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ORIGINAL PAPER

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Accelerated proliferation of epidermal keratinocytes by the transgenic expression of the platelet-activating factor receptor

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Abstract **Transgenic mice overexpressing platelet-activating factor receptor (PAFR) have abnormal pigmentation of the ear and the tail, which can progress to melanocytic tumors as the mice age. Histologically, epidermal hyperproliferation and increases in dermal melanocytes are evident. Examination of these transgenic mice at various ages revealed hyperproliferation of the epidermis even 2 weeks after birth which developed as the mice aged. Dermal melanocytes also increased in number with growth. Expression of the PAFR transgene was found in keratinocytes and not in melanocytes, thereby suggesting that PAF does not play a direct role in proliferation of melanocytes. Topical application of a cream containing WEB2086, a specific PAFR antagonist, to the ear and the dorsal skin significantly suppressed the number of BrdU-positive cells in PAFR transgenic mice. These results suggest that PAF plays a modulatory role in the growth of epidermal keratinocytes. PAFR transgenic mice would be a useful model for investigations of skin diseases related to altered proliferation of epidermal keratinocytes including psoriasis.**

Key words PAF · PAFR · Differentiation · Proliferation · In situ hybridization

Introduction

Platelet-activating factor (PAF), a potent bioactive phospholipid mediator, has functions in various physiological processes [6, 11, 25]. In the skin, PAF has also been found to play a role in inflammatory and allergic skin diseases such as psoriasis, cold urticaria, urticaria pigmentosa, and contact dematitis [3, 4, 14, 17]. PAF exerts its biological effects by binding to a specific cell-surface PAF receptor (PAFR). PAFR has been reported to be present in platelets, neutrophils, monocytes, eosinophils, lymphocytes, vascular endothelial cells, smooth muscle cells, neurons and microglia [6, 11, 21, 25]. cDNAs or genome DNA of PAFR have been cloned from various species [1, 7, 9, 23, 34]. Travers et al. have shown PAFR mRNA expression in human keratinocytes [31], and we have found this expression in rat and human keratinocytes but not in human melanocytes [28]. As a means of improving our understanding of the physiological roles of PAF and PAFR in vivo, we developed transgenic mice overexpressing guinea-pig PAFR cDNA [10], under the regulation of the β-actin promoter and cytomegalovirus (CMV) enhancer [24]. These transgenic mice have a bronchial hyperreactivity to methacholine and an increased mortality after exposure to bacterial endotoxin. Unexpected altered phenotypes are epidermal and dermal hyperthickening with dermal melanosis. Electron microscopy has revealed the presence of dermal melanocytes with melanosomes at various stages of development suggesting an accelerated melanogenesis [10]. Some aged transgenic mice spontaneously develop melanocytic tumors in the dermis. In the study reported here, we investigated the skin phenotypes of PAFR transgenic mice, the macroscopic and microscopic changes taking place during growth of the mice, the localization of the transgene expression and the characteristics of the epidermal keratinocytes.

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Materials and methods

Materials

Restriction enzymes and DNA-modifying enzymes were obtained from Takara, Tokyo, Japan, and monoclonal antibodies to cytokeratin 8.60 from Sigma, St. Louis, Mo. and to filaggrin from Biogenesis, Poole, UK. A monoclonal antibody to epidermal protein markers cytokeratin K1 was a generous gift from Dr. D.R. Roop (Baylor College of Medicine, Tx.). A digoxigenin (DIG)-dUTP RNA labeling kit and a DIG nucleic acid detection kit were from Boeh-

ringer Mannheim Biochemica, Mannheim, Germany, and a cell proliferation kit was from Amersham, Buckinghamshire, UK. FITC-conjugated goat antirabbit IgG was obtained from Zymed, South San Francisco, Calif., and rhodamine-conjugated goat antirabbit IgG from Serotec, Oxford, UK. Other materials and reagents were of analytical grade.

Animals

Male PAFR transgenic mice were mated with BDF1 females (Charles River Japan, Yokohama, Japan) and their offspring were used for experiments. These mice were housed in communal cages with 13-h light periods in an air-conditioned room, and fed standard laboratory chow (MF; Oriental Yeast, Tokyo, Japan) *ad libitum*. The offspring were weaned at age 3 weeks. For genotyping, genomic DNA was isolated from the tips of the toes, and subjected to 30 cycles of PCR amplification (1 min at 94 °C, 2 min at 55 °C and 2 min at 72 °C). Primers specific to guinea-pig PAFR cDNA were forward 5′-ACCACACTCCTGTCAATC-3′ and reverse 5′-TCAGGATCAGGTCATGAT-3′. The PCR product was 290 bp long and was detected by gel electrophoresis.

Macroscopic observations and histology

To observe the macroscopic appearance at various ages of these mice, the animals were genotyped and identified 2 weeks after birth. Photographs of the left ear and the tail were taken every 2 weeks. A group of three transgenic mice and three control mice were used for this study. For microscopic examinations, the left ear was removed at 2, 6, 8, 12 weeks of age, after anesthetization with sodium thiopental (15 mg/kg body weight). The samples were fixed in 4% paraformaldehyde in phosphate-buffered saline (PFA/PBS), paraffin-embedded samples were sectioned at a thickness of 3 μm on a microtome, placed on a slide glass, and dried overnight at 37 °C. After deparaffinization with xylene, the sections were stained with hematoxylin and eosin. Toluidine blue staining was done to enable the mast cells to be counted, or the Fontana-Masson technique was used to identify melanin pigment. The thickness of the epidermis was measured using a micrometer. The number of melanocytes per 200 × high-power field and of mast cells per 400 × high-power field in the dermis were recorded. Sections from three animals were taken and ten visual fields per section were evaluated. Values are expressed as the number of cells per millimeter squared.

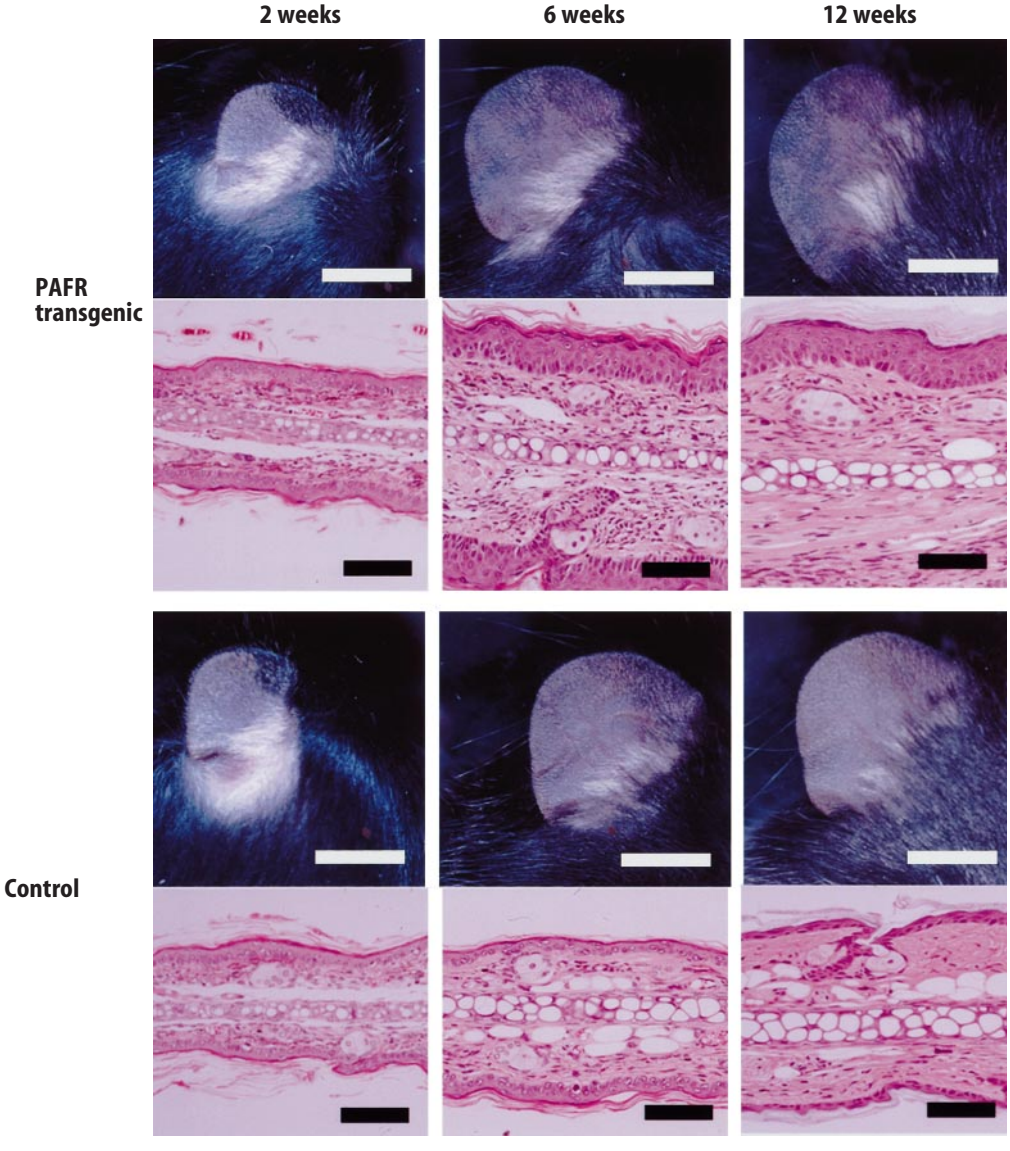

Fig. 1 Macroscopic appearance and the histology of the ear. Macroscopically, the patchy pigmentation of the skin is evident in 6 week-old transgenic mice. Microscopically, the epidermal layer is hyperproliferated at age 2 weeks, and increase in dermal melanocytes was noted at age 6 weeks. These changes show remarkably with the growth. *White scale bar*, 5 mm. *Black scale bar*, 100 μm

In situ hybridization

The skin tissue of 13-week-old PAFR transgenic mice and control littermates, and 46-week-old PAFR transgenic mice were fixed in 4% PFA/PBS for 8 h, embedded in paraffin, then cut at a thickness of 4 μm, mounted on aminopropyltriethoxysilane (APS)-coated glass slides (Matsunami, Tokyo, Japan), and dried at 37 °C overnight. After deparaffinization, the sections were treated with 0.2 *N* HCl for 15 min at room temperature, permeabilized in 10 μg/ml protease K for 10 min at 37 °C, postfixed in 4% PFA/PBS for 20 min, and soaked in PBS containing 2 mg/ml glycine twice for 15 min each time. Subsequently, the sections were acetylated with 0.25% acetic anhydride, dehydrated in a graded series of ethanol, and stored at –80 °C until use. In situ hybridization (ISH) was carried out as described previously but with several modifications [8, 27]. Briefly, a NcoI-AccI fragment of the coding region of guinea-pig PAF receptor cDNA (224 bp) was inserted into the *Sma*I site of pBluescript II. The plasmid was linearized either with *BamH*I to synthesize antisense cRNA or with *Bgl*II to synthesize sense cRNA by T7 or T3 RNA polymerase, respectively. The probes were labeled with DIG-11-UTP.

Sections were prehybridized for 2 h in 100 μl hybridization buffer consisting of 50% formamide, 10% dextran sulfate, 1 × Denhardt's solution, 10 m*M* Tris-HCl (pH 7.5), 5 × SSC, 0.02% sodium dodecylsulfate, 250 μg/ml yeast tRNA, 2% blocking reagent, and 0.1% sodium *n*-lauroyl sarcosinate. Hybridization was performed in hybridization buffer containing the labeled RNA probe (final concentration 2 μg/ml) in a humidified chamber for 18 h at 45 °C. The sections were then washed in 5 × SSC for 3 min, in 2 × SSC with 50% formamide for 30 min, in 2 × SSC for 20 min, and in 0.2 × SSC twice for 20 min, each time at 45 °C. The hybridization signals were visualized with 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium chloride, as outlined in the DIG nucleic acid detection kit protocol.

Cell proliferation assay

Three PAFR transgenic mice and four control littermates (age 13 weeks and weighing 20–25 g) were given the 5-bromo-2′-deoxyuridine (BrdU) labeling reagent (Amersham, Buckinghamshire, UK) intraperitoneally (3 mg/ml per 100 g body weight) and were euthanized by cervical dislocation 2 h later. The dorsal skin and the ears were removed, fixed overnight with formalin/methanol (1:1), paraffin-embedded, cut at a thickness of 3 μm, and deparaffinized with xylene. The sections were then soaked in 70% ethanol, washed briefly with PBS, treated with 0.5% hydrogen peroxide for 30 min at room temperature to inactivate endogenous peroxidase, washed with PBS three times for 5 min, and soaked in 1 *N* HCl for 30 min at room temperature. After washing with PBS three times for 5 min each time, the sections were blocked with 10% sheep serum/PBS for 30 min at room temperature, incubated in 100 μl nuclease/anti-BrdU antibody in deionized water (1:100) for 1 h in a moist chamber at room temperature, washed with PBS three times for 5 min each time, covered with 100 μl peroxidase anti-mouse IgG2a/diluent (15:1000) for 30 min at room temperature, and washed with PBS three times for 5 min each time. The signals were visualized by immersing the slides in a staining solution of substrate/intensifier and diaminobenzidine (DAB) in phosphate buffer. After washing in deionized water, the sections were dehydrated and mounted with DPX Mountment (Fluka Chemika, Switzerland). The number of BrdU-positive cells was determined in the basal layer of the interfollicular epidermis. Two sections from every tissue were taken, and cell numbers were measured in ten visual fields per section. Values were standardized as the number of cells per 100 basal cells.

Immunohistochemistry

Fresh ear and dorsal skin of the 13-week-old PAFR transgenic mice and control littermates were frozen with Tissue Tek OCT embedding compound (Miles Laboratories, Elkhart, Ind.) in liquid nitrogen-cooled isopentane. Frozen sections of 6 μm thickness were collected on APS-coated slides and dried. After a 5-min wash with PBS, the sections were incubated with a mouse anti-K10 and K11 monoclonal antibody (1:20), a mouse anti-K1 monoclonal antibody (1:500), or a mouse anti-filaggrin monoclonal antibody (1:200) for 18 h at room temperature in a humid chamber, then washed with PBS three times for 3 min each time. The bound primary antibodies were detected with an FITC-conjugated goat antimouse IgG (1:40), or a rhodamine-conjugated goat antimouse IgG (1:100) at 37 °C for 30 min in a humid chamber, in the dark. After washing three times with PBS, the sections were coverslipped with Fluoromount G (Southern Biotechnology Associates, Birmingham, Ala.). Immunostained sections were examined under a microscope equipped with an epi-illuminator containing blocks selective for green FITC and red rhodamine emissions (Olympus, Tokyo, Japan). Difference-interference-contrast images (Nomarski optics) were taken of the same sections. Daylight professional film (Fuji, Tokyo, Japan) was used for immunofluorescence microphotography.

Topical application of WEB2086 to mouse epidermis

Four PAFR transgenic mice and four littermates (aged 7 weeks and weighing 20–25 g) were shaved on the dorsal skin. They were treated daily with 4 mg WEB2086 cream (30 μg/mg) on the left

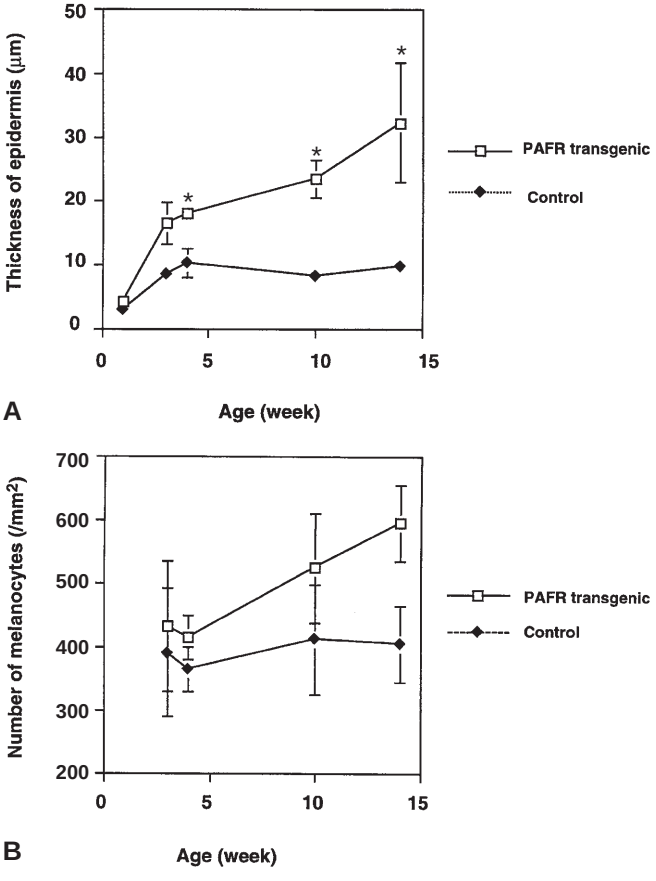

Fig. 2 A, B Changes in thickness of the epidermis and the number of dermal melanocytes of the ear. (**A**) Thickness of the epidermis of the transgenic mice continues to increase while that of control mice remained constant after 5 weeks. (**B**) The number of dermal melanocytes is constant in the control mice, but increased in the transgenic mice. Values are given as mean and S.D. from groups of three animals. * $P < 0.005$ versus another group, as determined by unpaired Student's *t* test

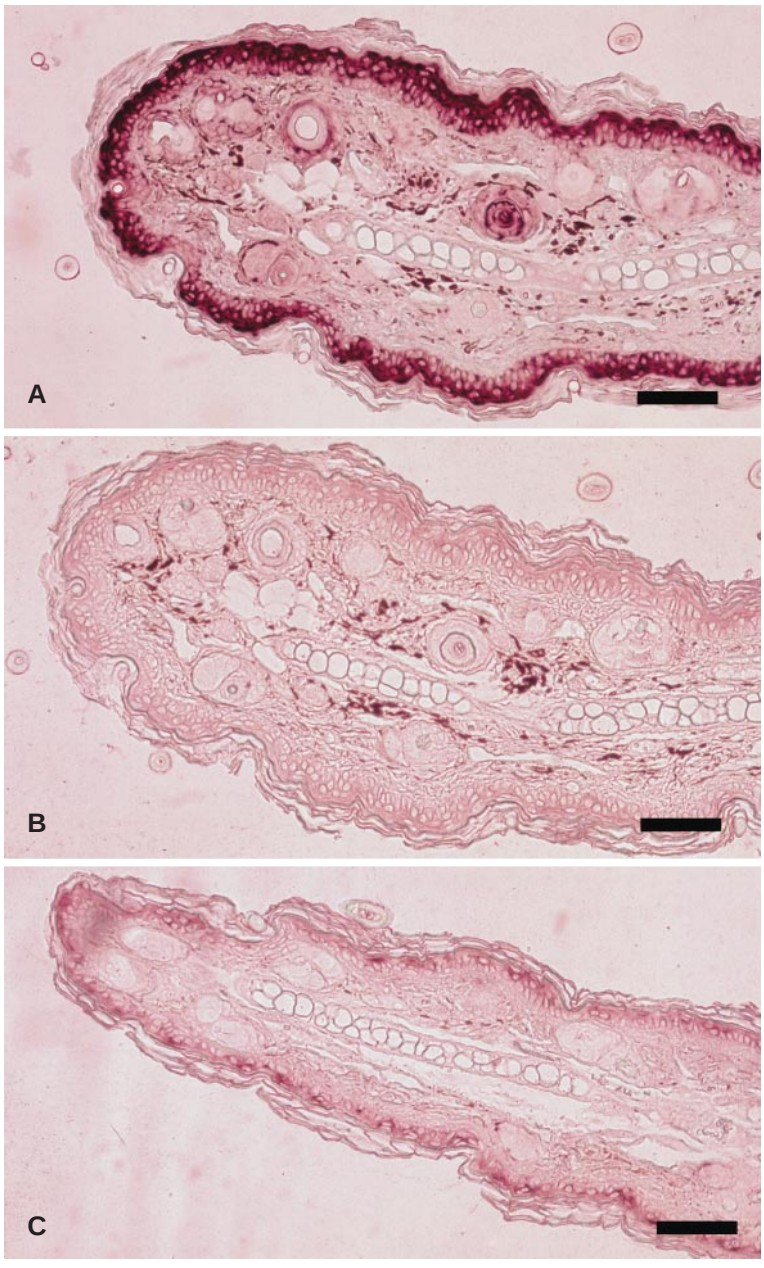

Fig. 3 A–C In situ hybridization of guinea pig PAFR mRNA in the ear. (**A**) Intense signals are seen in epidermal keratinocytes of the transgenic mouse, using the DIG-labeled antisense cRNA probe. (**B**) No signals are found with a sense probe. (**C**) Weak signal is seen in epidermis with the antisense probe in the control mouse, indicating cross hybridization of the probe to endogenous murine PAFR mRNA. *Scale bar*, 100 μm

side and 4 mg placebo cream on the right side of the dorsal skin and the ears for 7 days. They were injected intraperitoneally 2 h after the final treatment with the BrdU labeling reagent, and were sacrificed as described above. The removed skin and ears were either fixed with formalin/methanol (1:1) for the cell proliferation assay or frozen with Tissue Tek OCT embedding compound for the immunohistochemical analysis.

Results

Changes in the skin of PAFR transgenic mice during growth

The PAFR transgenic mice developed characteristic patchy pigmentation on the ears, the tail, and the external genital area, but not on the skin of other regions. The colors of the coat hair and eyes did not change. Macroscopically, the pigmentation had become apparent by 6 weeks after birth, as shown in Fig. 1. Microscopically, the epidermis was thickened and the number of melanocytes was increased in the transgenic mice, compared with the control littermates, as reported previously [10]. The melanocytes were identified by Fontana-Masson staining, and immunohistochemically using anti-S-100 antibodies (data not shown). Although the number of fibroblasts and mast cells also increased in the dermis of the transgenic mice, other inflammatory changes were not observed microscopically in the skin from 2 to 14 weeks of age (data not shown). The increase in epidermal thickness was already evident 2 weeks postnatally (Fig. 1). The control mice had one or two layers of keratinocytes, regardless of age. In the transgenic mice, the layers of keratinocytes increased with the

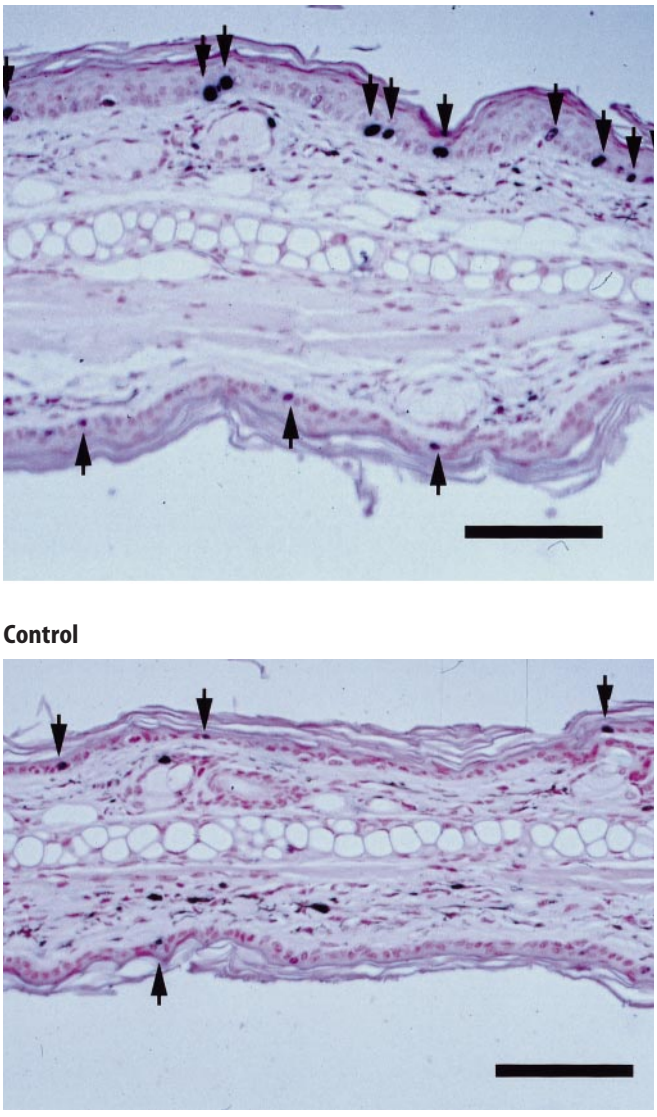

Fig. 4 Distribution of BrdU positive proliferating cells in epidermis of the ear. BrdU positive cells are increased in the basal layer of the epidermis in the transgenic mouse. Arrowheads mark BrdU positive nuclei of keratinocytes. *Scale bar*, 100 μm

age of the mice, reaching five or six layers at age 12 weeks (Fig. 1). The epidermis of the transgenic mice became about three times as thick as that of the control mice at age 3 and 14 weeks (Fig. 2A). These changes in epidermal thickness were also observed in the skin of the abdomen and tail (data not shown). The number of dermal melanocytes remained constant in the control mice, but increased in the transgenic mice with age (Fig. 2B).

PAFR transgene mRNA expression determined by ISH

In the skin of the transgenic mice, the transgene was predominantly expressed in epidermal keratinocytes, especially from the basal to the granular layer (Fig. 3A). Dermal melanocytes did not express the transgene (Fig. 3A). Melanocytic tumor cells from aged transgenic mice also did not express the transgene (data not shown). In the control mice, weak positive signals were found in the epidermis, indicating cross hybridization of the probe with endogenous murine PAFR (Fig. 3C). The specificity of ISH was verified by negative staining using the sense RNA probe (Fig. 3B).

Table 1 BrdU positive cells in the ear and the back skin. Values are means ± SD from groups of four transgenic and five control animals

	Ear, dorsal-side	Ear, ventral-side	Dorsal skin
Transgenic mice	6.34 ± 1.14 *	5.00 ± 0.69 *	3.93 ± 0.31 *
Control littermates	1.83 ± 0.61	1.40 ± 0.65	1.19 ± 0.23

*$P < 0.005$, vs control littermates, unpaired Student *t*-test

Accelerated proliferation of epidermal keratinocytes of PAFR transgenic mice

To examine the nature of acanthosis (keratinocyte hyperplasia), proliferating keratinocytes were identified using an *in vivo* BrdU uptake assay, as described in Materials and methods. BrdU-positive nuclei were observed in the basal layer of both PAFR transgenic and control mice (Fig. 4). The proportion of BrdU-positive cells was greater in PAFR transgenic mice than in control mice (Table 1), indicating accelerated proliferation of epidermal keratinocytes in the transgenic mice.

Normal differentiation of epidermal keratinocytes of PAFR transgenic mice

To examine the degree of epidermal differentiation of PAFR transgenic mice, the expression of the major differentiation markers, cytokeratin K1, cytokeratin K10, and filaggrin was determined by immunocytochemistry [15, 16, 20, 29, 30, 33]. In control mice, cytokeratin K1 was expressed predominantly in keratinocytes in the lower spinous layer (Fig. 5). In PAFR transgenic mice, however, cytokeratin K1 was expressed in keratinocytes in a broader area from the lower to the upper spinous layer. In both PAFR transgenic and control mice, filaggrin was expressed only in the granular layer (Fig. 5). Thus, in the control mice, there was a clear border between areas of K1 staining and filaggrin staining. In PAFR transgenic mice, the two areas overlapped, as shown superimposed in Fig. 5. The distributions of cytokeratin K10 and K1 expression in PAFR transgenic and the control mice were similar (data not shown).

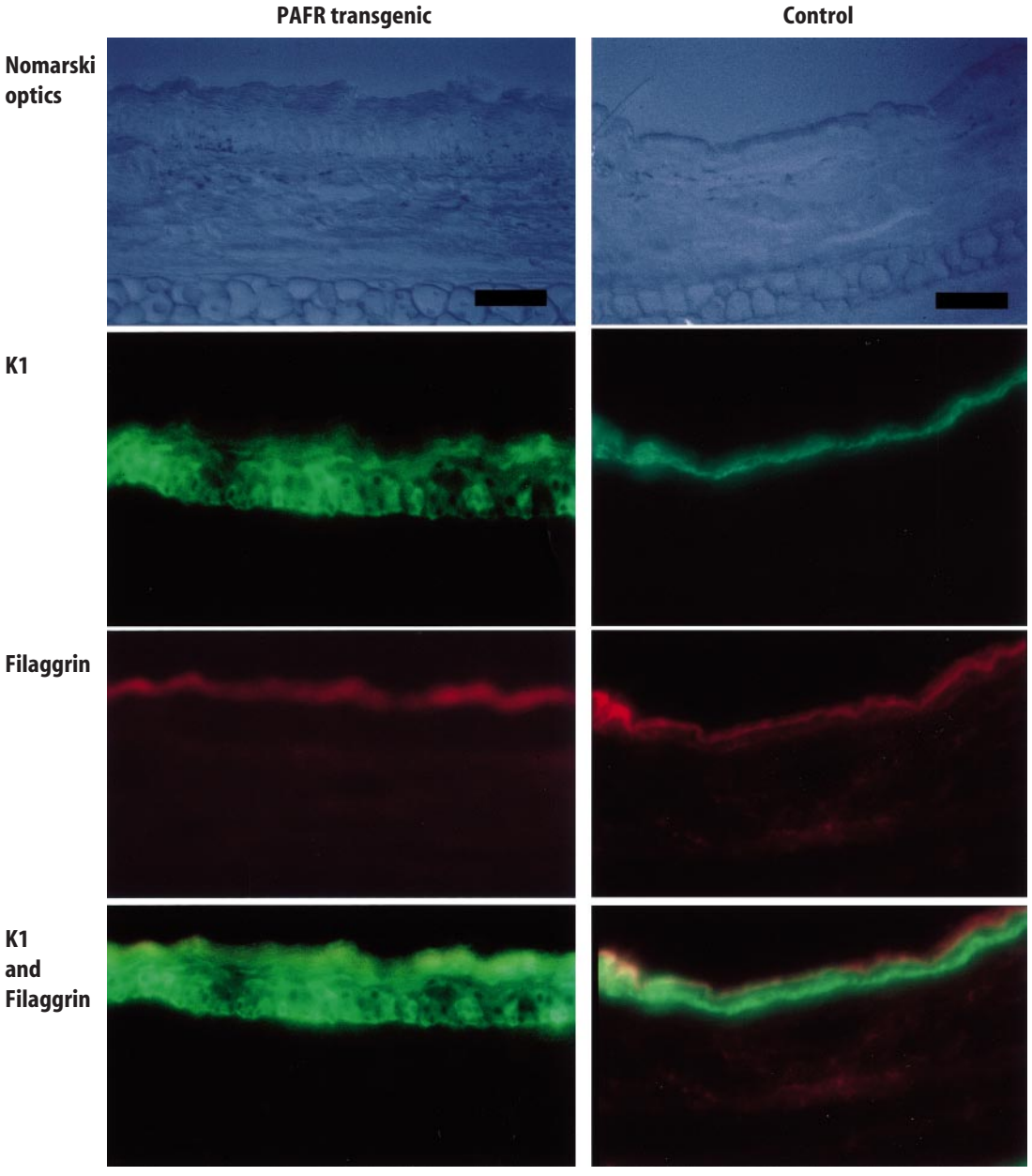

Fig. 5 Immunofluorescence labelings of the epidermas of the ear. Immunofluorescence signals for K1 (FITC, green) are found in the spinous layer and the signals for filaggrin (rhodamin, red) are in the granular layer in both transgenic and control mice. *Scale bar*, 50 μm

Inhibitory effect of WEB2086 on epidermal keratinocytes of PAFR transgenic mice

To elucidate the role of PAF in the proliferation of epidermal keratinocytes, we examined the inhibitory effect of a PAFR antagonist, WEB2086, on the intake of BrdU by the keratinocytes. The cream containing WEB2086 was applied topically to the skin of the mice. While the number of BrdU-positive keratinocytes significantly decreased in the WEB2086-treated regions of PAFR transgenic mice, no change was seen following the placebo treatment (Table 2). In the control mice, the same tendency towards a decrease in the number of BrdU-positive cells was observed, suggesting that PAF was also involved in keratinocyte proliferation in the control mice. The cream treatment did not affect the expression of cytokeratins K1, K10 and K11, or filaggrin. Microscopic observations revealed that the topical application of the placebo cream had no apparent effect on the epidermal histology (data not shown).

Discussion

We characterized skin abnormalities observed in PAFR transgenic mice. First, we quantified the thickness of the epidermis and the number of dermal melanocytes in PAFR transgenic mice at various ages and in control littermates.

Table 2 Topical application of WEB2086 to mouse epidermis. Values are means ± SD from groups of four transgenic and four control animals

	Ear, dorsal-side		Ear, ventral-side		Dorsal skin	
	WEB2086	Placebo	WEB2086	Placebo	WEB2086	Placebo
Transgenic mice	2.00 ± 1.12	6.34 ± 1.13	2.27 ± 0.57	5.00 ± 0.69	1.80 ± 0.37	3.93 ± 0.31
	*		*		*	
Control littermates	1.04 ± 0.99	1.83 ± 0.71	1.27 ± 0.67	1.52 ± 0.69	1.04 ± 0.16	1.20 ± 0.27

*$P > 0.005$, as determined by unpaired Student *t*-test

Next, the localization of the transgene in the skin was shown by ISH. The proliferation and the differentiation of epidermal keratinocytes were then investigated to characterize the nature of the epidermal keratinocytes of PAFR transgenic mice.

Cell proliferation was assayed by BrdU incorporation into DNA during the S-phase of the cell cycle. The number and the localization of the BrdU-positive cells reflects the characteristics of the proliferation. In normal human skin, BrdU-positive cells are usually found only in the basal layer and the proportion of positive cells is small [22]. In contrast, in basal cell carcinoma, BrdU-positive cells are increased in number and are present in other layers [22]. In seborrheic keratosis, a loss of polarity of BrdU-positive cells often occurs [22]. In PAFR transgenic mice, BrdU-positive cells were found only in the basal layer, but the proportion of positive cells was increased (Table 1). Thus, proliferation of the epidermal keratinocytes in the basal layer seems to be moderately accelerated in PAFR transgenic mice.

Epidermal differentiation was examined by immunocytochemistry, using specific markers. Cytokeratin K1 is a major early differentiation marker of mature epidermis [15, 20, 29, 33]. Filaggrin is thought to be responsible for organizing keratin intermediate filaments into bundles, is used as a marker of later stages of differentiation, and is expressed in the granular layer in normal murine and human epidermis [16, 30]. A reduction in filaggrin expression occurs in psoriatic regions where differentiation is dysregulated [12]. In the present study, both PAFR transgenic and control mice expressed K1 and K10 in the spinous layer and filaggrin in the granular layer. These findings indicate that the epidermis of the transgenic mice is well differentiated, and the cell cycle of epidermal keratinocytes is accelerated to induce acanthosis.

Other workers have noted that human and rat epidermal keratinocytes in the normal physiological state produce PAF [19, 32] and have PAFR [28, 31], suggesting that PAF acts in an autocrine manner. In WEB2086 cream-treated skin of PAFR transgenic mice, the number of BrdU-positive cells was decreased significantly compared with placebo cream-treated skin. This result suggests that PAF has a positive role in the proliferation of the epidermal keratinocytes in vivo, and that overexpression of PAFR leads to hyperplasia of keratinocytes.

Dermal melanocytes are increased in number and spontaneously transform into tumors during aging in PAFR transgenic mice [10]. However, the dermal melanocytes do not express detectable amounts of mRNA of the PAFR transgene. Thus, the hyperproliferating keratinocytes may produce factors that stimulate growth of dermal melanocytes directly or indirectly. Among the growth factors, stem cell factor is known to stimulate the proliferation of mast cells and melanocytes [2, 13, 18, 26, 27], although transgenic stem cell factor mice bare no melanoma [13]. In another report, basic fibroblast growth factor derived from human keratinocytes is described as a natural mitogen for melanocytes [5]. These growth factors might be independently or cooperatively involved in the proliferation of melanocytes and mast cells, and melanocytic tumor formation. The origin of PAF in the skin, and interaction of keratinocytes and melanocytes need to be clarified in future studies. PAFR transgenic mice have proved to be a pertinent model for the investigation of the pathogenesis of skin diseases with acanthosis, including psoriasis, melanin-containing tumors, and dermal melanocytosis, and the effectiveness of PAFR antagonist.

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References

1. Bito H, Honda Z, Nakamura M, Shimizu T (1994) Cloning, expression and tissue distribution of rat platelet-activating-factor-receptor cDNA. Eur J Biochem 221: 211–218
2. Geissler EN, Ryan MA, Housman DE (1988) The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. Cell 55: 185–192
3. Grandel KE, Farr RS, Wanderer AA, Eisenstadt TC, Wasserman SI (1985) Association of platelet-activating factor with primary acquired cold urticaria. New Engl J Med 313: 405–409
4. Guinot P, Summerhayes C, Berdah L, Duchier J, Revillaud RJ (1988) Treatment of adult systemic mastocytosis with a PAF-acether antagonist BN52063. Lancet 2: 114
5. Halaban R, Langdon R, Birchall N, Cuono C, Baird A, Scott G, Moellmann G, Mcguire J (1988) bFGF from human keratinocytes is a natural mitogen for melanocytes. J Cell Biol 107: 1611–1619

6. Hanahan DJ (1986) Platelet activating factor: a biologically active phosphoglyceride. Annu Rev Biochem 55: 483–509
7. Honda Z, Nakamura M, Miki I, Minami M, Watanabe T, Seyama Y, Okado H, Toh H, Ito K, Miyamoto T, Shimizu T (1991) Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. Nature 349: 342–346
8. Igarashi A, Nashiro K, Kikuchi K, Sato S, Ihn H, Grotendorst GR, Takehara K (1995) Significant correlation between connective tissue growth factor gene expression and skin sclerosis in tissue sections from patients with systemic sclerosis. J Invest Dermatol 105: 280–284
9. Ishii S, Matsuda Y, Nakamura M, Waga I, Kume K, Izumi T, Shimizu T (1996) A murine platelet-activating factor receptor gene: cloning, chromosomal localization and up-regulation of expression by lipopolysaccharide in peritoneal resident macrophages. Biochem J 314: 671–678
10. Ishii S, Nagase T, Tashiro F, Ikuta K, Sato S, Waga I, Kume K, Miyazaki J, Shimizu T (1997) Bronchial hyperreactivity, increased endotoxin lethality and melanocytic tumorigenesis in transgenic mice overexpressing platelet-activating factor receptor. EMBO J 16: 133–142
11. Izumi T, Shimizu T (1995) Platelet-activating factor receptor: gene expression and signal transduction. Biochim Biophys Acta 1259: 317–333
12. Kanitakis J, Ramirez-Bosca A, Reano A, Viac J, Roche P, Thivolet J (1988) Filaggrin expression in normal and pathological skin: a marker of keratinocyte differentiation. Virchows Arch A 412: 375–382
13. Kunisada T, Lu SZ, Yoshida H, Nishikawa S, Nishikawa S, Mizoguchi M, Hayashi S, Tyrrell L, Williams DA, Wang X, Longley BJ (1998) Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. J Exp Med 187: 1565–1573
14. Lavaud P, Rodrigue F, Carre C, Touvay C, Mencia HJ, Braquet P (1991) Pharmacologic modulation of picryl chloride-induced contact dermatitis in the mouse. J Invest Dermatol 97: 101–105
15. Leigh IM, Navsaria H, Purkis PE, McKay IA, Bowden PE, Riddle PN (1995) Keratins (K16 and K17) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro. Br J Dermatol 133: 501–511
16. Lynley AM, Dale BA (1983) The characterization of human epidermal filaggrin. A histidine-rich, keratin filament-aggregating protein. Biochem Biophys Acta 744: 28–35
17. Mallet AI, Cunningham FM, Daniel R (1984) Rapid isocratic high-performance liquid chromatographic purification of platelet activating factor (PAF) and lyso-PAF from human skin. J Chromatogr 309: 160–164
18. Mayer TC (1970) A comparison of pigment cell development in albino, steel, and dominant-spotting mutant mouse embryos. Dev Biol 23: 297–309
19. Michel L, Denizot Y, Thomas Y, Jean-Louis F, Heslan M, Benveniste J, Dubertret L (1990) Production of PAF-acether by human epidermal cells. J Invest Dermatol 95: 576–581
20. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors, and cultured cells. Cell 31: 11–24
21. Mori M, Aihara M, Kume K, Hamanoue M, Kohsaka S, Shimizu T (1996) Predominant expression of platelet-activating factor receptor in the rat brain microglia. J Neurosci 16: 3590–3600
22. Morimoto Y, Saga K, Bando M, Takahashi M (1991) In-vitro DNA synthesis of keratinocytes in normal human skin, psoriasis, seborrheic keratosis, Bowen's disease and basal cell carcinoma. Br J Dermatol 125: 9–13
23. Nakamura M, Honda Z, Izumi T, Sakanaka C, Mutoh H, Minami M, Bito H, Seyama Y, Matsumoto T, Noma M, Shimizu T (1991) Molecular cloning and expression of platelet-activating factor receptor from human leukocytes. J Biol Chem 266: 20400–20405
24. Niwa H, Yamamura K, Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene 108: 193–199
25. Prescott SM, Zimmerman GA, McIntyre TM (1990) Platelet-activating factor. J Biol Chem 265: 17381–17384
26. Qiu FH, Ray P, Brown K, Barker PE, Jhanwar S, Ruddle FH, Besmer P (1988) Primary structure of c-kit: relationship with the CSF-1/PDGF receptor kinase family-oncogenic activation of v-kit involves deletion of extracellular domain and C terminus. EMBO J 7: 1003–1011
27. Russell ES (1979) Hereditary anemias of the mouse: a review for geneticists. Adv Genet 20: 357–459
28. Shimada A, Ota Y, Sugiyama Y, Sato S, Kume K, Shimizu T, Inoue S (1998) In situ expression of platelet-activating factor (PAF)-receptor gene in rat skin and effects of PAF on proliferation and differentiation of cultured human keratinocytes. J Invest Dermatol 110: 889–893
29. Steinert PM, Roop DR (1988) Molecular and cellular biology of intermediate filaments. Annu Rev Biochem 57: 593–625
30. Steven AC, Bisher ME, Roop DR, Steinert PM (1990) Biosynthetic pathways of filaggrin and loricrin – two major proteins expressed by terminally differentiated epidermal keratinocytes. J Struct Biol 104: 150–162
31. Travers JB, Huff JC, Rola PM, Gelfand EW, Morelli JG, Murphy RC (1995) Identification of functional platelet-activating factor receptors on human keratinocytes. J Invest Dermatol 105: 816–823
32. Travers JB, Harrison KA, Johnson CA, Clay KL, Morelli JG, Murphy RC (1996) Platelet-activating factor biosynthesis induced by various stimuli in human HaCaT keratinocytes. J Invest Dermatol 107: 88–94
33. Tseng PC, Runge MS, Cooper JA, Williams RJ, Pollard TD (1984) Physical, immunochemical, and functional properties of *Acanthamoeba profilin*. J Cell Biol 98: 214–221
34. Ye RD, Prossnitz ER, Zou A, Cochrane CG (1991) Characterization of a human cDNA that encodes a functional receptor for platelet activating factor. Biochem Biophys Res Commun 180: 105–111