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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*

Altering 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 erythemal 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 (UV-chimeric). When the dorsal skin of UV-chimeric mice was challenged with innate inflammatory agents, the hypertrophy 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. Erythemal 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: 5471–5484.

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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 UVR 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<sup>–</sup> (CD45.1 alloantigen), and BALB/c mice were obtained from the Animal Resources Centre (Murdoch, WA, Australia). B6/IL10<sup>–/–</sup> mice were bred in-house (Centre for Cancer Research, 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 cm<sup>2</sup>). To administer UVR, mice were placed 20 cm beneath the UV lamps, and up to 8 kJ/m<sup>2</sup> UVR was delivered (8 kJ/m<sup>2</sup> UVR is equivalent to three or four minimal erythemal doses). When treated, the ears were covered with black plastic tape prior to UV irradiation. The UVB output by the lamps was measured using a UVX radiometer (UV Products, Upland, CA). Mice were 6–10 wk old at the time of irradiation, unless otherwise stated.

Isolation of BM cells for in vitro culture

BM cells were prepared by flushing the tibias and femurs of mice using a solution of 11 mM Na<sub>2</sub>glucose, 5.5 mM KCl, 137 mM NaCl, 25 mM Na<sub>2</sub>HPO<sub>4</sub>, 5.5 mM NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O containing 10% FCS. Bone debris was removed by passing the BM cells through cotton wool. The BM cells were cultured in RPMI 1640 (Thermo Scientific, Waltham, MA) supplemented with 10% FCS (Sigma-Aldrich, St. Louis, MO), 2 mM l-glutamine, 50 μM 2-ME, and 5 μg/ml gentamicin (Sigma-Aldrich) (RPMI-10) at a density of 8 × 10<sup>5</sup> cells/ml/well in 24-well plates. The medium was supplemented with 10 ng/ml GM-CSF + 10 ng/ml IL-4 (PeproTech, Rocky Hill, NJ) to promote inflammatory DC development or 200 ng/ml FLT3-L (PeproTech) to promote steady-state DC development. The medium of cells cultured in GM-CSF + IL-4 was replaced after 48 and 96 h during the 7 d of culture. The medium of FLT3-L–cultured cells was not replenished during 9 d of culture. All cultures were maintained at 37°C in a 5% CO<sub>2</sub>-humidified environment. Following culture, all nonadherent cells were harvested.

Adoptive transfer of DCs differentiated from BM into naive recipients for an in vivo priming assay

Cells were prepared and transferred into naive recipients as previously described (17). Briefly, BM cells cultured for 7 d (in GM-CSF + IL-4) were enriched to >95% CD11c<sup>+</sup> cells (confirmed by flow cytometry) using magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany), and 10<sup>6</sup> cells/ml in RPMI-10 were pulsed with 1 mM dihydrobenzene sulfonic acid–sodium salt (DNBS, MP Biomedicals, Santa Ana, CA) for 30 min at 37°C. Because of the reduced numbers of differentiated cells produced after culture for 9 d with FLT3-L (compared with GM-CSF + IL-4), these cells were cultured in GM-CSF + IL-4, enriched using positive selection, and pulsed with DNBS. Cells (10<sup>6</sup>) were injected i.v. into 0.9% saline (20 μl; Baxter, Deerfield, IL) into the ear pinnae of naive mice (n = 8 ears/group). Separate mice were injected with 20 μl 0.9% saline as a sensitization control. Seven days later, a recall immune response was performed by painting both sides of each ear with 10 μl 0.2% w/v DNFB (prepared in acetone for BALB/c mice or acetone 4:1 olive oil for C57BL/6 and chimeric mice) (Sigma-Aldrich). Ear thicknesses were determined before and 24 h after challenge with DNFB using a spring-loaded micrometer (Mitutoyo, Aurora, IL). The response to hapten priming was determined by subtracting the ear swelling measured in challenge-only mice (no sensitization).

Adoptive transfer of DCs differentiated from BM of UV-irradiated mice into OVA-sensitized recipients

DCs were differentiated as described in the previous section but were pulsed with 1 μg/ml OVA (Sigma-Aldrich) for 60 min at 37°C. Cells (2 × 10<sup>6</sup>) were injected i.v., in 0.9% saline (200 μl), into mice that were injected i.p. (7 d earlier) with 200 μl 0.9% saline solution containing 10 μg OVA and 2 mg aluminum hydroxide (Serva, Heidelberg, Germany). As a control (for cell injection), separate mice were injected with 200 μl 0.9% saline. Seven–days after i.v. injection with cells, mice were challenged with a 1% w/v OVA (prepared in 0.9% saline) delivered as an aerosol using a UltraNeb ultrasonic nebulizer (DeVilbiss Healthcare, Somerset, PA) for 30 min. Twenty-four hours later, the LNs draining the airways (ADLNs; posterior mediastinal, tracheobronchial, and parathymic) were harvested, and the total cell numbers were determined.

Phenotyping of BM, spleen, and LN cells

Single-cell suspensions of spleen and LN were prepared by physical disaggregation and passed through an FCS-washed 100-μm nylon filter (BD Biosciences, San Jose, CA). Spleen and BM cells suspension were lysed by RBCs using ammonium chloride prior to phenotyping. Cells were washed with 11 mM Na<sub>2</sub>glucose, 5.5 mM KCl, 137 mM NaCl, 25 mM Na<sub>2</sub>HPO<sub>4</sub>, 5.5 mM NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O containing 0.2% w/v BSA (Sigma-Aldrich), with all procedures performed at 4°C. To prevent nonspecific binding, cells were preincubated with anti-CD16/CD32 FcX Abs (Fc Block) for 5 min. For surface marker detection, cells were then incubated with Abs targeting CD11c, CD11b, MHC class II (I-A/I-E), CD19, CD4, CD45.2, CD45.1, CD103, CD40, CD86, B220, CD8α, CD3e, Gr1, and Ter119 for 30 min. Abs were labeled with FITC, PE, PE-Cy5, PE-Cy7, V450, allophycocyanin, allophycocyanin-Cy7, or biotin. Cells stained with biotinylated Abs were subsequently incubated with streptavidin–PE–Cy5 or streptavidin–allophycocyanin for 30 min. Appropriate isotype controls were included. Abs were purchased from BD Biosciences, eBioscience (San Diego, CA), or BioLegend (San Diego, CA). Data were acquired on a LSR II (BD Biosciences) or LSRFortessa (BD Biosciences) and analyzed using FlowJo software (version 8.8; TreeStar, Ashland OR).

BM chimeric mice

Eight-week-old recipient C57BL/6 (CD45.2 alloantigen) mice were gamma irradiated (2 × 550 rad) using a 137<sup>Cs</sup> source (Gammacell 3000 Elan; MDS Nordion, Ottawa, Canada). BM-ablated mice were injected i.v. with...
2 × 10^6 BM cells prepared from congenic B6.SJL-Ptprc^c (CD45.1 allo-antigen) mice that had either been exposed to 8 kJ/m² 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 K₂EDTA-coated Microtainer 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 after challenge with DNFB. The change in ear swelling attributed to sensitization was determined by subtracting the ear swelling measured in nonsensitized DNFB-challenged mice.

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

The skin-draining LNs (SDLNs; axillary, brachial, and inguinal) 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-10 containing 10 mM nonessential amino acids (Sigma-Aldrich) and 1 mM sodium pyruvate (Sigma-Aldrich). Cells (10^7) were aliquoted into wells of round-bottom 96-well plates and stimulated with 10 μg/ml plate-bound anti-CD3ε (BD Biosciences) and 1 μg/ml anti-CD28 (BD Biosciences) for 72 h. Alternatively, cells were left unstimulated. LN were prepared from 0.5 μl [3H]thyminidine (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, U.K.) after 48 h in culture. Twenty-four hours later, [3H]thyminidine 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.

**Statistical analysis**

Statistical analysis was performed using the Student t test or one-way ANOVA with Bonferroni correction. Differences were considered statistically significant when p < 0.05.

**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 10^6 cells were injected into the ear pinnae of naïve 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 naïve 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 were 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...
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+CD122+), T cells (CD3+CD4+, CD3+CD8+), 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 shaved 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
of CD3+CD4 and UV-chimeric mice (Fig. 3B). Prior to culture, the percentages ration by LN cells from nonchimeric mice, control-chimeric mice, and UV-chimeric mice (Fig. 3B). Prior to culture, the percentages of CD3+CD4− and 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/m²) 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<sup>hi</sup> 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<sup>hi</sup> 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<sup>+</sup>MHC<sub>classII</sub><sup>+</sup>). There was no difference in the number of DCs in skin tissue preparations from control-chimeric mice (1.49 ± 0.18 × 10<sup>7</sup>/cm², mean ± SEM, n = 3 mice from a single transplant cohort) and UV-chimeric mice (1.25 ± 0.24 × 10<sup>7</sup>/cm², 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<sup>hi</sup> DCs (Fig. 4A, gate II) in their SDLNs compared with control-chimeric mice (Fig. 4C). There were also reduced numbers of FITC<sup>lo/neg</sup> 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<sup>+</sup> cells in SDLNs were MHC<sub>classII</sub><sup>lo/neg</sup> (data not shown). Two populations of DCs were observed in the SDLNs: one expressing MHC<sub>classII</sub><sup>lo</sup> (Fig. 4E, gate V) and the other expressing MHC<sub>classII</sub><sup>hi</sup> (Fig. 4E, gate IV). The MHC<sub>classII</sub><sup>lo</sup> DCs were the predominant cells expressing FITC<sup>hi</sup> (Fig. 4E, gate IV). The FITC<sup>lo/neg</sup> DCs in SDLNs were CD8<sup>+</sup> <sup>+</sup>, confirming that these cells are migratory in origin (Fig. 4F). These FITC<sup>lo/neg</sup> DCs in SDLNs were <sup>90%</sup> donor origin (CD45.1 alloantigen, data not shown). Furthermore, the FITC<sup>lo/neg</sup> DCs in SDLNs were subdivided into CD103<sup>+</sup>CD11b<sup>hi</sup> and CD103<sup>-</sup> populations (Fig. 4G) (29–31). Hence, the DCs acquiring FITC and migrating to the SDLNs were predominantly subsets of migratory dermal DCs (20% CD103<sup>+</sup> DCs, 80% CD103<sup>-</sup>CD11b<sup>hi</sup> 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<sup>lo/neg</sup> 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<sup>lo/neg</sup> DCs within SDLNs that were CD103<sup>-</sup> between control-chimeric and UV-chimeric mice (Fig. 4G). In addition, similar percentages of SDLN FITC<sup>lo/neg</sup> DCs were CD103<sup>-</sup>CD11b<sup>hi</sup> in control-chimeric and UV-chimeric mice (Fig. 4G).

This result suggests that both dermal DC subsets have reduced migratory ability. FITC<sup>lo/neg</sup> 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<sup>lo/neg</sup> 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.

### 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 (× 10&lt;sup&gt;9&lt;/sup&gt;/l)</td>
<td>6.2 ± 0.1</td>
<td>6.0 ± 0.7</td>
<td>7.2 ± 1.3</td>
</tr>
<tr>
<td>RBCs (× 10&lt;sup&gt;12&lt;/sup&gt;/l)</td>
<td>9.3 ± 0.5</td>
<td>9.2 ± 0.1</td>
<td>9.5 ± 0.1</td>
</tr>
<tr>
<td>Platelets (× 10&lt;sup&gt;11&lt;/sup&gt;/l)</td>
<td>1006.0 ± 118.0</td>
<td>1044 ± 50.1</td>
<td>999.5 ± 31.3</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>10.9 ± 1.1</td>
<td>11.4 ± 0.4</td>
<td>13.2 ± 1.2</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>82.1 ± 1.3</td>
<td>81.1 ± 0.4</td>
<td>79.2 ± 2.0</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2.9 ± 0.2</td>
<td>2.5 ± 0.2</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.6 ± 0.2</td>
<td>3.4 ± 0.4</td>
<td>3.4 ± 0.7</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM (n = 3 mice from a single transplant cohort).
No alteration in LN cell profiles in FITC-painted control-chimeric and UV-chimeric mice

Considering there were fewer FITCh 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 FITCh (Fig. 4I). Furthermore, when the SDLN cell profiles of FITC-painted chimeric mice were examined, there were similar percentages of B cells (CD19+B220+) and T cells (CD3+CD4−, CD3+CD4+) 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 FITCh 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+ 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, FITCh CD11c+ 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 × 10⁷ 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 × 10⁷ cells, mean ± SEM, n = 10 mice from five independent transplant cohorts) and UV-chimeric mice (2.32 ± 0.26 × 10⁷ 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 DNFB 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 [3H]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 of four wells prepared from each of two mice taken from independent transplant cohorts. (C) SDLN cell profile. The percentages of CD11c+MHCclassII+ cells, CD19+B220+ B cells, CD3+CD4+ T cells, CD3+CD4− T cells, and CD11c+MHChistI+ 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/m² 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). (C) CD11c+MHCclassII+FITChi cells in SDLNs. The number of CD11c+MHCclassII+FITChi 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)
Dashed lines indicate CD11c+MHCclassII+ cells from control-chimeric mice. Solid lines indicate CD11c+MHCclassII+ cells from UV-chimeric mice.

Characterized for their activation marker expression. Representative flow cytometry plots from 11 mice from four independent transplant cohorts are shown.

Expression of activation markers for T cells is not reduced on dermal DCs differentiated from the BM of UV-chimeric mice.

The results above suggested that fewer FITC<sup>hi</sup> 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 FITC<sup>hi</sup> 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 FITC<sup>hi</sup> 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 FITC<sup>hi</sup> DCs being predominantly MHCclassII<sup>hi</sup> (Fig. 5).

Reduced priming ability of DCs differentiated from BM of UV-chimeric mice.

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 DNFB. 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...
was a reduced contact hypersensitivity response at 3 and 28 d. The response was measured following ear painting with DNFB. Four days after hapten sensitization, the contact hypersensitivity response was measured. It suggests that the systemic immunosuppression caused by the systemic immunosuppression caused by 8 kJ/m$^2$. Four days after UVR, the contact hypersensitivity response was reduced 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/m$^2$). 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/m$^2$ UVR subsides within 84 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/m$^2$ UVR, and 3 d later, the BM cells were harvested and cultured with GM-CSF + IL-4 for 7 d. After DNBS loading, BM-derived DCs were adoptively transferred into naive 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 $\pm$ 11.0% reduction, mean $\pm$ SEM, n = 3 independent experiments with eight ears measured per experiment) and UV-irradiated WT mice (62.4 $\pm$ 9.4% reduction, mean $\pm$ SEM, n = 3).

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**Figure 6.** Sixteen weeks after reconstitution, DCs cultured from the BM of UV-chimeric mice maintain reduced priming ability. (A) The BM of $>16$-wk reconstituted chimeric mice was harvested and cultured with FLT3-L or GM-CSF + IL-4 for DC differentiation. Cultured cells were pulsed with DNBS, and 3.5 $\times$ 10$^6$ cells (FLT3-L cultured) or 10$^6$ cells (GM-CSF + IL-4) were injected s.c. into the ear pinnae of naive mice (n = 8 ears/experiment). Seven days after injection, the ears of recipient mice were painted with DNFB, and the ear swelling was measured after 24 h. (B) Priming ability of DCs cultured from the BM of chimeric mice with FLT3-L. Mean $\pm$ SEM from a representative experiment is shown. (C) Priming ability of DCs cultured from the BM of chimeric mice with GM-CSF + IL-4. Open bars represent mice injected with DCs cultured from control-chimeric mice; filled bars represent mice injected with DCs cultured from UV-chimeric mice. Ear swelling for a representative experiment of two independent transplant cohorts. *p < 0.05, Student t test.

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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$^a$</td>
<td>6,807.3 $\pm$ 473.3</td>
<td>1,429.0 $\pm$ 321.9</td>
<td>450.3 $\pm$ 83.4</td>
<td>1,778.3 $\pm$ 200.4</td>
</tr>
<tr>
<td>Control-chimeric mice</td>
<td>6,795.6 $\pm$ 301.9</td>
<td>1,214.0 $\pm$ 230.5</td>
<td>515.5 $\pm$ 66.9</td>
<td>1,745.5 $\pm$ 200.5</td>
</tr>
<tr>
<td>UV-chimeric mice</td>
<td>7,990.1 $\pm$ 722.8$^*$</td>
<td>1,583.7 $\pm$ 161.9</td>
<td>617.3 $\pm$ 38.4$^*$</td>
<td>2,550.2 $\pm$ 288.9$^*$</td>
</tr>
<tr>
<td>FITC painted$^b$</td>
<td>8,206.1 $\pm$ 602.1$^*$</td>
<td>1,520.1 $\pm$ 147.3</td>
<td>586.1 $\pm$ 51.5</td>
<td>2,605.1 $\pm$ 265.2$^*$</td>
</tr>
<tr>
<td>Control-chimeric mice</td>
<td>17,379.8 $\pm$ 803.0$^{**}$</td>
<td>2,315.3 $\pm$ 210.8$^{**}$</td>
<td>1,044.4 $\pm$ 71.5$^{**}$</td>
<td>7,478.5 $\pm$ 404.6$^{**}$</td>
</tr>
<tr>
<td>UV-chimeric mice</td>
<td>15,756.2 $\pm$ 818.8$^*$</td>
<td>2,359.7 $\pm$ 167.4$^*$</td>
<td>1,021.1 $\pm$ 101.4$^*$</td>
<td>7,069.3 $\pm$ 386.2$^*$</td>
</tr>
</tbody>
</table>

$^a$Data represent mean fluorescence intensity ($\pm$ SEM) (n = 4 mice from two independent transplant cohorts).
$^b$Data represent mean fluorescence intensity ($\pm$ SEM) (n = 11 mice from four independent transplant cohorts).
$p < 0.05$ versus untreated, **p < 0.05 versus FITC painted, Student t test.
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 injected with 5-aza-dC 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 DBNS 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 different 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 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.
DCs differentiated from the BM of UV-chimeric mice are less immunogenic. When the SDLNs of UV-chimeric mice were examined, there were fewer migratory FITC<sup>hi</sup> DCs. Although this indicates an altered function of peripheral DCs in UV-chimeric mice, it is not known whether the lower numbers of migratory FITC<sup>hi</sup> DCs in the SDLNs reflect a reduced ability of cutaneous DCs to uptake FITC hapten, whether they have a reduced ability to migrate to SDLNs, and/or whether they have reduced survival. The finding that FITC<sup>hi</sup> DCs could still be detected in the SDLNs of UV-chimeric mice, but there was no change in the SDLN cell yield, suggests that peripheral DCs in UV-chimeric mice may also have a reduced capability to develop an immune response. It remains possible that, in UV-chimeric mice, there were not sufficient migratory F<sup>ITC</sup><sup>hi</sup> DCs within SDLNs to reach a threshold to induce LN hypertrophy. Despite this, these results confirm our earlier suggestions that UVR alters an early DC progenitor within the BM (17). Moreover, it also illustrates that the less immunogenic DCs that develop in vivo from the BM of UV-irradiated mice can subsequently seed peripheral sites and be responsible for reduced immune responses to inflammatory stimuli.

Because the rate of reconstitution of control-chimeric and UV-chimeric mice might influence the ability of mice to initiate an immune response, engraftment kinetics were investigated. However, there was no difference in the reconstitution of control-chimeric and UV-chimeric mice with regard to cell yields or the percentage of leukocyte cell types within the blood, the secondary lymphoid organs, or the skin. Thus, the different immune responses observed in control-chimeric and UV-chimeric mice did not reflect altered reconstitution of hematopoietic pathways, but rather differences in the functional capacity of the BM-derived immune cells.

In the 16-wk reconstituted UV-chimeric mice, changes to the DC progenitors within the BM (assessed in a priming assay with in vitro-differentiated DCs) were as strong as measured in BM harvested 3 d after UVR. This suggests that UVR induces 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 UV-irradiated site (local UV-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 (37–41), this suggested that UVR may affect an early DC progenitor within the BM. Because diminished immune responses were observed in UV-chimeric mice that had been reconstituted for > 16 wk, it is possible that UVR affects LT-HSCs (11). Although there are variations on how to define LT-HSCs phenotypically, these cells are best characterized by their functional ability to provide long-term hematopoietic renewal (> 16 wk) (42).

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. 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 inflamed 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 erythematous 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.

References