from nonirradiated mice.

**UV irradiation of mice reduces the priming ability of bone marrow-derived CD11c<sup>+</sup> cells via an indomethacin-inhibitable pathway**

To determine whether UV-induced prostanooids were involved in changes to the activity of the bone marrow-derived CD11c<sup>+</sup> cells, slow-release indomethacin pellets or placebo pellets were introduced into mice s.c. 4 d prior to UV irradiation. Three days after UV irradiation (7 d after indomethacin was administered to mice), bone marrow cells were isolated and cultured with GM-CSF and IL-4. Indomethacin (10 μM) was included in the culture medium of bone marrow cells isolated from the mice with indomethacin pellets.

CD11c<sup>+</sup> cells cultured from the bone marrow of UV-irradiated, indomethacin-treated mice stimulated higher contact hypersensitivity responses than did CD11c<sup>+</sup> cells cultured from UV-irradiated mice with placebo pellets inserted (see Fig. 6A for a representative of two experiments). The contact hypersensitivity responses were not significantly different for CD11c<sup>+</sup> cells cultured from bone marrow of UV-irradiated and nonirradiated, indomethacin-treated mice. There was no consistent change in ear swelling induced by CD11c<sup>+</sup> cells cultured from mice with placebo pellets inserted, compared with CD11c<sup>+</sup> cells cultured from indomethacin-treated mice. In confirmation of the results of Fig. 4, ear swelling was reduced 24 h post-DNFB painting of ears of mice previously injected with CD11c<sup>+</sup> cells cultured from UV-irradiated mice. A third experiment was performed without the addition of indomethacin during the culture of bone marrow cells from indomethacin-treated mice. Very similar contact hypersensitivity responses were observed (data not shown).

**Increased myelopoiesis is not responsible for reduced bone marrow-derived CD11c<sup>+</sup> cell priming ability**

The effect of s.c. indomethacin pellets on UV-induced myelopoiesis was determined. The increased percentage of Gr1<sup>+</sup>CD11b<sup>+</sup> cells, as previously reported for UV-irradiated mice (Fig. 1), was maintained in mice administered indomethacin pellets prior to UV irradiation (Fig. 6B). Furthermore, in comparison with non-irradiated, indomethacin-treated mice, there was a lower percentage of Ter119<sup>+</sup>CD11b<sup>+</sup> erythroid cells in bone marrow from mice that had an indomethacin pellet implanted and then were subsequently UV irradiated.

**Indomethacin does not act via an IL-10- or IL-12-dependent mechanism to reverse the UV-mediated reduction of CD11c<sup>+</sup> cell priming ability**

Bone marrow cells were isolated from mice that had been administered placebo or indomethacin pellets, with or without subsequent UV irradiation. Supernatants were harvested from 1) bone marrow cells cultured for 24 h with or without LPS and 2) cells cultured for 7 d with IL-4, GM-CSF, and with and without LPS for the final 24 h. LPS stimulated freshly isolated bone marrow cells from UV-irradiated mice to produce higher IL-10 levels (Table II). Indomethacin pellets inserted into nonirradiated and UV-irradiated mice did not alter the levels of IL-10 (Table II) or IL-12 (data not shown) secreted by freshly isolated bone marrow cells. Similarly, indomethacin pellets inserted into mice did not alter the LPS-induced IL-10 and IL-12 secretion by 7-d cultured bone marrow cells (data not shown). In the absence of LPS stimulation, bone marrow cells isolated from mice with placebo or indomethacin pellets, with or without UV exposure, produced very similar low levels of IL-10 and IL-12 (data not shown).

**PGE<sub>2</sub> reduces the priming ability of bone marrow-derived CD11c<sup>+</sup> cells**

To determine if PGE<sub>2</sub> was the prostanoid whose production was inhibited by indomethacin, slow-release PGE<sub>2</sub> pellets were s.c. inserted into mice 3 d prior to bone marrow harvest. CD11c<sup>+</sup> cells cultured from the bone marrow of PGE<sub>2</sub>-treated mice and subsequently loaded with DNBS, stimulated reduced contact hypersensitivity responses to DNFB, compared with CD11c<sup>+</sup> cells cultured from UV-irradiated mice and PGE<sub>2</sub>-treated mice. This finding suggests that PGE<sub>2</sub> may be responsible for, or contribute to, the effects of UV irradiation on bone marrow-derived CD11c<sup>+</sup> cells. The PGE<sub>2</sub> pellets in mice did not stimulate myelopoiesis (data not shown) or regulate LPS-induced cytokine production by bone marrow cells in culture (data not shown).

**Discussion**

In this study, UV irradiation of skin reduced the immune competency of bone marrow-derived CD11c<sup>+</sup> cells by a mechanism reversible by indomethacin and a mechanism that could be simulated by PGE<sub>2</sub> administration. Our model involved delivering an erythematous UV dose to the skin of mice. After 3 d, the bone marrow was harvested and cultured for CD11c<sup>+</sup> cell development,
and subsequently, the CD11c<sup>+</sup> cells were tested for their functional ability in vivo. CD11c<sup>+</sup> cells cultured from the bone marrow of UV-irradiated mice had a reduced ability to prime T cells in naive mice such that when subsequently challenged with the priming Ag, there was a reduced contact hypersensitivity response. Furthermore, these CD11c<sup>+</sup> cells from the bone marrow of UV-irradiated mice induced a long-lived suppression of the memory response to DNFB. Thus, we have shown for the first time, to our knowledge, that UV irradiation of skin can affect the functional ability of bone marrow-derived CD11c<sup>+</sup> cells.

Because of the ability of UV to inhibit bone marrow-derived CD11c<sup>+</sup> cells to prime an immune response, we initially proposed that UV affects CD11c<sup>+</sup> cell maturity and costimulatory molecule expression. However, these cells were not resistant to maturation by LPS.

**FIGURE 5.** Reduced contact hypersensitivity in mice that received LPS-stimulated CD11c<sup>+</sup> cells from bone marrow of UV-irradiated mice despite upregulation of costimulatory molecule expression. Bone marrow cells isolated from nonirradiated (open bars) and UV-irradiated mice (closed bars) were cultured with IL-4 and GM-CSF for 7 d with or without LPS added during the final 24 h (half the medium was replaced with medium containing 2 μg/ml LPS). CD11c<sup>+</sup> cells were purified and pulsed for 30 min with DNBS at 37°C. Ears of naive mice were injected with 10<sup>6</sup> CD11c<sup>+</sup> cells (n = 8 ears per experiment). As a control, some mice were injected with 0.9% saline (hatched bar). After 7 d, ears were painted with DNFB and ear swelling measurements taken after 24 h. Ear swelling for a representative of two independent experiments, mean + SEM. A, Ear swelling for a representative of two independent experiments, mean + SEM. *p < 0.05.

**FIGURE 6.** Indomethacin negates the effect of UV irradiation of mice on development of CD11c<sup>+</sup> bone marrow cells with reduced priming ability but not UV-induced myelopoiesis. Indomethacin or placebo pellets were implanted s.c. into the backs of mice. At 4 d later, mice were either UV irradiated (closed bars) or left not irradiated (open bars), and their bone marrow was isolated 3 d later. A, Bone marrow cells were cultured with IL-4 and GM-CSF for 7 d with indomethacin (10 μM) included in the cultures of cells from indomethacin-treated mice. CD11c<sup>+</sup> cells were purified and pulsed for 30 min with DNBS at 37°C. Ears of naive mice were injected with 10<sup>6</sup> CD11c<sup>+</sup> cells (n = 8 ears per experiment). As a control, some mice were injected with 0.9% saline (hatched bar). After 7 d, ears were painted with DNFB and ear swelling measurements taken after 24 h. Ear swelling for a representative of two independent experiments, mean + SEM. B, Freshly isolated bone marrow cells were analyzed for their expression of the cell surface markers Gr1, CD11b, B220, and Ter119 using flow cytometry. Data represent mean + SEM, n = 9 independent experiments containing one mouse per group per experiment. *p < 0.05.

CD86 on CD11c<sup>+</sup> MHC class II<sup>+</sup> cells, as determined by flow cytometry. The shaded area depicts cells not exposed to LPS, whereas solid lines represent bone marrow cells from UV-irradiated mice. Broken lines represent bone marrow cells from nonirradiated mice. A representative experiment is shown.
LPS, and despite the upregulation by LPS of CD40, CD80, and CD86 expression by CD11c+ MHC class II+ cells, the cells from UV-irradiated and nonirradiated mice remained functionally different. Moreover, there was no difference in costimulatory molecule expression by CD11c+ cells from the bone marrow of nonirradiated and UV-irradiated mice. In addition, bone marrow-derived CD11c+ cells from UV-irradiated mice did not produce lower amounts of IL-12 and higher levels of IL-10 than those produced by cells from nonirradiated mice. Further research is required, as these cells do not express the published properties of tolerogenic DCs (31, 32).

In experiments in which the ears of mice were rechallenged with DNFB, CD11c+ cells from the bone marrow of UV-irradiated mice induced a long-lasting suppression of the memory response to DNFB. Although subsequent exposures to DNFB amplified the immune response, we believe that this increased response is a result of the inflammation caused by DNFB. The persistent reduced response to DNFB is highly suggestive of tolerance induced by CD11c+ cells from the bone marrow of UV-irradiated mice. However, this could not be clearly demonstrated owing to the nature of our contact hypersensitivity assay.

Because of the ability of indomethacin, an inhibitor of prostanoïd production, to partially reverse the systemic immunosuppression induced by UV irradiation (8, 9) and the involvement of PGE2 in the regulation of DC activity (for review see Refs. 12, 33), we proposed that UV-induced PGE2 in the skin of mice was responsible for the UV-induced effects on bone marrow-derived CD11c+ cell function. To initially test this hypothesis, indomethacin pellets were implanted prior to UV irradiation of mice. Indomethacin treatment reversed the reduced Ag priming ability of bone marrow-derived CD11c+ cells from UV-irradiated mice. In the absence of UV irradiation, indomethacin had no consistent effect on bone marrow-derived CD11c+ cells. This finding suggested that prostanoïds induced by UV were responsible for the initiation of changes that affected CD11c+ cell development and their reduced Ag priming ability. An experiment in which indomethacin pellets were inserted before UV irradiation, but indomethacin was not included during bone marrow cell culture, supports the conclusion that the prostanoïd-dependent effects occurred in the mouse before bone marrow harvest.

In the next series of experiments, constant-release PGE2 pellets were administered to mice 3 d before bone marrow harvest. We selected a 10 μg/day dose for our model owing to its ability to induce serum IL-10 in mice (34), a cytokine known to be involved in UV-induced immunosuppression (5). CD11c+ cells from the bone marrow of PGE2-treated mice had poor priming ability, compared with those from bone marrow of mice given placebo pellets. Thus, PGE2 has functional similarities to UV but may be only one of several UV-induced prostanoïds involved. Further, it is difficult to compare the dose of PGE2 administered with that induced by UV irradiation. Other prostanoïds produced by COX-1 and COX-2 activity include PGD2 metabolites (35–37) and thromboxanes (38), all of which are able to influence the immune response. As indomethacin is a nonspecific COX inhibitor, PGE2 or a combination of these downstream mediators may be responsible for the UV effects on bone marrow-derived CD11c+ cell activity.

There are many stages at which UV-induced PGE2 and other prostanoïds may be important. We propose that PGE2 and prostanoïds produced by keratinocytes (11, 39), fibroblasts (11), mast cells, and possibly other cell types within UV-irradiated skin may contribute to the regulation of CD11c+ precursor cells within the bone marrow. Attempts were also made to measure the levels of PGE2 present in the bloodstream following UV irradiation. However, they could not be reliably determined owing to the PGE2 in serum having a 1/2 of <1 h (40).

In addition to the reduction in bone marrow-derived CD11c+ cell function, UV irradiation of mice also increased the PGE2 production by bone marrow cells. We propose that CD11c+ cells were not responsible, as they constitute <1% of freshly isolated bone marrow cells (S. Gorman, unpublished observations). Further, after 7 d in culture, the nonadherent cells comprise ~50% CD11c+ cells. However, there was no change in the PGE2 levels secreted.

### Table II. LPS-induced IL-10 production by bone marrow cells from mice administered indomethacin pellets 4 d prior to UV irradiation

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Placebo Pellets</th>
<th>Indomethacin Pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nonirradiated</td>
<td>UV-Irradiated</td>
</tr>
<tr>
<td></td>
<td>190.4 ± 14.4 6</td>
<td>289.9 ± 30.1 7</td>
</tr>
<tr>
<td></td>
<td>186.0 ± 11.1</td>
<td>352.9 ± 48.1 13</td>
</tr>
<tr>
<td>2</td>
<td>208.4 ± 45.3</td>
<td>269.6 ± 18.6</td>
</tr>
<tr>
<td></td>
<td>233.7 ± 8.9</td>
<td>352.8 ± 42.1 8</td>
</tr>
<tr>
<td>3</td>
<td>502.6 ± 8.6</td>
<td>701.4 ± 67.4</td>
</tr>
</tbody>
</table>

*aMice were administered placebo or indomethacin pellets 4 d before UV irradiation (8 kJ/m²). At 3 d later, bone marrow cells were harvested and cultured (8 × 10⁶ cells/ml) with LPS for 24 h before measuring IL-10 in the culture supernatant.  
  bMean ± SEM for supernatants from triplicate cultures.  
  p < 0.05 compared with nonirradiated.
by 7-d cultured cells from UV-irradiated and nonirradiated mice. These results suggest that a different cell type is responsible for the increased levels of PGE2 released by bone marrow cells from UV-irradiated mice. There are a number of possible sources of PGE2 in the bone marrow. Both CD11b+ and CD11b− bone marrow cells from UV-irradiated mice secreted greater quantities of PGE2. However, as CD11b− cells represent ∼70% of freshly isolated bone marrow (Fig. 1), they are the major contributors to PGE2 production. These CD11b− cells may include mesenchymal stem cells (MSCs), which are multipotent nonhematopoietic progenitor cells that constitutively secrete PGE2. These cells are most frequently associated with bone marrow (41) but can also be isolated from adipose tissue, amniotic fluid, and a number of fetal tissues (42). MSCs can inhibit the immunity provided by a number of cell types, including NK cells, B cells, and DCs (43, 44). The secretion of PGE2 may be one of the mechanisms by which MSCs exert their immunomodulatory effects (45). Following coculture of MSCs with peripheral blood monocytes, the monocytes failed to gain an immature DC phenotype (CD14+CD1a+) (46). PGE2 was the underlying mechanism involved. The activity of MSCs may contribute to the reduced competency of CD11c+ cells derived from bone marrow of UV-irradiated mice. Further studies will be performed to characterize bone marrow CD11b− cells and the involvement of MSCs.

Myeloid-derived suppressor cells have been described, which although phenotypically heterogeneous, commonly express Gr1+CD11b+ (47, 48). These cells are induced by proinflammatory mediators such as PGE2 and are able to inhibit innate and adaptive immune responses. As myeloid cells (Gr1+CD11b+) were increased in the bone marrow of UV-irradiated mice, the numbers of myeloid-derived suppressor cells may also have increased. The inclusion of pellets containing indomethacin in our model did not inhibit the UV increase in Gr1+CD11b+ cells but reversed the UV-associated reduction in the function of bone marrow-derived CD11c+ cells. This observation suggests that the increase in percentage of Gr1+CD11b+ cells in bone marrow is not responsible for the UV-induced effect on CD11c+ cells. However, whether UV irradiation is able to induce a functional change in Gr1+CD11b+ cells remains to be determined. Gr1+CD11b+ cells in the bone marrow were responsible for almost all LPS-induced IL-10 production. Hence, we propose that LPS-induced IL-10 by freshly isolated bone marrow cells from UV-irradiated mice correlates with increased myelopoiesis, and this increase in IL-10 may not be involved in changes to CD11c+ cell priming ability. During UV-induced inflammation, Langerhans cells and DCs migrate to the lymph nodes, with the bone marrow providing a source of cells to replenish those in the periphery. This study shows that the immune competency of CD11c+ cells derived from the bone marrow of UV-irradiated mice is reduced, and this result provides what we believe is a novel mechanism of UV-induced systemic immunosuppression.

Disclosures
The authors have no financial conflicts of interest.

References

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