Phorbol 12-Myristate 13-Acetate–Induced Development of Functionally Active Mast Cells in W/W' but not SI/Si' Genetically Mast Cell-Deficient Mice

By John R. Gordon and Stephen J. Galli

The normal skin and other tissues of adult genetically mast cell-deficient WBB6F1-/-W/W' or WCBB6F1-SI/Si' mice contain less than 1.0% the number of mast cells present in the corresponding tissues of the congenic normal (+/+) mice. We previously reported that mature mast cells developed locally in the skin of W/W', but not SI/Si', mice at sites of chronic idiopathic dermatitis. We now report that the repeated application of phorbol 12-myristate 13-acetate (PMA) to the ear skin of either W/W' or +/- mice induces both dermatitis and a striking and dose-dependent increase in the number of dermal mast cells. The number of dermal mast cells at sites treated for 6 weeks with 5 μg PMA, three times per week, was 39 ± 7/mm² and 305 ± 34/mm² for W/W' and +/- mice, respectively; the corresponding values for vehicle-treated skin were 1.5 ± 1.0/mm² and 145 ± 8/mm², respectively.

A double dose of mutant genes at the W or SI locus of the mouse can produce a constellation of phenotypic abnormalities that includes a macrocytic anemia, a lack of hair pigmentation, sterility, and a profound mast cell deficiency. However, the mechanisms responsible for these abnormalities in W or SI mutant mice are different. A large body of evidence indicates that the abnormality in W mutant animals, such as the WBB6F1-W/W' (W/W') mouse, reflects a defect in the stem cells that generate the affected lineages. For example, transplantation of WBB6F1-W/W' mice with bone marrow cells derived from the congenic normal (WBB6F1 +/-) animals cures both the anemia and the mast cell deficiency of the W/W' recipients.

Recently, certain W locus mutations have been shown to be closely linked to or actually involve the gene for the putative tyrosine kinase receptor c-kit. Because c-kit exhibits significant homology to the receptors for colony-stimulating factor-1 (CSF-1) and platelet-derived growth factor (PDGF), the stem cell defect in W/W' mice probably reflects the expression by the affected lineages of quantitative and/or qualitative abnormalities of the c-kit product. By contrast, the defect responsible for the mast cell deficiency of SI mutants, such as the WCBB6F1-SI/Si' (SI/Si') mouse, lies in a microenvironmental factor(s) required for the maturation/differentiation of this lineage. Thus, even though SI/Si' mice are themselves anemic and profoundly mast cell-deficient, SI/Si' bone marrow cells can cure the anemia and mast cell deficiency of WBB6F1-W/W' mice. Furthermore, while transplantation of congenic normal (+/+) bone marrow cells into SI/Si' mice has no effect on the mutants' anemia or mast cell deficiency, introduction of 3T3 fibroblasts of WCBB6F1 +/- origin into WCBB6F1-SI/Si' mice results in the local development of mast cells in association with the adoptively transferred fibroblasts.

We have been searching for additional insights into the expression of genetically determined mast cell deficiencies by identifying and analyzing settings in which this abnormality can be reversed in vivo. We showed previously that the cutaneous mast cell deficiency of W/W' mice underwent repair at sites of chronic idiopathic dermatitis. The mast cells that developed at sites of dermatitis were indistinguishable from those in the skin of the +/- mice by light or transmission electron microscopy, or by staining with the heparin-binding fluorescent dye, berberine sulfate. Thus, they resembled normal mature dermal mast cells. SI/Si' mice also developed a chronic idiopathic dermatitis; however, in this mutant, dermatitis was not associated with the development of recognizable mast cell populations.

Chronic idiopathic dermatitis theoretically provides an opportunity to search for factors that locally overcome the stem cell abnormality responsible for the mast cell deficiency of W/W' mice, eg, changes in the expression of c-kit by cells in the mast cell lineage, alterations in local levels of the as yet unidentified c-kit ligand, or the local production of other factors that influence mast cell proliferation or development. However, the etiology of chronic idiopathic dermatitis is unknown, the condition affects only a minority of susceptible animals, and mice generally do not exhibit dermatitis before the age of 8 months. Therefore, we decided to search for a chemically defined agent capable of predictably inducing mast cell development in the skin of W/W' mice.

We decided to begin our investigation with the phorbol ester, phorbol 12-myristate 13-acetate (PMA) for three reasons. In normal mice, the repeated epicutaneous applica-

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tion of PMA (or PMA-containing preparations such as croton oil) induces a chronic dermatitis associated with epidermal hyperplasia and dermal thickening.\textsuperscript{13-15} Repeated applications of croton oil or PMA can also cause hyperplasia and dermal mast cellosis.\textsuperscript{8,11,12} In addition, the interaction of PMA with protein kinase C directly activates many cell types that can also respond to signals mediated through cell-surface receptors for specific ligands.\textsuperscript{16,17} We found that the repeated application of PMA induced a striking and dose-dependent increase in the number of dermal mast cells in \textit{W/W}\textsuperscript{\textsuperscript{	extsuperscript{\circ}}} mice. \textit{SI/SI}\textsuperscript{\textsuperscript{	extsuperscript{\circ}}} mice treated with PMA developed a dermatitis similar to that of PMA-treated \textit{W/W}\textsuperscript{\textsuperscript{	extsuperscript{\circ}}} mice, but demonstrated no change in their dermal mast cell deficiency. Some of our results have been reported in abstract form.\textsuperscript{18}

**MATERIALS AND METHODS**

**Animals.** Mast cell-deficient \textit{WBB6F}_{1}-\textit{W/W}\textsuperscript{\textsuperscript{\circ}} mice and the congenic normal ({}+/+) mice [(\textit{WB}/\textit{Rej}–\textit{W}+/+ \texttimes \textit{C57BL}/6\textit{J}-\textit{W}/+\texttimes \textit{F}_{1}/\textit{W}/+\texttimes \textit{C57BL}/6\textit{J}–\textit{W}/+] \textit{F}_{1}/\textit{W}/+, +/+ mouse and mast cell-deficient \textit{WCB6F}_{1}–\textit{SI/SI}\textsuperscript{\textsuperscript{\circ}} mice and the congenic normal ({}+/+) mice [(\textit{WC}/\textit{Rej}–\textit{SI}+/+ \texttimes \textit{C57BL}/6\textit{J}–\textit{SI}/+\texttimes \textit{F}_{1}/\textit{SI}/+\texttimes \textit{C57BL}/6\textit{J}–\textit{SI}/+\texttimes \textit{SI}/+, +/+ mouse] from Jackson Laboratories, Bar Harbor, ME, at 4 to 8 weeks of age. The mice were housed in the Beth Israel Hospital (BIH) Animal Care Facility, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care. The animals were maintained in accordance with guidelines established by the BIH Institutional Animal Care and Use Committee, and guidelines prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Resource Council.\textsuperscript{19}

**Treatment of animals.** PMA (Sigma Chemical Co, St Louis, MO) was dissolved in acetonitrile (HPLC grade; Fisher Scientific Co, Fair Lawn, NJ) at appropriate concentrations and stored at \textminus70°C. Three times per week, each mouse was lightly anesthetized with ether, and 5 μL of phorbol ester solution was applied evenly by micropipet over the entire dorsal surface of the animal’s left ear, while 5 μL of acetonitrile alone was applied similarly to the right ear. In some experiments, the animals were treated on the left ear with PMA but received no acetonitrile treatment on the right ear.

**Assessment of the effects of PMA treatment.** At various intervals during the course of the experiments, the thickness of PMA-treated or acetonitrile-treated (control) ears was measured with a micrometer as previously described, and the results were expressed in inches \times 10^{-4}.\textsuperscript{20} At various intervals after initiating treatment, the mice were killed with ether, and their ears were amputated; a central 1 mm section was removed. The marrow was excised from the bones, and the cells were released by peritoneal lavage with 10 mL of Hanks’ basal salt solution (HBSS; Gibco Ltd, Grand Island, NY) containing 1% bovine serum albumin (Pentex, fraction V; Miles Diagnostics, Kankakee, IL). Cytocentrifuge preparations of the peritoneal cells and the 1 μm tissue sections were stained with Giemsa solution, and the numbers of mast cells were determined by microscopic examination of the slides.

**Quantitation of tissue mast cells.** The quantitation of mast cells in the tissue sections was performed as previously described.\textsuperscript{21,22} Briefly, the Giemsa-stained 1 μm sections were examined by light microscopy at 400×, and the numbers of mast cells present were recorded. The same tissue section was then projected onto a piece of paper with a Leitz Type XC Xenon slide projector (Leitz, Rockleigh, NJ), and the outlines of the epidermis, dermis, and cartilage were traced. The area of the dermis was determined by analyzing the projected images using a MOP-3 digitizer (Zeiss, New York, NY) interfaced to an HP-86 computer (Hewlett-Packard Co, Palo Alto, CA). The area of the dermis was taken as the total area, on the dorsal aspect, between the dermal-epidermal junction and the superficial surface of the cartilage. The numbers of mast cells are expressed as the number of cells per mm\textsuperscript{2} of dermis ± SEM. The statistical significance of differences in the mean values for groups was calculated using Student’s \textit{t} tests (two-tailed; paired test for left ear versus right ear comparison, unpaired for comparisons between groups).

**Assessment of the functional maturity of the PMA-induced cutaneous mast cells.** In order to demonstrate that the PMA-induced mast cells present in the ear skin of the treated mice were functionally active, we tested their ability to mediate IgE-dependent passive cutaneous anaphylaxis, a classic type I hypersensitivity response.\textsuperscript{23} We injected, into both ears of \textit{W/W}\textsuperscript{\textsuperscript{\circ}} or +/+ mice that had been treated on only one ear with 5 μg of PMA for 6 weeks, either 20 μL of phosphate-buffered saline (PBS) or -50 ng of monoclonal IgE anti-DNP antibody (as ascites containing the monoclonal antibody H1-DNP-(-2625)) in 20 μL of PBS. After 24 hours, the mice were challenged intravenously with 100 μg of DNP\textsubscript{2626} human serum albumin (HSA) in 200 μL of a PBS solution containing 1.0% Evans Blue dye (Sigma). The ear swelling responses of the mice (Δ = experimental value – baseline measurement taken before antigen challenge) were measured at 10, 20, and 30 minutes, and at 1, 2, and 4 hours after antigen challenge and expressed as Σ Δ x 10^{-6} inch, as previously described.\textsuperscript{24} At 4 hours, the mice were killed and specimens of the ears were processed for 1 μm Epon-embedded, Giemsa-stained sections as above.

**Culture of bone-marrow-derived mast cells.** Donor \textit{WCB6F}_{1}+/+ mice were sacrificed with ether, and their tibiae and femora were removed. The marrow was excised from the bones, and the cells were maintained in long-term culture in the presence of conditioned medium containing IL-3 (supernatants of concanavalin A-stimulated spleen cells), as previously described.\textsuperscript{25,26} After 5 weeks, at least 97.0% ± 3.0% of cells that remained in the cultures (99% viability by Trypan blue exclusion) were identifiable as mast cells, as determined by counting with neutral red.\textsuperscript{27} Bone marrow-derived, cultured mast cells (BMMC) such as these are best regarded as immature mast cells that express certain similarities to “mucosal” type mast cells (reviewed in references 26 through 28). BMMC, however, can acquire multiple phenotypic features of mature “connective tissue-type” mast cells (CTMC) upon transfer into anatomical sites in \textit{WBB6F}_{1}-\textit{W/W}\textsuperscript{\textsuperscript{\circ}} mice, such as the skin or peritoneal cavity, where CTMC occur in normal mice.\textsuperscript{26,27,28} For intradermal injection (20 μL per site), BMMC were resuspended in HBSS to provide 5× 10\textsuperscript{5} mast cells per 20 μL volumes.

**Effect of PMA treatment on the survival of transplanted BMMC in \textit{WBB6F}_{1}-\textit{SI/SI}\textsuperscript{\textsuperscript{\circ}} mice.** The left ears of \textit{WCB6F}_{1}-\textit{SI/SI}\textsuperscript{\textsuperscript{\circ}}, \textit{WCB6F}_{1}-+/+, and \textit{WBB6F}_{1}-\textit{W/W}\textsuperscript{\textsuperscript{\circ}} mice were treated with 5 μg of PMA 3 times weekly for 2 weeks. After 2 weeks, 5× 10\textsuperscript{5} BMMC were injected into the dermis of the left and right ears of the \textit{WCB6F}_{1}-\textit{SI/SI}\textsuperscript{\textsuperscript{\circ}} mice, and into the right ears only of the \textit{WBB6F}_{1}-\textit{W/W}\textsuperscript{\textsuperscript{\circ}} animals. The PMA treatments were continued, as above, for an additional 6 weeks (ie, for 8 weeks in total), and then the animals were killed and their ears processed for histology.

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Repeated epicutaneous application of PMA induces dermatitis in WBB6F₁/W' and +/+/mice. We first established the dose requirements for the induction of dermatitis by PMA in WBB6F₁/W' and -/+ + mice. Varying doses of PMA were applied, three times weekly, to the left ears of W/W mouse and +/+ mice, and acetone alone was applied similarly to the right ears. After 6 weeks, the ears were processed for histology and analyzed to determine the effect of the treatment. As previously reported for normal mice, histologic examination showed that repeated epicutaneous application of PMA induced epidermal hyperplasia and a substantial thickening of the dermis with an associated dermal infiltrate of inflammatory cells, consisting predominantly of neutrophils. The extent of the chronic dermatitis induced by the PMA treatment, as reflected both in total ear thickness (Fig 1) and the histologic findings, was dependent on the dose applied. PMA (10 µg) did not induce significantly greater reactions than did the application of 5 µg of PMA (Fig 1). Treatment with acetone alone had no detectable effect on ear thickening (Fig 1). At each dose of PMA tested, the effects on ear thickness observed in W/W (Fig 1A) or congenic +/+ (Fig 1B) mice were statistically indistinguishable (P > .05 for all comparisons).

We also applied 5 µg of PMA or 5 µL of acetone to the ears of another group of mice, as above, but for either 3 or 6 weeks. Again, acetone had no effect on ear thickness, whereas PMA treatment resulted in significant thickening. There were no significant differences in the effect on ear thickness of PMA given for 3 or 6 weeks, in either WBB6F₁-W/W' or -/+ + mice (data not shown).

Repeated epicutaneous application of PMA is associated with the development of mast cells in the dermis of both WBB6F₁/W' and the congenic +/+ mice. Treatment of control ears with acetone had no significant effect on the number of dermal mast cells; the ears of the untreated W/W' mice had 1.3 ± 1.0 mast cells/mm², while those treated with acetone for 3 or 6 weeks had 1.7 ± 0.9 and 1.5 ± 1.0 mast cells/mm², respectively. These values are similar to those published previously for W/W' mice; the number of mast cells in the untreated (123 ± 17/mm²) or acetone-treated (145 ± 8/mm²) ears of the WBB6F₁ +/+ mice are also similar to those reported previously.

By contrast, treatment with repeated applications of PMA was associated with dose-dependent increases in the numbers of dermal mast cells in both the genetically mast cell-deficient W/W' (Fig 2A) and the congenic normal (+/+ ) animals (Fig 2B). Thus, no significant increases in dermal mast cell numbers were observed in the ears of mice that had been treated with only 5 ng of PMA for 6 weeks, but significantly increased numbers of mast cells were observed at all higher doses of PMA tested. The coefficients of correlation for the doses of PMA applied to the ears and the numbers of dermal mast cell per square millimeter of dermis after 6 weeks of treatment were highly significant (for W/W' mice, r = .946, P < .01; for +/+ mice, r = .828, P < .05).

Although the extent of ear thickening reached its plateau 3 weeks after initiation of PMA treatments, the numbers of identifiable mast cells only began to increase at 3 weeks, and increased substantially by 6 weeks. At 3 weeks after initiation of treatment with PMA (5 µg per application), the number of mast cells per linear unit of dermis was increased significantly in both W/W' mice (PMA, 0.88 ± 0.12 mast cells/mm length of dermis; acetone, 0.18 ± 0.16 mast cells/mm length of dermis) compared to untreated W/W' mice (0.12 mast cells/mm²).
mm length of dermis, \( P < .03 \) and congenic +/- mice (PMA, 36.6 \( \pm \) 1.3 mast cells/mm length of dermis; acetone, 17.5 \( \pm \) 0.8 mast cells/mm length of dermis, \( P < .001 \)). However, when the PMA-induced dermal thickening was taken into account and the data were expressed as the number of mast cells/mm\(^2\) of dermis (Fig 3), there were no significant differences at this time in the values for the ears of the PMA- and acetone-treated animals. At 6 weeks after the initiation of treatment, the numbers of mast cells per square millimeter of dermis were markedly elevated in both the W/W\(^+\) (Fig 3A) and congenic +/- (Fig 3B) animals. The numbers of mast cells in the ears of the PMA-treated W/W\(^+\) mice actually exceeded the numbers that developed in the ears of W/W\(^+\) mice with severe chronic idiopathic dermatitis

Fig 2. Histologic sections were prepared for quantitation of mast cells in the PMA- or acetone-treated ears of the W/W\(^+\) (A) and congenic +/- (B) mice from the experiment shown in Fig 1. The results are expressed as the mean \( \pm \) SEM number of mast cells per square millimeter of dermis (note differences in scales for mast cell numbers in W/W\(^+\) and +/- mice). At doses \( \geq \)50 ng per application, PMA induced significant increases in the numbers of dermal mast cells in W/W\(^+\) or +/- mice (\( P < .01 \) [**] or \( .001 \) [***] for comparisons of PMA-treated left ears [■] to contralateral acetone-treated right ears [□]). The results obtained with 5 or 10 \( \mu \)g PMA did not differ significantly (\( P > .05 \)), either in W/W\(^+\) or +/- mice.

Fig 3. Kinetics of appearance of morphologically identifiable dermal mast cells in W/W\(^+\) (A) or congenic +/- (B) mice in the PMA-treated left ears (■) or contralateral acetone-treated right ears (□) at various intervals after initiation of treatment. Results are expressed as mean \( \pm \) SEM (note differences in scales for mast cell numbers in W/W\(^+\) and +/- mice). PMA-induced augmentation of mast cell numbers achieved significance (\( P < .05 \) [*], .01 [**], or .001 [***] for comparison of PMA-treated to contralateral acetone-treated ears of the same mice) at 4 weeks for W/W\(^+\) mice and at 5 weeks for +/- mice.
PMA INDUCES MAST CELLS IN W/W+ MOUSE SKIN

(10 µg of PMA, 45.8 ± 2.7 mast cells/mm²; severe chronic idiopathic dermatitis, 33.0 ± 3.5 mast cells/mm², reference 8). Little or no further increases in mast cell numbers were observed when PMA treatment was continued for 12 weeks (data not shown).

The mast cells present in the PMA-treated ears of both W/W+ and congenic +/+ mice were especially frequent in the more superficial aspects of the dermis (Fig 4). In the W/W+ animals, we often observed loose clusters of 2 to 4 mast cells (Fig 4B, insert) and rarely found mast cells undergoing detectable mitosis (Fig 4C through F). In both the W/W+ and congenic +/+ mice, the cytoplasmic granules of the dermal mast cells could be stained with the heparin-binding fluorescent dye, berberine sulfate, a characteristic feature of mature connective tissue-type mast cells.9,10,28

PMA-induced dermal mast cells are competent to mediate type I hypersensitivity responses. After sensitization with IgE anti-DNP antibody, the mast cells in the ears of PMA-treated WBB6F1/W/W+ (Fig 5A) or +/+ (Fig 5B) mice were able to orchestrate a passive cutaneous anaphylactic (PCA) response upon intravenous challenge with the antigen DNP, HSA. In contrast, such responses were not detectable after antigen challenge in the ears of W/W+ mice not treated with PMA, or in PMA-treated ears injected with medium instead of IgE. As expected, PCA reactions developed in the IgE-injected ears of +/+ mice not treated with PMA. However, the IgE-injected, PMA-treated ears of the +/+ mice responded even more strongly to antigen challenge than did the contralateral IgE-treated control (acetone-treated) ears.

The induction of mast cell development is a local, not a systemic, consequence of PMA treatment. In five WBB6F1/W/W+ mice that had developed dermal mast cells in ears treated for 6 weeks with PMA, we also examined biopsies of the tongue, forestomach, glandular stomach, duodenum, cecum, lungs and spleen, and examined the resident peritoneal cells in cytocentrifuge preparations. We found no mast cells whatsoever in any of these anatomical sites.

Repeated epicutaneous application of PMA induces dermatitis in WCB6F1-SI/SI4 mice, but is not associated with the development of dermal mast cells. Three or six weeks of treatment with PMA (5 µg per application, three times per week) induced thickening of the ears of WCB6F1-SI/SI4 or +/+ mice (Fig 6A) that was very similar to the responses observed in PMA-treated WBB6F1/W/W+ or +/+ animals. The dermatitis induced by PMA in WCB6F1 mice was also similar to that induced by PMA in WBB6F1 mice by histology. However, PMA treatment was not associated with the development of mast cells in the WCB6F1-SI/SI4 mice (Fig 6B). After 3 or 6 weeks of treatment with 5 µg PMA, there was still less than 1 mast cell/mm² of dermis in the SI/SI4 mice; even 12 weeks of high-dose PMA treatment (10 µg PMA, three times weekly) did not induce mast cell development in the treated ears of SI/SI4 mice (less than 1 mast cell/mm² of dermis). In contrast, PMA did induce increased levels of mast cells in the WCB6F1 +/+ mice (Fig 6B).

PMA treatment of WCB6F1-SI/SI4 mice does not render the dermal microenvironment of these mice hospitable for mast cell growth/maturation. IL-3-dependent BMCMC

Fig 4. Histology of WBB6F1 -/+ (A) and WBB6F1 -/- W/W+ (B through F) ears after treatment three times weekly for 6 weeks with PMA (5 µg PMA per application in 5 µL acetone). (A) In the PMA-treated WBB6F1 -/+ specimen, more than 10 mast cells (->) are present in the dermis immediately beneath the epidermis (a). Occasional neutrophils (>) are also present. (B) A section from the subepidermal dermis of a PMA-treated WBB6F1 -/- W/W+ mouse containing at least 10 mast cells (->) and a neutrophil (>). Inset: a lower magnification view of a subepidermal area of the same ear as in B showing a cluster of three mast cells (>). (C through F) Serial 1 µm sections of the same subepidermal area in a PMA-treated WBB6F1 -/- W/W+ mouse showing two mast cells near a small blood vessel (C; long solid arrow). The upper mast cell is undergoing mitosis (early telophase; small arrows indicate the two groups of chromosomes); the lower mast cell (C through F; >) is not. All panels taken from 1 µm Epon-embedded, Giemsa-stained sections, all photographed at the same original magnification (x = 630), except for the inset in B (x = 250). Scale bar in A = 10 µm.
of WBB6F1r+/+ origin maintained in suspension culture share phenotypic features with mucosal mast cells; but upon injection into the skin of mast cell-deficient WBB6F1r/W/W" mice, the injected population gradually acquires the phenotypic characteristics of mature CTMC.10 Bone marrow transplantation from the congeneric +/+ mice also can repair the cutaneous mast cell deficiency of WBB6F1r/W/W" mice.3,29 By contrast, transplantation of congeneric +/+ bone marrow cells into WCB6F1r/S1/Sld mice has no effect on the mast cell deficiency of this mutant.14,5

The preceding series of experiments showed that PMA treatment induced dermatitis in S1/Sld mice, but not the development of dermal mast cells from mast cell precursors of S1/Sld origin. However, we also investigated whether PMA-induced dermatitis rendered the S1/Sld dermal microenvironment tolerant of the survival and/or maturation of BMCMC, which are more mature than the circulating mast cell precursor. We treated the left ears of WCB6F1r-S1/Sld mice with PMA for 2 weeks to induce an inflammatory response, then injected 5 × 10^3 BMCMC of WCB6F1r+/+ origin into the dermis of both ears of these animals. The left ears were then treated with PMA for an additional 6 weeks in order to maintain the inflammatory response. The mice were then killed, and their ears were processed for histology. To provide a positive control group of mice whose dermal microenvironment was competent for mast cell development, we injected aliquots of the same WCB6F1r+/+ BMCMC into the untreated ears of WBB6F1r/W/W" mice, then processed the transplanted ears for histology 6 weeks later.

As shown in Table 1, morphologically typical dermal mast cells were present in the ears of WBB6F1r-W/W" mice 6 weeks after the injection of WCB6F1r+/+ BMCMC. In contrast, mast cells were not identified after the injection of WCB6F1r+-/+ BMCMC into either the untreated or the chronically PMA-treated ears of the WCB6F1r-S1/Sld mice. In accord with our findings in earlier experiments, PMA treatment was associated with the development of increased numbers of mast cells in the skin of WBB6F1r-W/W" and WCB6F1r+/+ mice, but not in the skin of WCB6F1r-S1/Sld mice.

**DISCUSSION**

Repeated epicutaneous application of PMA to the ear skin of genetically mast cell-deficient WBB6F1r-W/W" mice resulted in a striking and dose-dependent augmentation of the number of dermal mast cells at sites of treatment. This finding reflected a local effect of the agent: there were no changes in the mast cell deficiency of the contralateral acetone-treated (control) ears of W/W" mice treated on one ear with PMA, nor did mast cells appear in multiple other anatomical sites of these animals. The W/W" mast cells that developed at sites treated with PMA resembled the normal dermal mast cells of the congeneric +/+ mice by light microscopy and by staining with the heparin-binding fluorescent dye, berberine sulfate. Moreover, the mast cells in PMA-treated W/W" skin were able to generate a PCA reaction. To our knowledge, this represents the first evidence that mast cells of W/W" origin can express IgE-dependent mediator release and orchestrate an immediate hypersensitivity reaction.
PMA INDUCES MAST CELLS IN W/Wv MOUSE SKIN.

There are several possible mechanisms by which PMA treatment might reverse the dermal mast cell deficiency of the W/Wv mouse. Sonoda et al.13 showed that the expanded population of dermal mast cells in the PMA-treated back skin of C57BL/6-bg+/bg→ C57BL/6-+/+ chimeric mice was derived from both C57BL/6-+/+ mast cells or their precursors already present in the skin, as well as from C57BL/6-bg+/bg' mast cell precursors recently derived from the bone marrow. In our experiments, PMA might have directly influenced the recruitment and development of circulating W/Wv mast cell precursors at the treated site and/or affected the small number of mast cells or their precursors normally present in the skin of the mutants. If they occurred, such effects either might have been independent of biochemical pathways ordinarily involving the receptor c-kit, or might have triggered the c-kit-dependent pathway at a point downstream to c-kit itself. Alternatively, PMA might have induced cells not in the mast cell lineage to produce large amounts of a factor(s) that promotes mast cell development (eg, IL-3, IL-4, other leukocyte-derived cytokines, and/or the yet unidentified ligand for c-kit, reviewed in reference 28) and/or might have increased the sensitivity of cells in the mast cell lineage to factors influencing their proliferation or differentiation/maturation. Note that while the molecular nature of the W and W' mutations have not yet been reported,4,5 immature mast cells of W/W' origin can be generated in vitro when W/W' hematopoietic cells are maintained in media containing high levels of IL-3 and other growth factors.10,29,31

The development of increased numbers of morphologically recognizable mast cells at sites of PMA application in W/Wv mice required prolonged periods of treatment. Taguchi et al reported that a single application of PMA transiently augmented the histamine content and histidine decarboxylase activity of W/Wv skin.32 Because this effect was not associated with the development of recognizable mast cells at the treated sites and was greatly attenuated in irradiated mice, the finding was thought to reflect the infiltration into PMA-treated sites of leukocytes with histamine synthetic ability.32 When we examined the ear skin of W/Wv mice up to 36 hours after a single epicutaneous application of 10 μg of PMA, no change in the animals' dermal mast cell deficiency was detectable.33 In the present study, increased numbers of mast cells were only marginally detectable after 3 weeks of treatment with PMA at 5 μg per application. However, by 6 weeks after the beginning of PMA treatment at 5 μg per application, numbers of mast cells per square millimeter dermis in the skin of W/Wv mice exceeded those in contralateral control (acetone-treated) ears by greater than 25-fold.

**Table 1. Failure of Chronic Treatment With PMA to Render the Dermis of WCB6F1-SI/Sld Mice Tolerant to the Survival of Mast Cells of WCB6F1-+/+ Origin**

<table>
<thead>
<tr>
<th>Genotypes and Ear</th>
<th>PMA*</th>
<th>BMCMC†</th>
<th>No. Mast Cells/mm² of Dermis‡</th>
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<tr>
<td>R</td>
<td>−</td>
<td>−</td>
<td>145 ± 14</td>
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Abbreviations: L, left; R, right.

*The left ears (+) were treated with 5 μg of PMA, three times weekly for 2 weeks before injection of BMCMC, then treatment was continued for an additional 6 weeks. The right ears (−) received no treatment.

†Some ears (+) were injected intradermally with 5 × 10⁶ BMCMC of WCB6F1-+/+ origin. The other ears (−) received no injection.

‡All mice were killed for determination of mast cell numbers 6 weeks after injection of BMCMC in groups 1 and 2.

**Fig 6.** Ear thicknesses (A) and numbers of dermal mast cells in the ears (B) of WCB6F1-SI/Sld or -+/+ mice treated with 5 µg PMA three times weekly for 6 weeks (●), treated similarly with acetone alone (△), or not treated (○). Significant differences between values in treated and untreated ears are indicated: P < .01 (**).
PMA-induced mast cell hyperplasia also occurs in normal mice. In our experiments, both WBB6F1-+/-+ and WCB6F1-+/-+ mice developed significantly increased numbers of dermal mast cells at sites treated repeatedly with PMA. By contrast, genetically mast cell-deficient WCB6F1-SI/SI mice did not develop mast cells at sites of PMA-induced dermatitis, even when treated with high doses of the agent (10 µg per application) were continued for 12 weeks. These findings and those of our previous study indicate that a microenvironmental factor defective in the SI/SI mouse, presumably the product of the SI locus, must be adequately expressed to permit detection of the effects of either chronic idiopathic dermatitis or chronic PMA treatment on mouse mast cell populations in vivo.

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Phorbol 12-myristate 13-acetate-induced development of functionally active mast cells in W/Wv but not Sl/Sld genetically mast cell-deficient mice

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