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Altered Immunity and Dendritic Cell Activity in the Periphery of Mice after Long-Term Engraftment with Bone Marrow from Ultraviolet-Irradiated Mice

Royce L. X. Ng,* Naomi M. Scott,* Deborah H. Strickland,† Shelley Gorman,* Michele A. Grimbaldeston,‡ Mary Norval,‡ Jason Waithman,* and Prue H. Hart*

Alterations to dendritic cell (DC) progenitors in the bone marrow (BM) may contribute to long-lasting systemic immunosuppression (>28 d) following exposure of the skin of mice to erythema UV radiation (UVR). DCs differentiated in vitro from the BM of mice 3 d after UVR (8 kJ/m²) have a reduced capacity to initiate immunity (both skin and airways) when adoptively transferred into naive mice. Studies in IL-10−/− mice suggested that UV-induced IL-10 was not significantly involved. To investigate the immune capabilities of peripheral tissue DCs generated in vivo from the BM of UV-irradiated mice, chimeric mice were established. Sixteen weeks after reconstitution, contact hypersensitivity responses were significantly reduced in mice reconstituted with BM from UV-irradiated mice (UVR-chimeric). When the dorsal skin of UV-chimeric mice was challenged with innate inflammatory agents, the hypotrophy induced in the draining lymph nodes was minimal and significantly less than that measured in control-chimeric mice challenged with the same inflammatory agent. When DCs were differentiated from the BM of UV-chimeric mice using FLT3 ligand or GM-CSF + IL-4, the cells maintained a reduced priming ability. The diminished responses in UV-chimeric mice were not due to different numerical or proportional reconstitution of BM or the hematopoietic cells in blood, lymph nodes, and skin. Erythermal UVR may imprint a long-lasting epigenetic effect on DC progenitors in the BM and alter the function of their terminally differentiated progeny. The Journal of Immunology, 2013, 190: 000–000.

Eposure of skin to UV radiation (UVR) can induce a systemic immunosuppression in both mice and humans (i.e., a reduced immune response to Ags applied to non-irradiated tissue sites) (reviewed in Refs. 1, 2). Detrimental consequences include facilitating the development of skin cancers (3) and reduced responses to Ags associated with vaccination and microbial infection (1). Of benefit, UVR exposure may reduce the symptoms and incidence of inflammatory autoimmune and allergic diseases (4–6). UV chromophores include DNA, tryptophan, 7-dehydrocholesterol, and membrane lipids of skin cells, predominantly keratinocytes, as well as trans-urocanic acid of the stratum corneum (reviewed in Refs. 2, 6). Upon absorption of UV photons, multiple pathways are activated in skin cells, and immune mediators are produced, which, in turn, stimulate the migration of “altered” dendritic cells (DCs), mast cells (in defined settings), and other immune cells to the draining nodes. The roles of activated migrating and resident cells in the nodes, as well as in more distant tissues, in the processes of systemic immunosuppression are not clear but may involve further production of soluble mediators and/or regulatory cells (reviewed in Ref. 6).

DCs are heterogeneous cells responsible for linking innate and adaptive immunity and are widely accepted as key initiators of T cell immunity in vivo (7). The DCs that originate from progenitors within the bone marrow (BM) are gamma-radiation sensitive (8). In contrast, Langerhans cells, a unique type of DC that is located predominantly in the epidermis and lymph nodes (LNs) (9), are derived from an epidermal progenitor in the absence of inflammation and are resistant to gamma radiation (8, 10). All hematopoietic cells, including BM-derived DCs, are downstream products of long-term hematopoietic stem cells (LT-HSCs). LT-HSCs are multipotent cells capable of providing long-term replenishment of the entire hematopoietic pathway (11). Although LT-HSCs are viewed as a critical component in the response to infection or inflammation (12, 13), a number of studies described the long-term impact of inflammation on the function of LT-HSCs in terms of their ability to self-renew, engraft, and differentiate (14–16). It remains less well understood whether inflammation-associated alterations to LT-HSCs can also affect the function of downstream leukocytes and, of particular interest in our studies, DCs.

We reported previously that DCs differentiated in vitro from the BM of UV-irradiated BALB/c mice, and then transferred into naïve recipients, have reduced priming ability as determined using an Ag-specific recall response (17). This suggests that signals from UV-irradiated skin alter the hematopoietic cell compartment such that,
upon differentiation, BM-derived DCs have reduced priming ability. Because the BM acts as a reservoir for DC progenitors to seed the periphery, we hypothesized that differentiating DCs from the BM of UV-irradiated mice may be a major contributor to UVR-induced systemic immunosuppression. However, although in vitro culture of BM cells serves as a valuable tool for generating large numbers of DCs for functional experiments, caveats of in vitro culture include a nonphysiological cytokine environment in which supraphysiological levels of growth factors are used. Hence, we sought to use an in vivo system to test whether peripheral DCs derived from BM progenitors of UV-irradiated mice confer a reduced immune response to future inflammatory stimuli.

To test the effect of UV irradiation on the functional capacity of DCs generated in vivo, we established chimeric mice reconstituted with donor BM from either UV-irradiated mice (UV-chimeric) or nonirradiated mice (control-chimeric). Contact hypersensitivity responses to 2,4-dinitro-1-fluorobenzene (DNFB) were reduced in UV-chimeric mice compared with control-chimeric mice. Moreover, hapten application to the skin or UVR exposure caused diminished inflammatory responses, as evidenced by reduced cell yields in draining LNs. These different functional responses between control-chimeric mice and UV-chimeric mice were not due to different numerical or proportional reconstitution of the BM or the leukocyte populations of blood, secondary lymphoid organs, or skin. When the BM cells of UV-chimeric mice that were reconstituted for 16 wk (16-wk reconstituted UV-chimeric mice) were cultured with FLT3 ligand (FLT3-L) or GM-CSF + IL-4 for the propagation of DCs, these cells had reduced immunogenicity similar to that observed for DCs differentiated from the BM of recently UV-irradiated nonchimeric mice. Collectively, these results indicate that UVR can induce a long-lasting change, possibly epigenetic, to DC progenitors within the BM. We propose that DCs differentiated from these progenitors, which exit the BM to seed the periphery, may be major contributors to UV-induced systemic immunosuppression.

Materials and Methods

Mice

C57BL/6 (CD45.2 alloantigen), B6.SJL-Ptprc–/– (CD45.1 alloantigen), and BALB/c mice were obtained from the Animal Resources Centre (Murdoch, WA, Australia). B6/IL10−/− mice were bred in-house (Centre for Cancer Biology, Adelaide, SA, Australia) and backcrossed against C57BL/6 mice purchased from The Jackson Laboratory (Bar Harbor, ME) every 2 y. Experiments were performed in female mice unless otherwise stated. All experiments were performed with the approval of the Telethon Institute for Child Health Research Animal Ethics Committee or the Institute of Medical and Veterinary Science 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 UVR with 65% UVB (280–320 nm) and peak emission at 313 nm was used. Twenty-four hours prior to irradiation, a uniform area of dorsal skin of mice was shaved (8 cm²). To administer UVR, mice were held in Perspex compartments that were covered with 0.2 mm polyvinyl chloride plastic to eliminate wavelengths < 290 nm. The compartments were placed 20 cm beneath the UV lamps, and up to 8 kJ/m² UVR was delivered (8 kJ/m² UVR is equivalent to three or four minimal erythemal dose for Child Health Research Animal Ethics Committee or the Institute of Medical and Veterinary Science Animal Ethics Committee according to the guidelines of the National Health and Medical Research Council of Australia). Although, in vitro culture of BM cells serves as a valuable tool for generating large numbers of DCs for functional experiments, caveats of in vitro culture include a nonphysiological cytokine environment in which supraphysiological levels of growth factors are used. Hence, we sought to use an in vivo system to test whether peripheral DCs derived from BM progenitors of UV-irradiated mice confer a reduced immune response to future inflammatory stimuli.

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2.0 × 10^6 BM cells prepared from congenic B6.SJL-Ptprca^c (CD45.1 allo-antigen) mice that had either been exposed to 8 kJ/m^2 UVR 3 d prior or not irradiated. RBCs were lysed with ammonium chloride prior to injection. Age-matched mice that had not been gamma irradiated were used as a control for chimeric mice (nonchimeric). Alternatively, gamma-irradiated C57BL/6 mice were injected i.v. with an equal mixture (1:1) of a total of 2 × 10^6 BM cells from mice irradiated 3 d earlier with UVR (CD45.1) and from nonirradiated (CD45.2) mice. The ratio of donor cells was confirmed using flow cytometry. Mice were maintained on polymyxin B sulfate and neomycin trisulfate salt hydrate (both from Sigma-Aldrich) for 2 wk either side of gamma irradiation and cell reconstitution.

**Hematology tests**

Sixteen weeks after gamma irradiation and reconstitution of chimeric mice, 250 μl blood was collected in K$_2$EDTA-coated Micratainer tubes (BD, Franklin Lakes, NJ) and analyzed using the Advia 120 hematology system (Siemens Healthcare Diagnostics, Tarrytown, NY) to measure the concentration and percentage of circulating leukocytes.

**Contact hypersensitivity assay**

The shaved ventral surface of C57BL/6 mice was painted with 50 μl 0.5% v/v DNFB (Sigma-Aldrich) diluted in acetone 4:1 olive oil. Four days later, both sides of each ear were painted with 10 μl 0.2% v/v DNFB prepared in acetone 4:1 olive oil. Ear thicknesses were determined before and 24 h after challenge with DNFB. The change in ear swelling attributed to sensitization was determined by subtracting the ear swelling measured in non-irradiated DNFB-challenged mice.

**Accessory cell–independent in vitro T cell–proliferation assay**

The skin-draining LNs (SDLNs; axillary, brachial, and inguinal) were harvested from mice, physically disaggregated, and resuspended to 5 × 10^5 cells/ml in RPMI-10 containing 10 mM nonessential amino acids (Sigma-Aldrich) and 1 mM sodium pyruvate (Sigma-Aldrich). Cells (10^5) were aliquoted into wells of round-bottom 96-well plates and stimulated with 10 μg/ml plate-bound anti-CD3e (BD Biosciences) and 1 μg/ml anti-CD28 (BD Biosciences) for 72 h. Alternatively, cells were left unstimulated. LN cells were pelleted at 400 x g and 0.25 μl [1H]thymidine (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, U.K.) after 48 h in culture. Twenty-four hours later, [1H]thymidine incorporation was measured using a 1450 MicroBeta TriLux beta counter (PerkinElmer, Waltham, MA).

**FITC uptake and cell-migration assay**

The shaved dorsal surface of mice was painted with 100 μl 0.5% w/v FITC (Sigma-Aldrich) prepared in acetone 1:1 dibutylphthalate. After 16 h, the SDLNs were removed, and single-cell suspensions were prepared as described above.

**Skin cell preparations**

Leukocytes were isolated from skin as previously described (19). Mice were euthanized and perfused with 10 ml PBS. Dorsal skin sections (1 cm × 2 cm) were taken after hair removal by clipping and chemical depilation with Veet cream (Reckitt Benckiser, Slough, U.K.). Skin sections were cut into fine pieces and incubated for 90 min at 37°C in RPMI 1640 medium containing 2% FCS, 3 mg/ml collagenase (Worthington Biochemical, Lakewood, NJ), and 5 μg/ml DNase (Roche, Penzberg, Germany). Cells were filtered through 110-μm nylon mesh and stained for flow cytometric analysis. Abs targeting CD11c and MHC class II were used to identify DCs. Propidium iodide was used to detect dead cells. A known number of Spherex blank calibration particles (BD Biosciences) was added to each sample to determine cell numbers/cm² of skin.

5-Aza-2‘-deoxycytidine

5-Aza-2‘-deoxycytidine (5-aza-dC; Sigma-Aldrich), a DNA methylation inhibitor, was dissolved in DMSO at 5 mg/ml before further dilution in saline. DMSO was similarly diluted as the vehicle control. Female mice were injected i.p. with 5-aza-dC at 0.2 mg/kg 60 min before UV irradiation (8 kJ/m²). Injections of the same amount were performed 24 and 48 h later, and BM was harvested 3 d after UVR exposure.

**Results**

**DCs differentiated from the BM of UV-irradiated mice using FLT3-L or GM-CSF + IL-4 have reduced immunogenicity**

Our previous results describing poorly immunogenic DCs differentiated from the BM of UV-irradiated BALB/c mice were obtained using GM-CSF + IL-4 as the differentiative growth factors (17). To test whether UV irradiation of mice alters BM-derived DCs generated in steady-state culture conditions, the BM cells of UV-irradiated BALB/c mice were cultured with FLT3-L for 9 d (Fig. 1A). As performed previously, BM was obtained 3 d post-UVR exposure (17). After 9 d, the cells were pulsed with DNBS (1 mM), and 10^5 cells were injected into the ear pinnae of naive mice. To determine the response to in vivo priming, after 7 d the ears of the recipient mice were painted with DNFB (hydrophobic equivalent of DNBS), and the ear swelling was measured 24 h later. The ear swelling observed in mice injected with FLT3-L-differentiated DCs cultured from the BM of UV-irradiated mice was significantly less than that measured in mice injected with DCs differentiated from the BM of nonirradiated mice (56% reduction, Fig. 1B). In a parallel experiment, DCs differentiated from the BM of UV-irradiated mice using GM-CSF + IL-4 induced a similarly diminished ear swelling response (46% reduction, Fig. 1C). The results illustrate that DCs from the BM of UV-irradiated mice differentiated under steady-state or inflammatory conditions have a reduced ability to prime naive T cells in vivo.

The phenotype of DCs generated using FLT3-L or GM-CSF + IL-4 was compared. When BM cells were cultured with FLT3-L, a higher proportion of cells was physically smaller than was cells cultured with GM-CSF + IL-4 (data not shown). In addition, after culture with FLT3-L, an increased percentage of CD11c+ cells was CD11b– B220+ (i.e., likely plasmacytoid DCs) (Fig. 1D) (20–22). There was no difference in the percentage of CD11c+ cells that was CD11b– B220+ in FLT3L cultures from the BM of nonirradiated mice (21.7 ± 7.6%, mean ± SEM, n = 3 independent experiments) and UV-irradiated mice (23.6 ± 4.1%, mean ± SEM, n = 3 independent experiments). Following culture with GM-CSF + IL-4, a low percentage of CD11c+ cells exhibited a plasmacytoid DC phenotype from the BM of nonirradiated mice (2.7 ± 1.0%, mean ± SEM, n = 3 independent experiments) and UV-irradiated mice (1.5 ± 0.5%, mean ± SEM, n = 3 independent experiments). The morphological and phenotypic differences in DCs cultured using these two differentiative conditions are as previously reported (23–26). However, irrespective of the culture conditions used for DC differentiation, a similarly reduced immunogenicity was observed in DCs cultured from the BM of UV-irradiated mice (Fig. 1B, 1C).

**Chimeric mouse model for the generation of immune cells from the BM of UV-irradiated mice**

To address any artifacts potentially introduced by our culture system for DC propagation, we used a chimeric mouse model to enable in vivo differentiation of BM progenitors harvested from UV-irradiated and nonirradiated mice. Using chimeric mice that were reconstituted for >16 wk (>16-wk reconstituted chimeric mice), we determined whether UVR reduced the immunogenicity of the peripheral DC compartment, which had differentiated from BM-derived progenitors and then migrated to peripheral tissues. As a result of the benefits of using allelic markers, it was confirmed in C57BL/6 mice that UVR reduced the immunogenicity of DCs differentiated in vitro from BM (data not shown). To generate chimeric mice, BM-ablated CD45.2 mice were reconstituted with BM cells from 3-d post UV-irradiated (UV-chimeric) or nonirradiated (control-chimeric) CD45.1 mice (Fig. 2A).

The ability of the transferred hematopoietic progenitors to reconstitute the BM and the peripheral secondary lymphoid organs was determined. Total cell yields of the BM, spleen, and LNs...
Pooled axillary, brachial, inguinal, mesenteric, and cervical LNs were measured over a 16-wk period. There was no significant difference in the cell yields obtained from these lymphoid organs between control-chimeric and UV-chimeric mice at any of the time points tested (Fig. 2B). Both control-chimeric and UV-chimeric mice had restored BM, spleen, and LN to levels observed in non-chimeric mice by 2, 4, and 8 wk postreconstitution, respectively (Fig. 2B). The time required for reconstitution of these lymphoid organs was similar to previous reports (27).

The BM cell profiles of 16-wk reconstituted control-chimeric and UV-chimeric mice were examined. There was a similar percentage of myeloid cells (Gr1*) in the BM of control-chimeric mice (27.7 ± 1.0%, mean ± SEM, n = 3 mice from independent transplant cohorts) and UV-chimeric mice (27.7 ± 1.8%, mean ± SEM, n = 3). Furthermore, there were similar percentages of erythroid cells (Ter119*) in the BM of control-chimeric mice (18.1 ± 2.0%, mean ± SEM, n = 3) and UV-chimeric mice (19.2 ± 0.6, mean ± SEM, n = 3).

The cell profiles of the secondary lymphoid organs were also investigated. Similar percentages of B cells (CD19*CD21*) and T cells (CD3*CD4*, CD3*CD4*), and CD11c* cells were observed in the LN (Fig. 2C) and spleen (data not shown) of control-chimeric and UV-chimeric mice at all time points tested. By 16 wk after reconstitution, all of the tested cell types within the LN (Fig. 2C) and spleen (data not shown) of chimeric mice were similar to the percentages observed in age-matched nonchimeric mice. After 16 wk, >97% of cells examined expressed CD45.1 alloantigen, indicating the repopulation of the hematopoietic system with donor cells (for LNs, Fig. 2D). There was no significant difference in the amount of CD45.1 expressed by any of these cell types between chimeric mice at any time point tested (for LNs, Fig. 2D).
Blood profiles were performed after 16 wk to confirm similar reconstitution of circulating leukocytes in chimeric mice. There was no significant difference in the concentration or percentage of circulating RBCs, platelets, neutrophils, lymphocytes, monocytes, or eosinophils between control-chimeric and UV-chimeric mice (Table I). In addition, circulating blood cells in chimeric mice were reconstituted to levels measured in nonchimeric mice. Together, these results suggest that UVR does not alter the reconstitution potential of immune cell progenitors in the BM.

Reduced contact hypersensitivity response in UV-chimeric mice

The ability of control-chimeric and UV-chimeric mice to develop cell-mediated immunity was tested using a contact hypersensitivity assay. Sixteen weeks after reconstitution, the shaven ventral skin of control-chimeric and UV-chimeric mice was painted with DNFB. Four days later, DNFB was applied to the ears of the mice, and the ear swelling was measured after 24 h. The ear swelling observed in UV-chimeric mice was less than in control-chimeric mice (Fig. 3A). The contact hypersensitivity response measured in the control-chimeric mice was not significantly different from that measured in the nonchimeric mice. Considering the design of the chimeric mice, BM-derived cells in UV-chimeric mice must be responsible for the reduced contact hypersensitivity response.

T cells within LNs of UV-chimeric mice do not have reduced proliferative ability

A contact hypersensitivity response is dependent on hapten-bearing APCs activating hapten-specific T cells (28). To determine whether...
Table I. Blood screen profile from 16-wk reconstituted chimeric mice

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Nonchimeric Mice</th>
<th>Control-Chimeric Mice</th>
<th>UV-Chimeric Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs ($\times10^9/l$)</td>
<td>$6.2 \pm 0.1$</td>
<td>$6.0 \pm 0.7$</td>
<td>$7.2 \pm 1.3$</td>
</tr>
<tr>
<td>RBCs ($\times10^{12}/l$)</td>
<td>$9.3 \pm 0.5$</td>
<td>$9.2 \pm 0.1$</td>
<td>$9.5 \pm 0.1$</td>
</tr>
<tr>
<td>Platelets ($\times10^9/l$)</td>
<td>$1006.0 \pm 118.0$</td>
<td>$1044 \pm 50.1$</td>
<td>$999.5 \pm 31.3$</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>$10.9 \pm 1.1$</td>
<td>$11.4 \pm 0.4$</td>
<td>$13.2 \pm 1.2$</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>$82.1 \pm 1.3$</td>
<td>$81.1 \pm 0.4$</td>
<td>$79.2 \pm 2.0$</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>$2.9 \pm 0.2$</td>
<td>$2.5 \pm 0.2$</td>
<td>$3.0 \pm 0.2$</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>$2.6 \pm 0.2$</td>
<td>$3.4 \pm 0.4$</td>
<td>$3.4 \pm 0.7$</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM ($n = 3$ mice from a single transplant cohort).

The reduced contact hypersensitivity response observed in UV-chimeric mice was a consequence of reduced T cell–proliferative capacity, their ability to proliferate in vitro was tested. The SDLNs (axillary, brachial, and inguinal) of chimeric mice were harvested, and single-cell suspensions were prepared and incubated for 72 h with the accessory cell–independent, stimulatory combination of plate-bound anti-CD3e and soluble anti-CD28.

There was no difference in the level of $[^3]$H]thymidine incorporation by LN cells from nonchimeric mice, control-chimeric mice, and UV-chimeric mice (Fig. 3B). Prior to culture, the percentages of CD3$^+$CD4$^+$ and UV-chimeric mice (Fig. 3B). Prior to culture, the percentages of CD3$^+$CD4$^+$ T cells were similar in cell preparations from control-chimeric and UV-chimeric mice (Fig. 3C). These results suggest that there is not an intrinsic deficiency in T cell proliferation that explains the reduced contact hypersensitivity response in UV-chimeric mice.

Reduced LN hypertrophy in response to innate immune challenge in UV-chimeric mice

The reduced contact hypersensitivity response in UV-chimeric mice may be explained by functionally different BM-derived cutaneous DCs. Using FITC as a model Ag, we investigated the capacity of skin DCs to acquire Ag, migrate to draining LNs, and induce LN hypertrophy. Sixteen weeks after reconstitution, the shaved dorsal skin of chimeric mice was painted with FITC. The cell yields from the SDLNs of control-chimeric mice and age-matched nonchimeric mice increased significantly 16 h after FITC application to skin (Fig. 3D). In contrast, there was no significant increase in the cell numbers of the SDLNs of UV-chimeric mice after FITC application. The ability of an alternate stimulus to induce LN hypertrophy was tested by delivering UVR (2 kJ/m$^2$) to the shaved dorsal skin of chimeric mice. After UV irradiation (24 h), the SDLN cell yields of nonchimeric and control-chimeric mice increased significantly (Fig. 3E). The total cell number in the SDLNs of UV-chimeric mice did not increase in response to UVR. These results demonstrate that the immune mechanisms that drive LN hypertrophy are reduced in UV-chimeric mice.

Reduced number of migratory FITC$^{hi}$ DCs in SDLNs of UV-chimeric mice

The reduced LN hypertrophy observed in UV-chimeric mice after topical FITC application could be due to reduced numbers of DCs in the skin (before challenge), an impaired ability of cutaneous DCs to acquire FITC and subsequently migrate to the draining LN, or a decreased potential of FITC$^{hi}$ migratory DCs to induce LN hypertrophy when within the LN. First, the number of cutaneous DCs in the chimeric mice was determined. In this series of experiments, a stricter interpretation of DCs was used (CD11c$^+$MHCclassII$^+$). There was no significant difference in the number of DCs in skin tissue preparations from control-chimeric mice ($1.49 \pm 0.18 \times 10^{7}$/cm$^2$, mean ± SEM, $n = 3$ mice from a single transplant cohort) and UV-chimeric mice ($1.25 \pm 0.24 \times 10^{7}$/cm$^2$, $n = 4$ mice from a single transplant cohort).

Second, to determine whether cutaneous DCs in UV-chimeric mice had a reduced ability to acquire FITC in the skin and subsequently migrate to the draining LNs, SDLN DCs (Fig. 4A, gate I) were examined for FITC expression (Fig. 4A, gates II and III). Prior to FITC application, there was no difference in the number of DCs in the SDLNs of control-chimeric and UV-chimeric mice (Fig. 4A, gate I; Fig. 4B, -FITC). Sixteen hours after FITC application to dorsal skin, the number of DCs in the SDLNs of UV-chimeric mice was significantly lower than in the SDLNs of control-chimeric mice (Fig. 4B, +FITC). UV-chimeric mice had significantly lower numbers of FITC$^{hi}$ DCs (Fig. 4A, gate II) in their SDLNs compared with control-chimeric mice (Fig. 4C). There were also reduced numbers of FITC$^{lo/neg}$ DCs (Fig. 4A, gate III) in the SDLNs of UV-chimeric mice compared with control-chimeric mice (Fig. 4D).

Third, the migratory DCs within the SDLNs of FITC-painted chimeric mice were investigated. Greater than 85% of CD11c$^+$ cells in SDLNs were MHCclassII$^{hi/int}$ (data not shown). Two populations of DCs were observed in the SDLNs: one expressing MHCclassII$^{hi/int}$ (Fig. 4E, gate V) and the other expressing MHCclassII$^{lo}$ (Fig. 4E, gate IV). The MHCclassII$^{hi}$ DCs were the predominant cells expressing FITC$^{hi}$ (Fig. 4E, gate IV). The FITC$^{hi}$ DCs in SDLNs were CD8$^+$, confirming that these cells are migratory in origin (Fig. 4F). These FITC$^{hi}$ DCs in SDLNs were >90% donor origin (CD45.1 allotype, data not shown). Furthermore, the FITC$^{hi}$ DCs in SDLNs were subdivided into CD103$^+$CD11b$^{int/hhi}$ and CD103$^+$ populations (Fig. 4G) (29–31). Hence, the DCs acquiring FITC and migrating to the SDLNs were predominantly subsets of migratory dermal DCs (20% CD103$^+$ DCs, 80% CD103$^+$CD11b$^{int/hhi}$ DCs). These results suggest that, in UV-chimeric mice, fewer dermal DCs acquire FITC in the skin and migrate to the SDLNs. The migratory FITC$^{hi}$ dermal DCs were investigated further (against a background of the different absolute numbers shown in Fig. 4B–D). There was no difference in the percentage of FITC$^{hi}$ DCs within SDLNs that were CD103$^+$ between control-chimeric and UV-chimeric mice (Fig. 4G). In addition, similar percentages of SDLN FITC$^{hi}$ DCs were CD103$^+$CD11b$^{int/hhi}$ in control-chimeric and UV-chimeric mice (Fig. 4G). This result suggests that both dermal DC subsets have reduced migratory ability. FITC$^{hi}$ DCs (of confirmed migratory origin) were still present in the SDLNs of UV-chimeric mice (Fig. 4C), but they failed to induce a significant increase in the number of cells within the SDLNs (Fig. 3D). This suggests that the BM-derived DCs that replenish the skin of UV-chimeric mice have a reduced ability to migrate to the SDLNs and to stimulate LN hypertrophy once within the SDLNs. It is not known whether the smaller number of FITC$^{lo/neg}$ DCs in the SDLNs of UV-chimeric mice (Fig. 4D) results from an impaired ability of migratory DCs to transit to SDLNs. However, all LN leukocytes were maintained in the same proportion for both control-chimeric and UV-chimeric mice.
No alteration in LN cell profiles in FITC-painted control-chimeric and UV-chimeric mice

Considering there were fewer FITC<sup>hi</sup> DCs migrating from skin to SDLNs in UV-chimeric mice compared with control-chimeric mice (Fig. 4C), the SDLN cell profiles were investigated. There was no difference in the percentage of DCs in the SDLNs of control-chimeric and UV-chimeric mice (Fig. 4H). In addition, there was no disparity in the percentage of DCs in SDLNs that were FITC<sup>hi</sup> (Fig. 4I). Furthermore, when the SDLN cell profiles of FITC-painted chimeric mice were examined, there were similar percentages of B cells (CD19<sup>+</sup>B220<sup>+</sup>) and T cells (CD3<sup>+</sup>CD4<sup>+</sup>, CD3<sup>+</sup>CD4<sup>+</sup>) in control-chimeric and UV-chimeric mice (Fig. 4H). These results illustrate that a proportionally similar cell profile (infiltrating and resident) was maintained in the SDLNs of FITC-painted control-chimeric and UV-chimeric mice, despite the difference in the absolute number of migratory FITC<sup>hi</sup> DCs.

UVR induces a cell-intrinsic effect on BM-derived DCs

To determine whether the effects of UVR on BM-derived DCs were cell intrinsic, chimeric mice were generated using a 1:1 mixture of BM cells from nonirradiated (CD45.2) and UV-irradiated mice (CD45.1). When the dorsal skin of 16-wk reconstituted mixed chimeric mice was painted with FITC, there was a similar contribution of CD45.2 and CD45.1 cells to the leukocyte compartment of SDLNs (Fig. 4J, total LN cells). However, when the CD11c<sup>+</sup> DCs in these FITC-painted mixed chimeric mice were examined, there were fewer DCs of CD45.1 origin (i.e., from the BM of UV-irradiated mice) within the SDLNs (Fig. 4J, FITC<sup>hi</sup> CD11c<sup>+</sup> cells). Moreover, when the numbers of cells in the SDLNs of the mixed chimeric mice were determined 16 h after FITC application (2.52 ± 0.26 × 10<sup>7</sup> cells, mean ± SEM, n = 3 mice from a single transplant cohort), this value was between that observed for the SDLN yields of control-chimeric mice (3.13 ± 0.23 × 10<sup>7</sup> cells, mean ± SEM, n = 10 mice from five independent transplant cohorts) and UV-chimeric mice (2.32 ± 0.26 × 10<sup>7</sup> cells, mean ± SEM, n = 10 mice from five independent transplant cohorts) (Fig. 3D). This suggests a cell-intrinsic alteration in DCs derived from the BM of UV-irradiated mice.

FIGURE 3. Reduced contact hypersensitivity responses and SDLN hypertrophy in response to inflammatory stimuli in UV-chimeric mice. Control-chimeric and UV-chimeric mice were given 16 wk for reconstitution. (A) Contact hypersensitivity assay. Mice were sensitized to DNBC on their shaved ventral surface. The ears of mice were challenged with DNFB 4 d later, and the ear swelling was determined after 24 h (n = 8 ears/group). The hatched bar represents age-matched nonchimeric mice, the open bar represents control-chimeric mice, and the filled bar represents UV-chimeric mice. Ear swelling for a representative experiment of two independent transplant cohorts is shown (mean ± SEM). *p < 0.05, one-way ANOVA. (B) Proliferation of LN T cells. The cells from the SDLNs (axillary, brachial, and inguinal) of naive chimeric mice were incubated for 72 h with plate-bound anti-CD3e and soluble anti-CD28 or were left untreated. Cell suspensions were pulsed with [H]<sub>3</sub>thymidine during the final 24 h of culture. Hatched bars represent age-matched nonchimeric mice; open bars represent control-chimeric mice; and filled bars represent UV-chimeric mice. Data represent mean ± SEM from four wells prepared from each of two mice taken from independent transplant cohorts. (C) SDLN cell profile. The percentages of CD11c<sup>+</sup>MHC<sub>classII</sub> cells, CD19<sup>+</sup>B220<sup>+</sup> B cells, CD3<sup>+</sup>CD4<sup>+</sup> T cells, CD3<sup>+</sup>CD4<sup>+</sup> T cells, and CD11c<sup>+</sup>MHC<sub>classII</sub> cells in the SDLNs of control-chimeric and UV-chimeric mice were determined. Data represent mean ± SEM from four mice from two independent transplant cohorts. (D) LN cell numbers after FITC application. The shaved dorsal skin of chimeric mice was painted with FITC (+FITC) or left untreated (-FITC). The cell yields of SDLNs were determined 16 h later. For -FITC, data represent mean ± SEM from six mice from two independent transplant cohorts. For +FITC, data represent mean ± SEM from 10 mice from five independent transplant cohorts. *p < 0.05 versus untreated (-FITC) mice, Student t test. (E) LN cell numbers after UV irradiation. The shaved dorsal skin of chimeric mice was irradiated with 2 kJ/m<sup>2</sup> UVR (+UV) or left nonirradiated (-UV). The cell yields of SDLNs were determined 24 h later. Data represent mean ± SEM from six mice from two independent transplant cohorts. *p < 0.05 versus nonirradiated (-UV), Student t test.
FIGURE 4. Reduced migration of CD11c+MHCclassII+ cells from skin to draining LNs in UV-chimeric mice. The shaved dorsal skin of >16-wk reconstituted chimeric mice was painted with FITC (+FITC) or left unpainted (-FITC), and the SDLNs were harvested 16 h later. (A) Gating strategy. The CD11c+MHCclassII+ cells in SDLNs of +FITC mice (gate I) were gated for FITChi (gate II) and FITClow/neg expression (gate III). (B) CD11c+MHCclassII+ cells in SDLNs. The SDLNs were harvested from -FITC and +FITC mice, and the number of CD11c+MHCclassII+ cells was determined (i.e., gate I). For -FITC, mean ± SEM from three mice from two independent cohorts. For +FITC, mean ± SEM from eight mice from four independent transplant cohorts. (C) CD11c+MHCclassII+FITHi cells in SDLNs. The number of CD11c+MHCclassII+FITHi cells (i.e., gate II) in the SDLNs of +FITC mice was determined. Mean ± SEM from eight mice from four independent transplant cohorts. (D) CD11c+MHCclassII+FITClow/neg cells in SDLNs. The number of CD11c+MHCclassII+FITClow/neg cells (i.e., gate III) in the SDLNs of +FITC mice was determined. Open bars represent control-chimeric mice; filled bars represent UV-chimeric mice. Mean ± SEM from eight mice from four independent transplant cohorts. (E) CD11c+MHCclassIIhi (Figure legend continues)
The results above suggested that fewer FITChi migratory cutaneous DCs in UV-chimeric mice may be responsible for reduced LN hypertrophy. Whether decreased expression of activation markers on these DCs contributed to reduced hypertrophy was investigated. In the SDLNs at 16 wk postreconstitution, there was no significant difference in the expression of MHC class II, CD40, CD80, or CD86 on DCs from control-chimeric and UV-chimeric mice (Fig. 5). This was observed before (Fig. 5, top panels) and after (Fig. 5, middle panels for DCs, bottom panels for FITChi DCs) FITC application. The mean fluorescence intensity values from multiple experiments for these activation markers on DCs in SDLNs are shown in Table II. As expected, activation marker expression was significantly increased on migratory FITChi DCs (32, 33). Similar to the results described above (Fig. 4E), the expression of MHC class II on DCs seems to be bimodal, with the FITChi DCs being predominantly MHCclassIIhi (Fig. 5).

To confirm that DCs differentiated from the BM were implicated in the reduced contact hypersensitivity and inflammatory responses observed in UV-chimeric mice, the BM of 16-wk reconstituted chimeric mice was harvested for in vitro differentiation of DCs using FLT3-L or GM-CSF + IL-4. The harvested cells were tested for their in vivo priming ability (Fig. 6A). After pulsing cells with DNBS, cells were injected into the ears of naive mice, and 7 d later, the ears were challenged with DNPB. The ears of mice injected with DCs cultured from the BM of 16-wk reconstituted UV-chimeric mice using either FLT3-L (Fig. 6B) or GM-CSF + IL-4 (Fig. 6C) had reduced swelling (recall response) compared with mice injected with cells generated from the BM of control-chimeric mice. The suppression in hapten priming for DCs differentiated from the BM of UV-chimeric mice using the different growth factors (Fig. 6B, 6C) was similar to that observed for DCs differentiated from the BM of UV-irradiated nonchimeric mice (Fig. 1B, 1C). This suggests that UVR alters DC progenitors within

**FIGURE 5.** Similar activation marker expression by CD11c+MHCclassII+ cells in control-chimeric and UV-chimeric mice. Chimeric mice reconstituted for >16 wk were either left untreated (-FITC) or painted with FITC (+FITC), and SDLNs were harvested 16 h later. CD11c+MHCclassII+ cells in SDLNs of untreated chimeric mice (top panels) and FITC painted mice (middle panels for CD11c+MHCclassII+, bottom panels for CD11c+MHCclassII+FITChi) were characterized for their activation marker expression. Representative flow cytometry plots from 11 mice from four independent transplant cohorts are shown. Dashed lines indicate CD11c+MHCclassII+ cells from control-chimeric mouse. Solid lines indicate CD11c+MHCclassII+ cells from UV-chimeric mouse. Shaded graphs represent isotype controls.
the BM, and the effect on DC progenitors detected after 3 d is maintained when the BM is transplanted into marrow-ablated mice for >16 wk.

**UV-induced suppression of a contact hypersensitivity response persists for >28 d**

The previous results suggest a long-lasting effect of UVR on DC progenitors within the BM (Fig. 6). It was necessary to test the validity of the chimeric mouse model and confirm whether UVR effects on BM progenitors were long-lasting in nonchimeric mice. Hence, the duration of UVR-induced systemic suppression of contact hypersensitivity responses was tested in nonchimeric mice. The ventral skin of nonchimeric C57BL/6 mice was sensitized to DNFB at 3, 28, and 84 d post–UV irradiation (8 kJ/m²). Four days after hapten sensitization, the contact hypersensitivity response was measured following ear painting with DNFB. There was a reduced contact hypersensitivity response at 3 and 28 d post–UV irradiation compared with nonirradiated mice (Fig. 7A). By 84 d after UVR, the contact hypersensitivity response was of a similar magnitude to that measured in nonirradiated mice, which suggests that the systemic immunosuppression caused by 8 kJ/m² UVR subsides within 84 d. The longevity of the UVR-induced effects on the contact hypersensitivity responses supports the involvement of BM progenitors, as well as those altered by UVR, in replenishing immune cells in the periphery for ≥28 d.

**Reduced priming ability of BM-derived DCs from UV-irradiated IL-10−/− mice**

IL-10 was reported to be involved in UVR-induced systemic immunosuppression (34). To test whether IL-10 was involved in the generation of BM-derived DCs with reduced priming ability from UV-irradiated mice, IL-10−/− mice were exposed to 8 kJ/m² UVR, and 3 d later, the BM cells were harvested and cultured with GM-CSF + IL-4 for 7 d. After DBNs loading, BM-derived DCs were adoptively transferred into naïve wild-type (WT) mice to test their in vivo priming ability. There was reduced priming by DCs differentiated from the BM of UV-irradiated IL-10−/− mice compared with nonirradiated IL-10−/− mice (Fig. 7B). A similar reduced priming was seen in BM-derived DCs from age-matched UV-irradiated WT mice. There was no significant difference in the reduced DC priming ability between UV-irradiated IL-10−/− mice (44.2 ± 11.0% reduction, mean ± SEM, n = 3 independent experiments with eight ears measured per experiment) and UV-irradiated WT mice (62.4 ± 9.4% reduction, mean ± SEM, n = 3).

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**Table II. Activation marker expression on CD11c+MHCclassII+ cells in SDLNs of chimeric mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MHC Class II</th>
<th>CD40</th>
<th>CD80</th>
<th>CD86</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>6,807.3 ± 473.3</td>
<td>1,429.0 ± 321.9</td>
<td>450.3 ± 83.4</td>
<td>1,778.3 ± 200.4</td>
</tr>
<tr>
<td>Control-chimeric</td>
<td>6,795.6 ± 301.9</td>
<td>1,214.0 ± 230.5</td>
<td>515.5 ± 66.9</td>
<td>1,745.5 ± 200.5</td>
</tr>
<tr>
<td>UV-chimeric</td>
<td>5,990.1 ± 722.8</td>
<td>1,583.7 ± 161.9</td>
<td>617.3 ± 38.4</td>
<td>2,550.2 ± 288.9*</td>
</tr>
<tr>
<td>FITC painted</td>
<td>8,206.1 ± 602.1*</td>
<td>1,520.1 ± 147.3</td>
<td>586.1 ± 51.5</td>
<td>2,605.1 ± 265.2*</td>
</tr>
</tbody>
</table>

aData represent mean fluorescence intensity (± SEM) (n = 4 mice from two independent transplant cohorts).

bData represent mean fluorescence intensity (± SEM) (n = 11 mice from four independent transplant cohorts).

*p < 0.05 versus untreated, **p < 0.05 versus FITC painted, Student t test.
WT and IL-10 induced suppression of BM-derived DC function. BM was harvested from the BM of UV-irradiated mice compared with mice injected with DCs differentiated from the BM of nonirradiated mice (Fig. 7C). This result suggests that DCs differentiated from the BM of UV-irradiated mice can be delivered to sites other than skin to regulate systemic immune responses.

**DCs differentiated from the BM of UV-irradiated mice do not have reduced immunogenicity**

Epigenetic effects of UVR were investigated by i.p. injection of 5-aza-dC 1 h before and on two subsequent days after UV irradiation of shaved skin of 8-wk-old BALB/c mice. BM was taken 3 d after UVR, and the immunogenicity of DCs differentiating during a 7-d culture of BM cells was examined. The significantly reduced swelling measured in the ears of mice injected with DCs differentiated from the BM of UV-irradiated mice was not seen when the UV-irradiated mice were injected with 5-aza-dC (Fig. 8A).

**Reduced immunogenicity of BM-derived DCs from progeny of UV-irradiated mothers**

Because the gestational period is particularly susceptible to epigenetic perturbation (35), mice were UV irradiated (8 kJ/m²) on the day of evidence of successful conception (i.e., detection of a vaginal plug). Two independent cohorts provided five nonirradiated and three UV-irradiated mothers giving birth. Litter sizes were four babies/litter (40% female) and 5.6 babies/litter (23% female) for nonirradiated and UV-irradiated mothers, respectively. When the progeny were 6–9 wk of age, BM cells were pooled from two mice from the same litter for culture with GM-CSF + IL-4 for DC differentiation. For each experiment, there were two BM cell cultures from progeny of UV-irradiated mothers and an equal number from nonirradiated mothers. After 7 d in culture, the DCs were loaded with DNBS and adoptively transferred into new mice for assay of their in vivo priming ability. If the progeny were male, the recipient mice in the in vivo priming assay were male. Significantly reduced swelling was measured in the ears of mice that received DCs differentiated from the BM of progeny of UV-irradiated mothers (Fig. 8B shows a representative experiment). When the results of three independent in vivo–priming assays (from two cohorts) were combined, the ability of DCs differentiated from the BM of mice delivered from UV-irradiated mothers to prime immune responses was 33 ± 2% (mean ± SEM) less than that of DCs differentiated from the BM of UV-irradiated mice (GM-CSF + IL-4) were pulsed with OVA and injected i.v. into mice previously sensitized with OVA. Seven days later, the recipient mice were challenged with aerosol containing OVA, and the ADLNs were harvested 24 h later. The open bar represents mice injected with DCs differentiated from the BM of nonirradiated mice; the filled bar represents mice injected with DCs differentiated from the BM of UV-irradiated mice. Data represent mean ± SEM of eight mice from two independent experiments. *p < 0.05, Student t test.

**FIGURE 7.** Investigating the impaired priming ability of DCs from the BM of UV-irradiated mice. (A) Long-lasting suppression of contact hypersensitivity responses to UV-irradiated mice. The shaved dorsal skin of WT C57BL/6 mice was administered 8 kJ/m² UVR. Three d (-3d), 28 d (-28d), and 84 d (-84d) post-UVR, the shaved ventral skin of mice was sensitized to DNFB. After 4 d, the ears of mice were painted with DNFB, and the ear swelling was measured 24 h later (n = 8 ears/group). The open bar represents mice not exposed to UVR prior to the contact hypersensitivity assay; the filled bars represent mice irradiated prior to DNFB sensitization. Ear swelling for a representative experiment, mean + SEM. *p < 0.05, one-way ANOVA. (B) IL-10 is not significantly involved in UV-induced suppression of BM-derived DC function. BM was harvested from WT and IL-10/− mice that were UV irradiated or not irradiated and cultured with GM-CSF + IL-4 for DC differentiation. Differentiated DCs were tested for their in vivo priming ability by adoptive transfer into WT recipients. The open bars represent mice injected with DCs differentiated from the BM of nonirradiated mice; the filled bars represent mice injected with DCs differentiated from the BM of UV-irradiated mice. Data represent mean ± SEM of three independent experiments (eight ears injected per experiment). *p < 0.05, Student t test. (C) DCs differentiated from the BM of UV-irradiated mice can regulate airway immune responses. DCs differentiated from the BM of UV-irradiated mice (GM-CSF + IL-4) were pulsed with OVA and injected i.v. into mice previously sensitized with OVA. Seven days later, the recipient mice were challenged with aerosol containing OVA, and the ADLNs were harvested 24 h later. The open bar represents mice injected with cells differentiated from the BM of nonirradiated mice; the filled bar represents mice injected with cells differentiated from the BM of UV-irradiated mice. The horizontal line represents the number of cells in the ADLNs of mice injected i.v. with saline. Data represent mean ± SEM of eight mice from two independent experiments. *p < 0.05, Student t test.
Discussion

Our previous studies using BM cells in culture suggested that UVR altered DC progenitors in the BM such that the differentiated DCs had reduced priming ability (17). We now confirm using chimeric mice that an erythemal dose of UVR delivered to shaved dorsal skin modulates DC progenitors within the BM and that this effect is long-lasting. Further, the cutaneous DCs differentiated from the BM of UV-chimeric mice are less capable of inducing a recall immune response and have altered responses to innate signals. Evidence is also presented that epigenetic alterations, including increased gene methylation, are involved in the mechanisms by which UVR alters DC progenitors in the BM.

When BM from UV-irradiated mice was used to generate chimeric mice (UV-chimeric mice), there was a diminished immune response in the recipient mice in vivo when a contact hypersensitivity response was observed in UV-chimeric mice that had been reconstituted for 16 wk after UVR exposure. This suggests that UVR affects a long-lasting effect on DC progenitors. To determine whether the generation of chimeric mice had amplified the effects of UVR on DC progenitors, immune responses were examined in WT C57BL/6 mice, 3, 28, and 84 d after UVR exposure. The UVR-induced effect on contact hypersensitivity responses measured only after 84 d was not significant. This is similar to a recent report (36), although these mice were sensitized at the UVR-irradiated site (local UVR-induced immunosuppression). All studies confirm the long-lasting effects of UVR.

Our initial studies showed that DCs differentiated from the BM of UV-irradiated mice using FLT3-L or GM-CSF + IL-4 have reduced priming ability. These are two established models for steady-state or inflammatory DC differentiation, respectively (23–26). Because FLT3-L and GM-CSF can regulate the development of early DC progenitors, immune responses were examined in WT C57BL/6 mice 3, 28, and 84 d after UVR exposure. The UVR-induced effect on contact hypersensitivity responses measured only after 84 d was not significant. This is similar to a recent report (36), although these mice were sensitized at the UVR-irradiated site (local UVR-induced immunosuppression). All studies confirm the long-lasting effects of UVR.

Recent studies showed that LT-HSCs can respond directly to inflammation and infection, rather than simply replenishing depleted hematopoietic cells (reviewed in Ref. 16). For instance, a number of laboratories reported that LPS-induced signaling through TLR4 (14, 43) and proinflammatory mediators, such as IFN (44, 45), can modulate LT-HSC function, with particular emphasis on their self-renewal potential and subsequent ability to
differentiate into downstream progenitors. This study shows that UVR can alter long-lived DC progenitors (still detected in the recipient mice >16 wk after reconstitution), and the differentiated DCs that seed the peripheral tissues can cause reduced immune responses in the host.

Because our results suggested that DC progenitors in the BM of UV-chimeric mice were altered, we further investigated the phenotype of the DCs in peripheral LNs. Because the activation markers MHC class II, CD40, CD80, and CD86 may be expressed at lower levels in poorly “immunogenic” DCs (reviewed in Refs. 46, 47), these markers were measured on DCs within SDLNs of either untreated or FITC-painted chimeric mice. FITC treatment increased the expression of these molecules on DCs in SDLNs, but there was no difference in expression between control-chimeric and UV-chimeric mice. This confirms the results of our previous study that suggested that the reduced immunogenicity of in vitro-differentiated DCs from UV-irradiated mice is not a product of altered maturation (17). The DCs differentiated from UV-chimeric mice may use other mechanisms to suppress the immune response (46, 47). However, this has yet to be determined in our studies.

Plasmacytoid DCs were described to be poor APCs and were implicated in tolerance (reviewed in Ref. 48). However, there was no alteration in the percentage of CD11c+CD11b- B220+ plasmacytoid DCs in FLT3-L cultures using BM cells of UV-irradiated mice. Furthermore, only 2% of CD11c+ cells differentiated using GM-CSF + IL-4 express a plasmacytoid DC phenotype. In light of these findings, suppressed priming ability was still observed in DCs cultured from UV-irradiated mice in both differentiative conditions. This indicates that a mechanism not involving plasmacytoid DC differentiation is responsible for reducing the priming ability of these DCs cultured from UV-irradiated mice.

A PGE2-mediated mechanism may be responsible for this long-lasting change to DC progenitors within the BM of UV-chimeric mice. In our previous study, the downregulation of the priming function of DCs cultured from the BM of UV-irradiated mice was blocked by the administration of indomethacin 4 d prior to UV irradiation (17). In addition, the UV-induced effect could be reproduced by administration of pellets releasing 10 μg/d PGE2 to mice (17). However, it is unknown whether PGE2 has a direct effect on DC progenitors within the BM or whether the effect is mediated through another cell type, such as mesenchymal stem cells or osteoblasts. The effect of inflammation on DC progenitors within the BM was also reported in animal models of inflammatory airway disease (49). Similar to the UVR-induced skin inflammation model, prostanoids were a key mediator involved in signals from the inamed airways to DC progenitors in the BM. It is possible that the induction of less immunogenic DCs from the BM could represent a homeostatic response to avoid overzealous inflammation. The longevity of the effect is surprising, and it will be important to use molecular tools to determine the nature of the imprinting on DC progenitors.

Our experiments suggest that the prolonged effects of UVR may reflect an epigenetic alteration involving increased DNA methylation. Experiments using 5-aza-dC, an inhibitor of DNA methyltransferase (50) and, thus, an inhibitor of DNA methylation, demonstrated that the effects of UVR on BM DC progenitors involved methylation of critical promoters/genes. We propose that, because PGE2 can stimulate DNA methyltransferase activity (51) and PGE2 may be responsible for the change to DC progenitors within the BM of UV-irradiated mice (17), 5-aza-dC inhibited PGE2-induced DNA methyltransferase activity in the UV-irradiated mice. In turn, UVR was unable to stimulate the methylation and silencing of (uncharacterized) genes involved in attenuated immune responses. The studies of UV-irradiated mothers suggested that UVR had an epigenetic effect on cells in the embryo. The effects of UVR maintained in the chimeric mice for >16 wk also support an epigenetic basis for changes in DC progenitors in the BM of UV-irradiated mice.

UV-induced IL-10, secreted from skin-resident cells (e.g., keratinocytes and mast cells) and infiltrating cells (e.g., regulatory T cells), was implicated in the processes of limiting excessive UV-induced inflammation at the affected skin site, as well as promoting systemic immunosuppression (34, 52). Furthermore, regulatory DCs may suppress responses by upregulated IL-10 production (46). However, our results indicate that IL-10 is not an important mediator of the effects of UVR on the differentiation of poorly priming DCs from BM. The DCs differentiated from the BM of UV-irradiated mice could regulate systemic immune responses other than those dependent on skin DCs. After i.v. delivery of OVA-loaded DCs, responses to OVA delivered to the airways were reduced significantly in those mice injected with DCs differentiated from the BM of UV-irradiated mice. Mechanistically, studies with mixed chimeras suggested that CD11c+ DCs differentiated from BM of UV-irradiated mice had cell-intrinsic altered properties, with a diluted effect in the mixed-chimeric mice. Studies in the mixed-chimeric mice suggested that, in a competitive environment, the DCs that differentiate from the BM of UV-irradiated mice have a reduced ability to migrate to SDLNs upon topical inflammatory challenges.

In summary, an erythemal dose of UVR can induce a long-lasting effect on early DC progenitors within the BM. Subsequently, the terminally differentiated DCs that develop from these progenitors, and seed the periphery, have a reduced ability to initiate a response to Ags and inflammatory stimuli. With BM involvement, we propose that UVR may modulate immune responses for several weeks. The modulation of the immune system by UVR exposure may have clinical benefits in diseases such as multiple sclerosis and asthma. Further elucidation of the mechanisms involved in UVR-induced modulation of the immune system could provide a means to alleviate, and possibly prevent, these conditions. More generally, this study teaches us about terminally differentiated progeny from the BM following UVR-induced skin inflammation.

Disclosures
The authors have no financial conflicts of interest.

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15. photochem. photobiol. 327: 316.


