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Prostaglandin E_2 imprints a long-lasting effect on dendritic cell progenitors in the bone marrow

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ABSTRACT

Dendritic cells (DCs) that differentiate in vitro from the bone marrow (BM) of mice with prostaglandin E_2 (PGE_2)-associated inflammation of the skin, airways, or peritoneal cavity poorly initiate immune responses. To remove in vitro differentiation and allow BM-derived DCs to seed the periphery under steady-state conditions, as well as study the molecule proposed responsible, chimeric mice were engrafted for >16 wk with BM cells from mice exposed to PGE_2. Serial PGE_2-chimeric mice were established with BM cells from the primary chimeric mice. Immune responses in the airways and skin of the PGE_2-chimeric mice and serial PGE_2-chimeric mice were significantly attenuated. After inflammatory challenges by intranasal LPS, topical fluorescein isothiocyanate, and intraperitoneal alum, DCs, macrophages, and neutrophils trafficked poorly in PGE_2-chimeric mice and serial PGE_2-chimeric mice. Injection of BM-differentiated DCs from nonchimeric mice restored the reduced immune responses of PGE_2-chimeric mice. DCs from BM of 16-wk-engrafted PGE_2-chimeric and serial PGE_2-chimeric mice resembled cells differentiated from BM exposed to PGE_2 for only 3 d, demonstrating the long-lasting effect of PGE_2 on DC progenitors. PGE_2 attenuates systemic immune responses by modulating myeloid cell progenitors in the BM such that BM-derived, terminally differentiated myeloid cells have poor trafficking ability to sites of need. *J. Leukoc. Biol.* 95: 225–232; 2014.

Introduction

Prostaglandin E_2 (PGE_2), a biolipid derived from the metabolism of arachidonic acid, has been associated with the resolution phase of inflammation [1]. More specifically, tissue resolution of inflammation is significantly delayed in mice given nonsteroidal anti-inflammatory drugs, which inhibit cyclooxygenase activity and, thus, PGE_2 production [2]. PGE_2 also attenuates immune responses by direct effects on various differentiated cells, including dendritic cells (DCs), macrophages, neutrophils, T lymphocytes, and NK cells [3] and stimulates the accumulation of cells (T regulatory cells and myeloid-derived suppressor cells) that actively reduce immune responses [3, 4]. The effects of PGE_2 on mature DCs may vary according to whether PGE_2 is produced exogenously or endogenously [5] and the number of colocated T cells [6].

It was first reported in the 1970s that exposure of the skin to UV radiation (UVR) stimulated systemic immunosuppression such that responses to antigens applied to nonirradiated sites in irradiated animals were reduced. This systemic suppressed state has been implicated in positive latitude gradients (lower latitude, more solar UVR, and less disease) observed for several chronic immune diseases such as multiple sclerosis and type 1 diabetes [7]. The long-lasting nature of this immunosuppression suggests bone marrow (BM) cell involvement and, more recently, using chimeric mice engrafted with BM cells from UV-irradiated mice, we have confirmed that the effects of UVR on BM cells are long-lasting and that DCs in the periphery of UV-chimeric mice have a reduced ability to initiate immune responses in the draining nodes [8].

Much evidence supports PGE_2 involvement in UVR-induced erythema [9], as well as in systemic immunosuppression [10–12]. PGE_2 binds to the prostaglandin E receptor subtype 4 (EP4) and signals increased receptor activator of NF-κB ligand (RANKL) expression in the epidermis and an elevation of T regulatory cell numbers in the draining lymph nodes [13]. We have shown previously that PGE_2 is important in the pathway by which UVR affects DC progenitors in the BM of UV-irradiated mice [14]. Not only did indomethacin, an inhibitor of PGE_2 production, given to the

Abbreviations: ADLN=airway draining lymph node, BM=bone marrow, CHS=contact hypersensitivity, DC=dendritic cell, DNFB=2,4-dinitro-1-fluorobenzene, EP4=prostaglandin E receptor subtype 4, HSC=hematopoietic stem cell, OVA=ovalbumin, PGE_2=prostaglandin E_2, SDLN=skin draining lymph node, UVR=UV radiation; WT=wild type

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mice 4 d before UVR prevent the differentiation of poorly immunogenic DCs from BM but also pellets releasing 9.5 μg of PGE_2 daily for 3 d replicated the effect of UVR on DC progenitors [14]. There was no relationship between myelopoiesis in the BM and the effects of UVR on DC progenitors; UVR, but not PGE_2, increased the percentage of $Gr1^+CD11b^+$ cells in the BM and indomethacin did not reduce the myelopoiesis induced by UVR [14].

Inflammation in the airways and peritoneal cavity, like UV-irradiated skin, via PGE_2 production, communicates to DC progenitors in the BM such that the differentiating DCs are less competent to prime immune responses upon transfer to new mice [15]. The BM-derived DCs are not myeloid-derived suppressor cells because they do not express the characteristic transcripts of these cells, namely mRNA for indoleamine dioxygenase, cyclooxygenase 2, or nitric oxide synthase 2. Further, they did not express increased IL-10 [15] or express Gr1 [16].

In the current study, the specific effects of PGE_2 per se, without other inflammation-associated molecules or processes, were examined on myeloid cell progenitors in the BM. By using chimeric mice, the effect of PGE_2 on BM cells from the donor mice was investigated away from changes of PGE_2 to mature cells in the periphery of the mice given the PGE_2 pellets, e.g., monocytes, macrophages, and DCs [3, 5]. BM cells from mice given PGE_2-releasing pellets for 3 d were used to generate the chimeric mice. At 16 wks later, BM cells from the primary chimeric mice were used to generate further chimeric mice (serial chimeric mice). Because PGE_2 can affect the self-renewal and differentiation of hemopoietic stem cells (HSCs) [17–21], the engraftment of the BM cells in the chimeric mice was tracked. There were no overt phenotypic differences detected in the chimeric mice due to PGE_2 administration. However, the DCs in the PGE_2-chimeric mice were immunologically less competent because of poor trafficking after they had been activated. This report highlights a previously unidentified pathway by which PGE_2 contributes systemically to resolution of inflammation.

MATERIALS AND METHODS

PGE_2 administration and establishment of chimeric mice

Mice were obtained from the Animal Resources Centre (Murdoch, Western Australia, Australia). All experiments were performed with the approval of the Telethon Institute for Child Health Research Animal Ethics Committee, according to the guidelines of the National Health and Medical Research Council of Australia. Female B6.SJL-Ptprca mice (CD45.1 alloantigen, 7–8 wk old) were implanted s.c. with two 0.1-mg PGE_2 pellets, with a combined constant release of 9.5 μg/d over 21 d (Innovative Research of America, Sarasota, FL, USA). Control mice were implanted s.c. with placebo pellets. BM was harvested 3 d after pellet implantation. No change by PGE_2 in myeloid ($Gr1^+CD11b^+$), lymphoid ($B220^+CD11b^-$), or erythroid ($Ter119^+CD11b^-$) cells was confirmed in BM to generate both the chimeric and serial chimeric mice [14, 15]. Eight-week-old C57BL/6J mice (recipients, CD45.2 alloantigen) were γ-irradiated (2×550 rads) using a ^{137}Cs source (Gammacell 3000 Elan; MDS Nordion, Ottawa, ON, Canada). These BM-ablated mice were injected i.v. with 2×10^6 BM cells from the congenic B6.SJL-Ptprca mice for the generation of PGE_2-chimeric or placebo-chimeric mice, respectively. For establishing serial chimeric mice, BM ablated (γ-irradiated) mice were injected with 2×10^6 BM cells that were prepared from the primary placebo- or PGE_2-chimeric mice (>16 wk reconstitution). Age-matched mice were used as a control for both the primary and the serial chimeric mice (nonchimeric). The reconstitution of hemopoietic cells in the BM, spleen and lymph nodes, and blood was tracked as described previously [8].

Immunological assays in the chimeric mice >16 wk after reconstitution

A systemic CHS assay was performed as published previously [8] using 2,4-dinitro-1-fluorobenzene (DNFB; Sigma-Aldrich, St. Louis, MO, USA) as the sensitizing hapten applied to ventral skin and subsequent hapten challenge to the ears. Experimental allergic airways disease was induced by i.p. injection of ovalbumin (OVA; Sigma-Aldrich) in alum (aluminum hydroxide suspension; Serva, Heidelberg, Germany), followed by OVA aerosol challenge on 3 consecutive d [15]. Blood (200 μl) was taken via tail vein 6 h after the second challenge for measures of OVA-specific IgG1 and IgE [15]. The mice were euthanized 24 h after the last challenge, and airway draining lymph nodes (ADLNs; mediastinal, tracheobronchial, and parathymic) and bronchoalveolar lavage fluid were collected for quantification of OVA-induced proliferation and inflammatory cell accumulation [15].

Intranasal PKH26 and LPS, topical FITC, and intraperitoneal alum

PKH26 diluted 1:100 in diluent C (part of PKH26 kit; Sigma-Aldrich) was delivered intranasally, 8 h before intranasal LPS (10 μg). The mediastinal lymph node was harvested after a further 40 h for physical disaggregation, counting, and analysis. Shaved dorsal skin was painted with 100 μl of 0.5% w/v FITC (Sigma-Aldrich) prepared in acetone 1:1 dibutyl phthalate, and cells in the skin-draining lymph nodes (SDLNs; axillary, brachial, and inguinal) examined after 16 h [8]. Alum (2 mg) in 200 μl of saline/mouse was injected i.p. At 1 and 3 d later, the cells in the peritoneal cavity were counted and identified on Diff-Quick-stained cytocentrifuge smears.

Adoptive transfer of BM-derived $CD11c^+$ cells into the ears of mice and assay of their priming ability

The culture of BM-derived cells with GM-CSF and IL-4 (each 10 ng/ml; Peprotech Inc., Rocky Hill, NJ, USA) for 6 d to promote differentiation of $CD11c^+$ cells and analysis of their priming ability in naive mice was performed as described previously [8, 14, 15].

RESULTS AND DISCUSSION

Engraftment of BM cells in PGE_2-chimeric mice

The rates of engraftment into γ-irradiated hosts of BM cells derived from mice implanted for 3 d with PGE_2-releasing or placebo pellets were not different. Cell yields in BM, spleen, and lymph nodes were similar to those measured in nonchimeric mice by 10 wk (**Fig. 1A**). With increasing time after injection of donor BM cells, the percentages of $CD19^+B220^+$ B cells (Fig. 1B), $CD3^+CD4^+$ T cells (Fig. 1C), and $CD11c^+$ DCs (Fig. 1D) in BM, spleen, and lymph nodes did not vary significantly and were similar to the percentages measured in nonchimeric mice by 10 wk (horizontal line). Greater than 90% $CD19^+B220^+$ B cells in the lymph nodes were CD45.1 by 2.5 wk, whereas $CD3^+CD4^+$ T

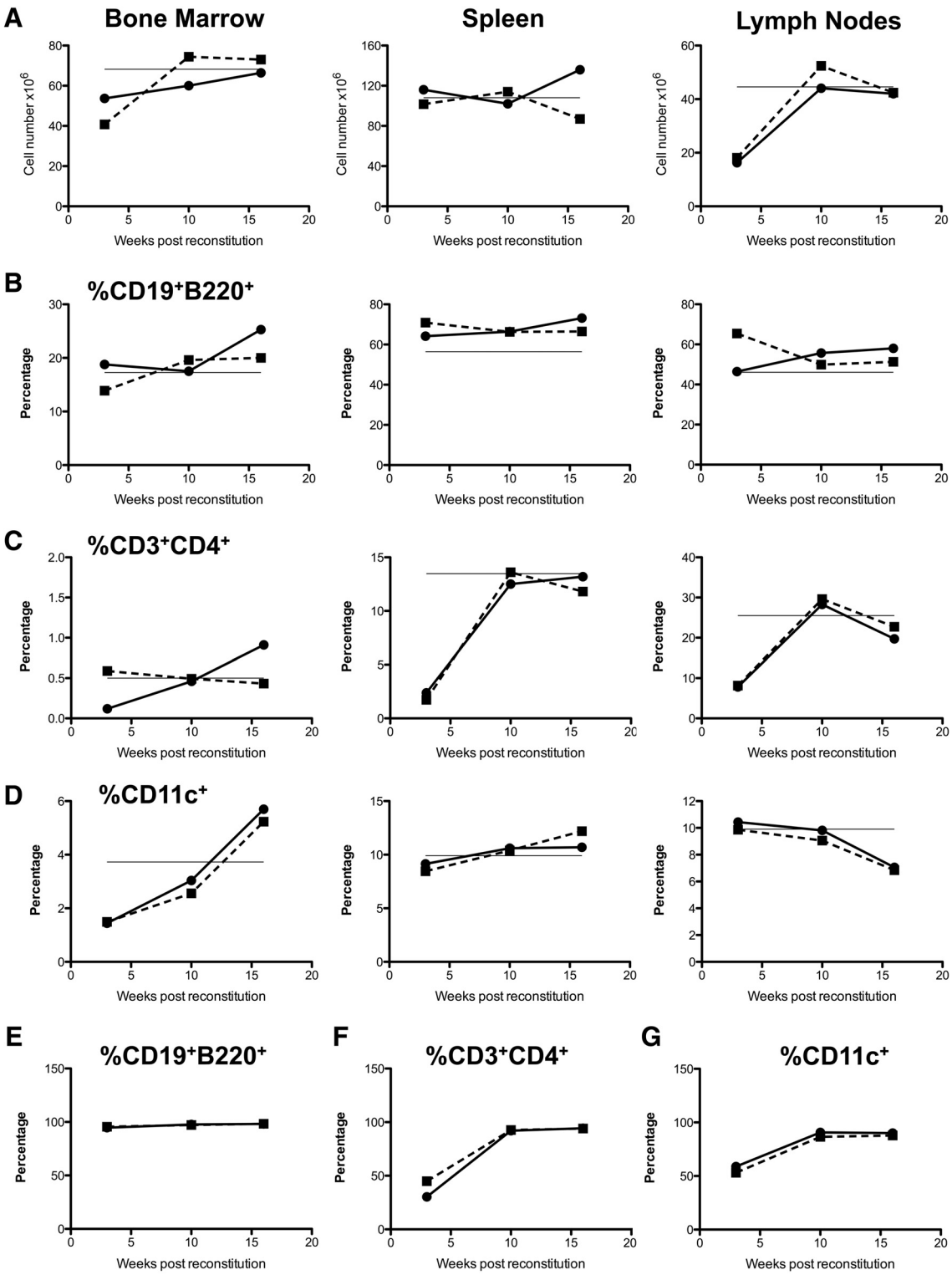


Figure 1. Reconstitution of γ-irradiated CD45.2 mice with BM cells from CD45.1 mice exposed to placebo or PGE_2-releasing pellets for 3 d. (A) Numbers of cells harvested from the BM, spleen, and lymph nodes with increasing time after injection of BM cells. (B–D) $CD19^+B220^+$ B cells (B), $CD3^+CD4^+$ T cells (C), and $CD11c^+$ DCs (D) as a percentage of cells recovered from the BM, spleen, and lymph nodes. The horizontal line shows the mean percentages of cells in the respective organs of age-matched nonchimeric mice (n=4 mice). (E–G) Percentages of $CD19^+B220^+$ B cells (E), $CD3^+CD4^+$ T cells (F), and $CD11c^+$ DCs (G) that express CD45.1 in the lymph nodes of chimeric mice. Results for the placebo-chimeric mice are shown by the solid line and circles; those for the PGE_2-chimeric mice are shown by the dashed line and squares. Two mice/group were examined after 2.5 wk and 1 mouse/group after 10 and 16 wk.

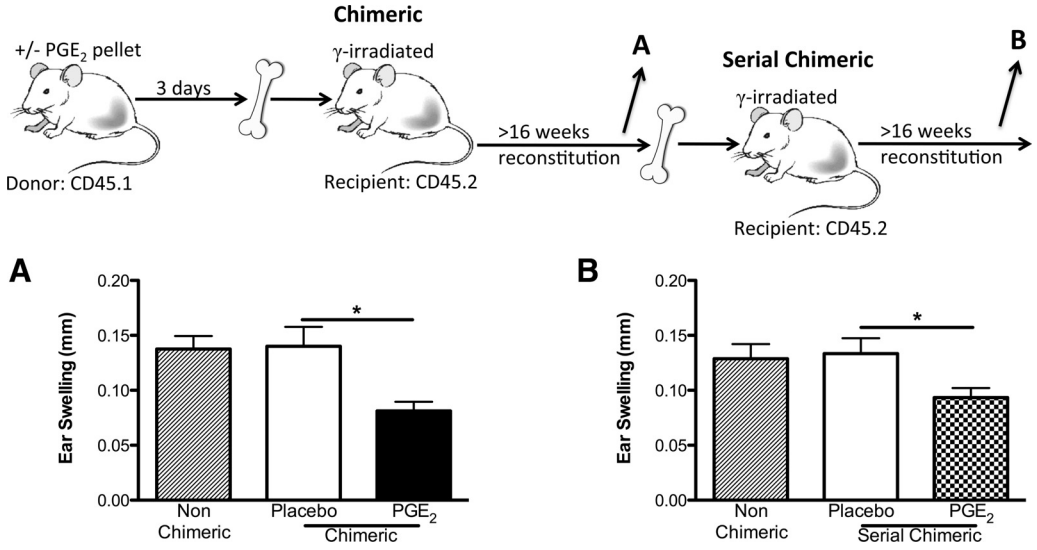


Figure 2. Reduced contact hypersensitivity responses in PGE_2-chimeric and serial PGE_2-chimeric mice. The establishment of the chimeric and serial chimeric mice is shown. Chimeric and serial chimeric mice (>16 wk since BM cell injection) were sensitized on their shaved abdominal skin with DNFB. Then 4 d later, the ears were challenged with DNFB, and a further 24 h later, the ear swelling was measured. (A) Ear swelling in placebo- and PGE_2-chimeric mice. (B) Ear swelling in serial placebo- and PGE_2-chimeric mice. Hatched bars represent ear swelling in age-matched nonchimeric mice. Data are means ± SEM (n=4 mice/group). *$P < 0.05$.

cells and $CD11c^+$ DCs were >90% CD45.1 after 10 wk (Fig. 1E–G). In the serial chimeric mice, very similar kinetics of engraftment was measured, and there were no differences in cell numbers/types recovered from the control and PGE_2-serial chimeric mice 16 wk after intravenous injection of BM cells from primary chimeric mice (data not shown). Blood cell profiles (both numbers and composition of white blood cells) in the placebo-chimeric mice and the PGE_2-chimeric mice were also very similar, as were those in the serial placebo-chimeric mice and serial PGE_2-chimeric mice and did not suggest any PGE_2-associated hematopoietic differences in cell recovery after γ-irradiation (data not shown).

Reduced immune responses in the PGE_2-chimeric mice

At 16 wk after each cohort of chimeric mice were injected with BM cells (original cohort >16 weeks, serial chimeric mice>32 weeks since BM were exposed to PGE_2; **Fig. 2**), systemic CHS responses (using DNFB on abdomen for sensitization and to ears for challenge) were assessed. There was no significant difference between the ear swelling in the nonchimeric mice and the placebo-chimeric or serial placebo-chimeric mice. However, there was a significantly reduced CHS response in the PGE_2-chimeric and serial PGE_2-chimeric mice (Fig. 2A and B).

The outcomes of sensitization to the allergen, OVA, were examined in the PGE_2- and placebo-chimeric mice (>16 weeks since injection of BM cells). Studies in the airways were important because any influence of radiation-resistant Langerhans cells was avoided [22]. Mice were sensitized to OVA with alum and challenged with aerosolized OVA. The number of cells harvested from the ADLNs 24 h later was significantly reduced in the PGE_2-chimeric mice (**Fig. 3A**). The proportion of B cells ($CD19^+B220^+$), T cells ($CD3^+CD4^+$), and DCs ($CD11c^+$) in the ADLNs did not change (data not shown). When equivalent numbers of cells from ADLNs were cultured with OVA for 4 d, proliferation as assessed by [^{3}H]thymidine uptake was identical (data not shown). Cells in the bronchoalveolar lavage fluid were also quantified, and there was a trend for fewer cells in the fluids from PGE_2-chimeric mice (Fig. 3B). No significant differences were detected in OVA-specific IgG1 and IgE levels in serum from the PGE_2- and placebo-chimeric mice taken 6 h after the second OVA aerosol challenge (data not shown).

Reduced responses to intranasal LPS and topical FITC in PGE_2-chimeric mice

PKH26 was delivered intranasally to label airway cells without cell activation. Then 8 h later, the mice were given intranasal LPS to stimulate migration of PKH26-labeled DCs

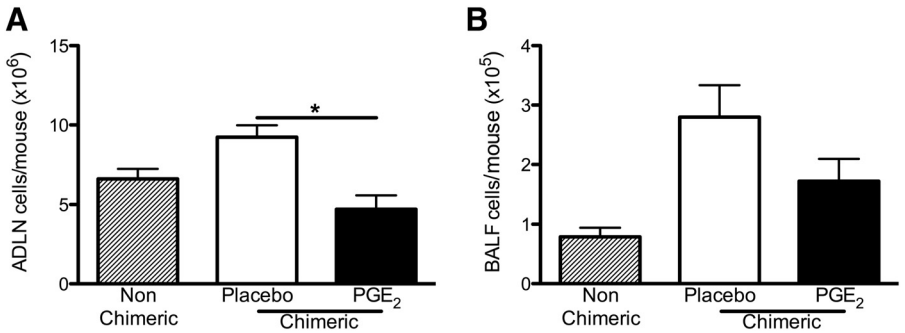


Figure 3. Reduced experimental allergic airway disease in PGE_2-chimeric mice. Mice were sensitized to OVA with alum on d 0 and 14, and then exposed to aerosolized OVA on d 21, 22, and 23. (A) Numbers of cells recovered from the ADLNs of chimeric and age-matched nonchimeric mice. (B) Numbers of cells measured in the bronchoalveolar lavage fluid (BALF). Data are means ± SEM (n=5 mice/group). *$P < 0.05$.

to the mediastinal lymph node. After 40 h, lymph nodes were disaggregated and compared with nodes from mice given intranasal PKH26, but not LPS. LPS stimulated a significant increase in cells isolated from the mediastinal lymph node in the placebo-chimeric, serial placebo-chimeric, and the age-matched nonchimeric mice (**Fig. 4A** and **B**). However, in the PGE_2-chimeric mice and the serial PGE_2-chimeric mice, there was no significant LPS-induced increase in the cells recovered from the mediastinal lymph node (Fig. 4A and B). When the basis for the lack of LPS stimulation of hypertrophy was investigated, in the nodes of the PGE_2-chimeric mice there were fewer DCs ($CD11c^+MHCclassII^+$ cells; Fig. 4C) and fewer $PKH26^+$ DCs (Fig. 4D) than measured in placebo-chimeric mice. The $PKH26^+$ $CD11c^+MHCclassII^+$ cells in the ADLNs of the placebo-chimeric mice did not express CD8α (data not shown), confirming that the $PKH26^+$ cells were migratory DCs and the dye had not been taken up by lymph node resident-$CD8\alpha^+$ DCs.

The response to topical FITC was also examined. Like PKH26, this molecule labels all cells at the site of application, but, unlike PKH26, it also activates immune cells and stimulates migration of DCs to the draining nodes. The number of cells in the SDLNs after 16 h increased significantly in the placebo-chimeric and nonchimeric mice (Fig. 4E). However, for the PGE_2-chimeric mice, FITC application did not significantly increase the cell yield from the SDLNs. Similar to the $CD11c^+MHCclassII^+PKH26^+$ $CD8\alpha^-$ cells in the ADLNs (Fig. 4C and D), the numbers of $CD11c^+MHCclassII^+FITC^+CD8\alpha^-$ cells were significantly reduced in the nodes of the PGE_2-chimeric mice (data not shown).

These data demonstrate that immune responses in the skin and airways that are initiated by DCs, but involve multiple cell types, are significantly attenuated in the PGE_2-chimeric, and serial PGE_2-chimeric mice. On further examination, DCs in the peripheral organs trafficked inefficiently to the draining lymph nodes (Fig. 4). Other changes may also contribute to reduced immunogenic properties of DCs in the PGE_2-chimeric and serial PGE_2-chimeric mice. In previous studies, adoptively transferred DCs differentiated from the BM of UV-irradiated mice [8] or mice with alum-induced peritoneal inflammation [15] suppressed immunity in presensitized mice. These outcomes suggested that the BM-derived DCs were regulatory by an "active" process. Functional studies after adoptive transfer of BM-derived DCs from mice with peritoneal inflammation also suggested that regulatory cells, possibly T regulatory cells, may be induced by these BM-derived DCs [15].

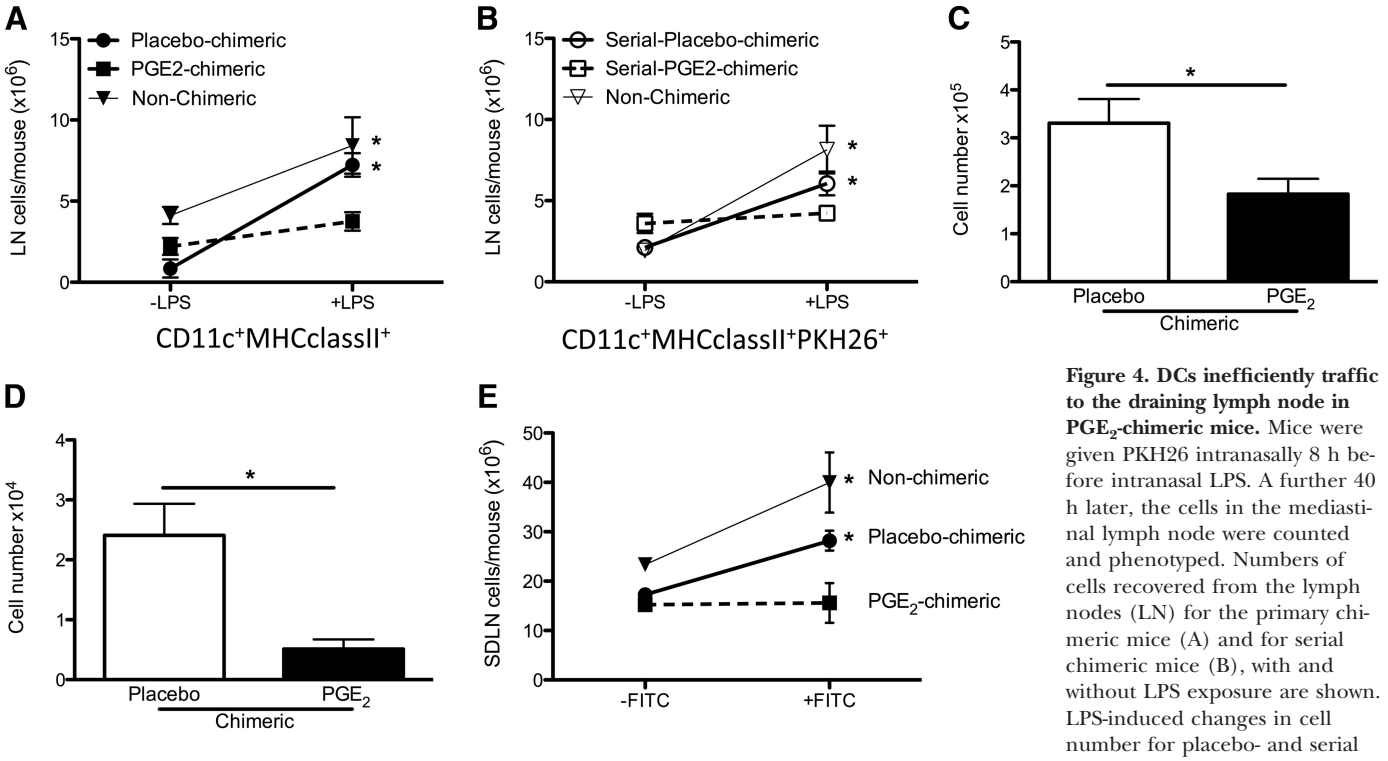


Figure 4. DCs inefficiently traffic to the draining lymph node in PGE_2-chimeric mice. Mice were given PKH26 intranasally 8 h before intranasal LPS. A further 40 h later, the cells in the mediastinal lymph node were counted and phenotyped. Numbers of cells recovered from the lymph nodes (LN) for the primary chimeric mice (A) and for serial chimeric mice (B), with and without LPS exposure are shown. LPS-induced changes in cell number for placebo- and serial placebo-chimeric mice are shown by a thick unbroken line and full and open circles, respectively. LPS-induced changes in PGE_2- and serial PGE_2-chimeric mice are shown by a thick broken line and full and open squares, respectively. LPS-induced changes in numbers of cells for age-matched nonchimeric mice are shown by a thin unbroken line and full and open triangles, respectively. $*P < 0.05$, significant increase in lymph node cell numbers with intranasal LPS. Data are means ± SEM for 3–4 mice/group from 3 to 4 independent experiments. (C and D) Absolute numbers of $CD11c^+MHCclassII^+$ cells (C) and $CD11c^+MHCclassII^+PKH26^+$ cells (D) in the mediastinal lymph node 40 h after intranasal LPS. Data are means ± SEM for 3–4 mice/group from 3−4 independent experiments. $*P < 0.05$. (E) Numbers of cells accumulating in the SDLNs of chimeric mice and age-matched nonchimeric mice, 16 h after topical application of FITC. Data are means ± SEM (n=4 mice/group from 4 independent experiments). $*P < 0.05$. Significant increase in lymph node cell numbers with topical FITC.

Macrophage and neutrophil migration to an inflammatory stimulus is reduced in PGE_2-chimeric mice

DCs, macrophages, and neutrophils share common progenitors during development in the BM [23, 24]. Migration of hematopoietic cells was measured, not to lymph nodes but instead into the peritoneal cavity after injection of the inflammatory stimulus, alum. After 3 d, the number of cells in the peritoneal cavity of the placebo-chimeric mice was significantly greater than the number measured in the PGE_2-chimeric mice (**Fig. 5A**). After 1 d, cells in the peritoneal cavity were approximately 50% neutrophils and 50% macrophages but after 3 d, macrophages comprised >80% cells in the peritoneal cavity, and this proportion did not vary significantly between placebo-chimeric and PGE_2-chimeric mice (Fig. 5B). After 3 d, there were significantly fewer macrophages and neutrophils in the peritoneal cavity of the PGE_2-chimeric mice than in the placebo-chimeric mice (Fig. 5B). Thus, macrophages and neutrophils may also traffic poorly in the PGE_2-chimeric mice. Common myeloid progenitors ($Lin^-Sca^-c\text{-}kit^+CD34^+Fc\gamma R^{lo}$) in the BM differentiate to granulocyte-monocyte progenitors ($Lin^-Sca^-c\text{-}kit^+CD34^+Fc\gamma R^{hi}$), from which neutrophils, DCs, and macrophages develop. Because there was reduced accumulation of macrophages and neutrophils in the peritoneal cavity of the PGE_2-chimeric mice at d 3, PGE_2 may affect granulocyte-monocyte progenitors or cells earlier in the DC differentiation process, possibly HSCs, where the fate of differentiating cells may be imprinted [17–21, 25].

Imprinted change maintained in bone marrow cells for >32 wk

When the PGE_2-chimeric mice and the serial PGE_2-chimeric mice had been reconstituted for >16 wk, their BM was harvested and cultured for DC differentiation. Loosely adherent $CD11c^+$ cells were harvested and loaded with a soluble form of DNFB [8, 15], and 10^6 cells/ear (or an equivalent volume of saline) were injected s.c. into nonchimeric, wild-type (WT) mice (8 ears/group). After a further 7 d, all ears were painted with DNFB, and their swelling was measured after 24 h. Allowing for swelling in the saline-injected, DNFB-painted ears, swelling due to injection of DNBS-loaded $CD11c^+$ cells from the PGE_2-chimeric mice (**Fig. 6A**) and the serial PGE_2-chimeric mice (Fig. 6B) was approximately 50% of that due to $CD11c^+$ cells from the placebo-chimeric mice (Fig. 6A) and the serial placebo-chimeric mice (Fig. 6B). The reduced immunogenicity of $CD11c^+$ cells differentiated from the BM of mice implanted s.c. with PGE_2 pellets that was first measured after 3 d [14] was maintained in $CD11c^+$ cells differentiated from BM of PGE_2-chimeric mice and serial PGE_2-chimeric mice (Fig. 6A and B).

To determine whether non-DCs have altered function in a CHS response in the PGE_2-chimeric mice, the ability of DCs from nonchimeric (WT) mice to overcome the poor immunity in PGE_2-chimeric mice was examined. In response to injection of antigen-loaded $CD11c^+$ cells from WT mice into the ears of placebo-chimeric and PGE_2-chimeric mice, there was identical swelling of the ears upon reexposure 7 d later to DNFB (Fig. 6C), which suggests a DC-intrinsic change in the PGE_2-chimeric mice. Thus, the reversal of the phenotype by injection of WT DCs into the PGE_2-chimeric mice suggested a selective effect of PGE_2 on myeloid (not lymphoid) progenitors in the BM.

Cells altered in the bone marrow of PGE_2-chimeric mice

HSCs are the most primitive of the hematopoietic progenitors and are subject to many forces that determine self-renewal, differentiation, and mobilization out of the BM niche [26]. Experiments suggest that HSCs can directly respond to proinflammatory cytokines released during infection and inflammation, and the responses can include lineage-specifying changes as measured in response to M-CSF [27]. We and others [14, 17–21, 28] propose that HSCs respond directly to PGE_2. Short-term ex vivo exposure of HSCs to PGE_2 enhanced their homing, survival, and proliferation and their competitive advantage for BM repopulation [18]. Thus, chemicals that enhance PGE_2 synthesis may increase HSC numbers, and those that inhibit PGE_2 production decrease them [17]. After radiation injury, PGE_2 increased HSC survival and hematopoietic recovery [21]. By increasing CXCR4 expression [18] and migration to CXCL12, an important molecule required for HSC maintenance in the BM [29], PGE_2 may reduce HSC mobilization. PGE_2 can also reduce HSC egress via signaling of EP4 [30], the major receptor for the PGE_2 on HSCs [28]. PGE_2 may also activate a nonhemopoietic cell population in

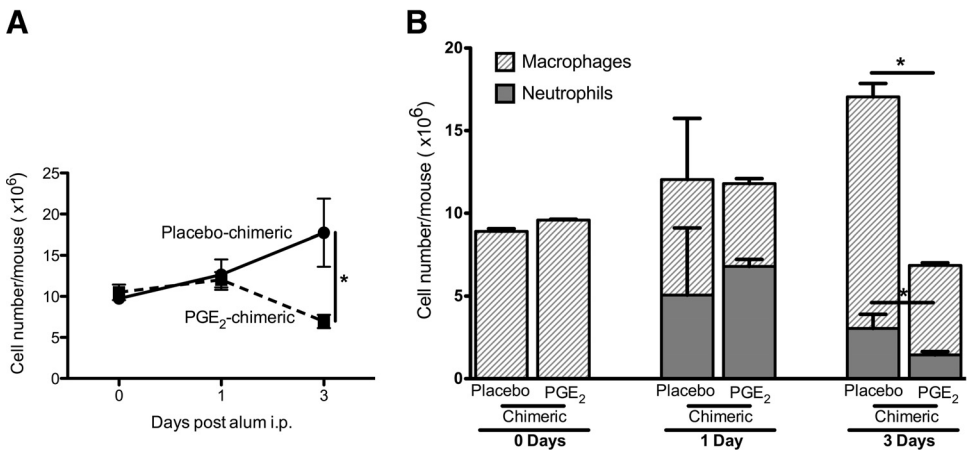


Figure 5. Cells in the peritoneal exudates of chimeric mice injected i.p. with alum. (A) Total cells recovered from the peritoneal cavity of chimeric mice 1 and 3 d after alum i.p. injection on d 0. Cells in exudates from placebo-chimeric mice are shown by an unbroken line and for PGE_2-chimeric mice by a broken line. Data are means ± SEM (n=5). *$P < 0.05$, significantly reduced cell numbers in peritoneal exudates from PGE_2-chimeric mice compared with those from placebo-chimeric mice. (B) Numbers of macrophages and neutrophils in the peritoneal exudates of the placebo- and PGE_2-chimeric mice before (0 d), and 1 and 3 d after alum injection. Data are means ± SEM (n=5 mice/group). *$P < 0.05$, significant alum-induced decrease in peritoneal cell numbers in PGE_2-chimeric mice.

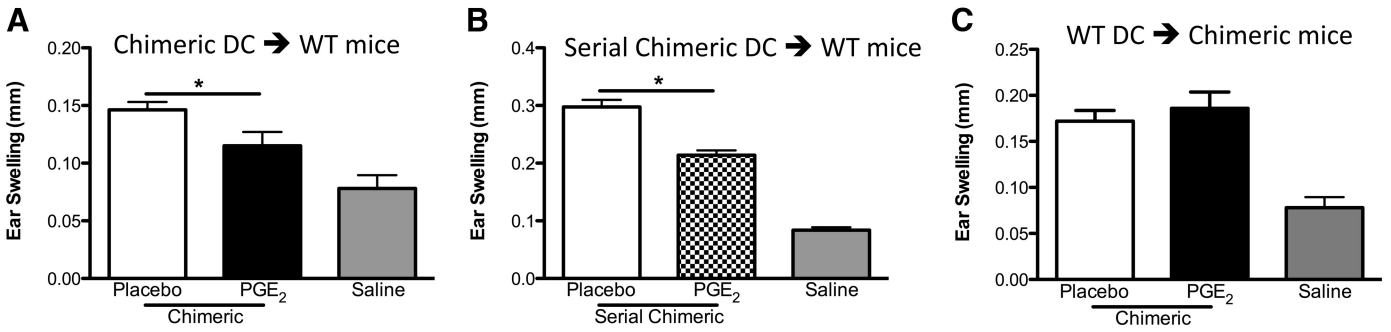


Figure 6. DCs differentiated from the BM of PGE_2-chimeric and serial PGE_2-chimeric mice have reduced priming ability. In placebo- and PGE_2-chimeric mice (A) and for serial placebo- and PGE_2-chimeric mice (B), BM cells from >16 wk reconstituted chimeric mice (pooled from 2 mice/group) were harvested and cultured with GM-CSF + IL-4 for DC differentiation. Cells were enriched for CD11c expression and pulsed with a soluble form of DNFB, and 10^6 cells were injected s.c into the ear pinnae of nonchimeric (WT) mice. Saline was injected into another group of WT mice. At 7 d after injection, the ears of all recipient mice were painted with DNFB, and the ear swelling was measured after 24 h. (C) Injection of DCs differentiated from the bone marrow of WT mice (n=3) into chimeric mice. BM cells from WT mice were cultured for DC differentiation, and 10^6 cells were injected s.c into the ear pinnae of placebo-chimeric or PGE_2-chimeric mice. At 7 d after injection, the ears of all recipient mice were painted with DNFB, and the ear swelling was measured after 24 h. Gray bars represent the ear swelling for WT mice injected with saline instead of cells. Data are means ± SEM (n=8 ears/group). *$P < 0.05$.

the BM to support HSC formation [28]. However, HSC expansion by PGE_2 may be short-lived with no residual effect measured 16 wk later [20]. It is notable that in all the studies reviewed above, the concentration of PGE_2 was generally 30 times greater than that administered to our mice, and our mice were exposed to PGE_2 for only 3 d.

PGE_2 and DC differentiation in the BM

Our studies suggest that DCs differentiating from BM of mice with PGE_2-associated inflammation have a reduced trafficking ability when activated, and thus immune responses in the draining nodes are significantly attenuated. Baseline PGE_2 may, however, be required for DC differentiation in the BM [31]. Demonstrated by use of indomethacin to remove PGE_2 production for ≥6 d, PGE_2 regulated the expression of Flt3 on DC-committed progenitor cells and allowed Flt3 ligand-mediated DC development [31]. In the present study, no differences in DC yield from BM cell cultures of mice administered PGE_2 were measured, and the function of DCs was assessed independently of Flt3 expression by use of GM-CSF, not Flt3-ligand, as the stimulus for DC differentiation in vitro. In addition, any irregularities due to the culture conditions were removed by studying BM DC differentiation in chimeric mice.

How PGE_2 may directly or indirectly affect DC, macrophage, and neutrophil progenitors in the BM and possibly HSCs is a matter for further study. PGE_2 can increase DNA methyltransferases [32], and we have shown that 5-aza-2′-deoxycytidine, a DNA methyltransferase inhibitor [33], can reverse alterations by UVRs to DC progenitors in the BM. With the rescue of the UVR-induced phenotype by 5-aza-2′-deoxycytidine, there was no reduction in immune competence of DCs differentiating from the BM of UV-irradiated mice [8]. In other studies, mice were UV-irradiated during the early gestational period when they are particularly sensitive to epigenetic perturbation [34]. A reduced ability of BM-derived DCs from progeny of the UV-irradiated mothers to initiate immune responses in naive mice was consistently measured [8].

The importance of these studies in PGE_2-chimeric and serial PGE_2-chimeric mice centers on the ability of PGE_2 to have long-lasting effects on BM macrophage, neutrophil, and DC progenitor cells. In the chimeric mice, DCs, macrophages, and neutrophils were allowed to differentiate and to seed the periphery under steady-state conditions. Responses to immune challenges were investigated only when the mice were fully engrafted (with no differences in the kinetics of engraftment observed). Because DCs are key to initiating responses to antigen challenges, their reduced ability to reach draining lymph nodes would support resolution of inflammation and encourage tissue homeostasis. The implications of reduced macrophage and neutrophil activity, particularly migratory capacity, are less clear. The actions of PGE_2 can be likened to those of TLR2 agonists that bind to hematopoietic stem and progenitor cells in the BM and affect the function of the macrophages they produce [35]. Our study and that of Yanez et al. [35] suggest that by affecting myeloid progenitors a form of "memory" for innate host defenses has been generated. As a corollary to these studies, medications that reduce PGE_2 production (e.g. nonsteroidal anti-inflammatory drugs) [1, 2] may reduce the effectiveness of this PGE_2-associated pathway of inflammation control. The studies support PGE_2 as the mediator involved in the systemic immunosuppressive effects of UVR and the outcomes published in UV-chimeric mice [8], as well as that after airway and peritoneal inflammation [15]. This study highlights an important immunosuppressive pathway of reduced immune activation by DCs differentiated from the BM of mice with tissue inflammation, principally by an attenuated ability to traffic to lymph nodes draining sites of antigen exposure. Although assays used in this study focused on DC-driven processes, the results also suggest that by affecting myeloid cell progenitors in the BM, PGE_2 attenuates the migration of other activated myeloid cells differentiating from the BM and thus dampens their responses to activating signals.

AUTHORSHIP

N.M.S. performed the experiments, helped to interpret the data, and wrote the manuscript; R.L.X.N., S.G., and J.W. helped to design and perform the experiments; M.N. helped in the design of the experiments; P.H.H. participated in de-

signing the study, interpreting data, and coordinating and performing the study and wrote the manuscript.

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DISCLOSURES

The authors declare no competing conflicts of interest.

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