We have demonstrated recently that phenylazonaphthol (PAN)-allergy-induced hyperpigmentation in brownish guinea pig skin is associated with the concomitant appearance of a melanogenic soluble factor(s) that activates the intracellular signal transduction system, including phosphatidylinositol turnover subsequent to ligand-receptor binding in cultured guinea pig melanocytes. In this study we have purified and characterized the PAN-induced melanogenic stimulating factor (PIMSF) that occurs in allergy-associated hyperpigmented skin. By successive column chromatography on TSK 2000SW, Mono Q, and octadecyl-NPR, the PIMSF was purified to homogeneity with a single band of apparent molecular mass of 7.9 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The specific bioactivity of PIMSF increased by 5,195-fold over the original skin homogenate. In cultured guinea pig melanocytes, this purified PIMSF had the potential of activating an intracellular signal transduction system such as inositol 1,4,5-trisphosphate formation and intracellular calcium levels through a pertussis toxin-sensitive G protein-coupled receptor. PIMSF consistently caused a rapid translocation of cytosolic protein kinase C (PKC) to membrane-bound PKC within 5 min of treatment with a return to the basal level after 120 min. The stimulating effects of PIMSF on proliferation and melanization of cultured guinea pig melanocytes were abolished completely by a PKC down-regulating agent (phorbol 12,13-dibutyrate). PIMSF was similar in molecular mass to rat growth-related oncogene α (GRO-α; molecular mass of 7.9 kDa) on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and had immunocross-reactivity with GRO-α upon Western immune blotting analysis. Further, the stimulatory effect of purified PIMSF on DNA synthesis of cultured guinea pig melanocytes was suppressed markedly by the addition of anti-rat GRO-α antibody, implying that the PIMSF is apparently identical to GRO-α. These findings suggest that PAN allergy provides a new mechanism of hyperpigmentation in which biological factors such as the GRO-α superfamily generated within allergy-induced skin stimulate melanocytes through activation of the PKC-related signal transduction pathway.

Phenylazonaphthol (PAN) allergy-induced late appearing hyperpigmentation is known as pigmented cosmetic dermatitis (1). We demonstrated previously that specific melanogenic factors produced after a time course of hyperpigmentation due to PAN allergy in brownish guinea pig skin are associated with the induction of epidermal hyperpigmentation by the allergen (2). Biological evidence of signal transduction profiles suggested that melanogenic stimulation induced by PAN allergy is mediated through protein kinase C (PKC) activation within melanocytes by soluble factors secreted during biologic processing subsequent to allergic reaction (2).

Evidence has accumulated demonstrating that chemical mediators such as arachidonic metabolites (3, 4) or growth factor-like substances (5) secreted during inflammation could be responsible for stimulating the function and population of melanocytes, leading to hyperpigmentation induced by several stimuli including ultraviolet (UV) light. However, few of these mediators are known as intrinsic cytokines responsible for hyperpigmentation in vivo. We demonstrated previously that endothelins (ET) secreted from human keratinocytes act as intrinsic mitogens and melanogens for human melanocytes in UVB-induced melanosis (6–8). Furthermore, UVA-induced melanosis is associated with other keratinocyte-derived growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF), secretion of which is specifically stimulated after exposure of human keratinocytes to UVA (9). It is thus likely that the paracrine linkage of cytokines between keratinocytes and melanocytes in the epidermis plays a constitutive role in stimuli-induced cutaneous pigmentation.

PAN allergy-induced hyperpigmentation clearly differs in its time course from ET or GM-CSF-associated pigmentation such as UVB or UVA melanosis, respectively (1, 10). Further, there is no similarity in the chromatographic properties between the relevant cytokines in that the allergy-evoked soluble factor exhibits an apparent molecular mass of about 8–9 kDa estimated by gel filtration, which is distinct from that of ET-1 (2.3–2.7 kDa) or GM-CSF (14.5–15.5 kDa).

Aside from studies focusing on the biological effects of exogenous added cytokines on human melanocytes (11), studies examining the paracrine linkage of cytokines between keratinocytes and melanocytes would provide new insight into cellular mechanisms involved with intrinsic that which eventually take part in allergy-induced melanosis. Our previous studies (7, 9) suggested that different stimuli, including UV irradiation, trigger keratinocytes in various ways to produce and secrete completely different spectra of growth factors. On the other hand, it is likely that melanocytes undergo proliferation...
and differentiation in a similar fashion when responding to different growth factors secreted from keratinocytes (12). In this study therefore, we have extended our previous work and have purified a 7.9-kDa polypeptide from guinea pig skin, and we have characterized its effects on melanocytes. We identified this factor to be a member of the growth-related oncogene α (GRO-α) family or very close to it. It seems likely that specific melanogenic factors, such as GRO-α, produced in allergic cutaneous inflammation are responsible for the induction of late appearing hyperpigmentation by PAN allergy.

**EXPERIMENTAL PROCEDURES**

**Materials**

Anti-ET-1 and anti-rat GRO-α antibodies were purchased from Immunobiological Laboratories Co., Ltd. (Gumma, Japan). Anti-bFGF was obtained from Oncogene Science Inc. (Manhasset, NY). Fetal calf serum and minimal essential medium (MEM) were purchased from Life Technologies, Inc. Other chemicals were of reagent grade.

**Melanocyte Culture**

Guinea pig melanocytes were established from the flank skin of newborn brownish guinea pigs. Briefly, epidermal sheets were peeled from the skin, incubated with 1.5 units/ml dispase at 37 °C for 2 h and at room temperature for another 2 h. The epidermal cells were then dispersed by treatment with 0.05% trypsin and 0.02% EDTA at 37 °C for 30 min. The suspension of epidermal cells was filtered through sterilized gauze to remove stratum corneum layers; after centrifugation at 800 × g for 10 min, the suspension was cultured in 250-cm² Falcon dishes with MEM supplemented with 10% fetal calf serum, 10 mM nonessential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.5 mg/ml Fungizone, 16 mM phenol 12-myristate 13-acetate (PMA), and 10 μg/ml cholera toxin. After 24–48 h of primary culture, the medium was replaced with new medium containing 100 μg/ml Geneticin, and the culture was continued for 7–10 days to remove contaminating fibroblasts. After removal of fibroblasts, melanocytes were cultured in medium without Geneticin for 45–60 days with medium exchange every 3 days. For the first 2 months, melanocytes were subcultured by trypsin treatment every week. After the melanocytes began to grow well, melanocyte cultures were carried out in MEM (supplemented with 10% fetal calf serum, 10 mM nonessential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin) (MCM) excluding PMA and cholera toxin.

**Assay of Melanogenesis**

Melanocytes were cultured in the MCM excluding PMA and cholera toxin for more than 1 week. After trypsin treatment they were seeded into 24-well culture plates at a concentration of 1–2 ¥ 10⁵ cells/ml in the MCM. After a 24-h culture, the medium was exchanged for MEM (without fetal calf serum) supplemented with antibiotics, and fraction samples or purified PAN-induced melanogenic stimulating factor (PIMSF) was added at a 100 μM/l or 1 mg/ml concentration, respectively. For assay of melanin formation (12, 13), L-[3,5-3H]tyrosine was diluted in Hanks’ solution and added to 0.5 μCi/ml/well in culture for 24 h at 37 °C. Incorporated labeled tyrosine was counted by a liquid scintillation counter.

**DNA Synthesis**

DNA synthesis of melanocytes was measured by the incorporation of [3H]thymidine (Amersham, Bucks, U. K.). Melanocytes were plated into 24-well culture plates at a concentration of 1–2 ¥ 10⁴ cells/well in the MCM excluding PMA and cholera toxin. After a 24-h culture, the medium was exchanged for MEM (without fetal calf serum) supplemented with antibiotics; 50 μl of chromatographic fractions from skin extracts was added to the melanocyte cultures for 24 h at 100 μl/ml concentration, and the cells were pulse labeled with 1.0 μC/ml [3H]thymidine for the last 4 h of the 24-h incubation period. After three washings with PBS(−), the cells were lysed with 2 N NaOH at 37 °C for 15 min and neutralized with an equal volume of 2 N HCl. Acid-insoluble materials were precipitated by the addition of 4 volumes of ice-cold 10% trichloroacetic acid, collected on glass microfilters, washed three times with 10% trichloroacetic acid, once with ethanol, and then dried. Incorporated labeled thymidine was counted by a liquid scintillation counter.

**Measurement of Mass Contents of Ins(1,4,5)P₁**

For inositol 1,4,5-trisphosphate ([1,4,5]P₃) assay (6), cells were seeded in 24-well culture dishes at a density of 3 ¥ 10⁴ cells/ml and cultured in MCM excluding PMA and cholera toxin for 24 h. The media were then aspirated. MEM containing 10 mM LiCl was added and incubated for 10 min at 37 °C before stimulation with skin extract. The stimulation by skin extract was terminated at the designated times by adding 10% perchloric acid after which the samples were kept on ice for 15 min. After neutralization with ice-cold 1.5 N KOH for 60 min on ice, the samples were centrifuged at 2,000 ¥ g for 10 min to remove the KClO₄ precipitate. The supernatant samples (100 μl each) were subjected to Ins(1,4,5)P₃ assay using an inositol trisphosphate assay kit (Amersham) (14, 15). The Ins(1,4,5)P₃ content was determined quantitatively from a calibration curve by using the binding protein specific for Ins(1,4,5)P₃ and [3H]Ins(1,4,5)P₃.

**Measurement of Intracellular Calcium Content**

The melanocytes (2 ¥ 10⁴ cells/dish) were loaded with the calcium indicator Fura-2/AM (2 μM) by incubation for 30 min at 37 °C in the MCM excluding PMA and cholera toxin. The cultured cells were washed twice with fresh medium and exposed in MEM (without fetal calf serum) to purified PIMSF at a 1 μg/ml concentration. Fluorescence images were obtained at alternating excitation wavelengths of 340 and 380 nm through a SIT vidicon camera and processed using an Argus-200 image analyzer (Hamamatsu Photonics Corp., Hamamatsu, Japan). The calibration of the fluorescent signal in terms of the intracellular Ca²⁺ concentration was performed using digital imaging microscopy as described previously (6). Pertussis toxin was added at a concentration of 100 ng/ml 15 h before incubation with 1 μg/ml concentration of purified PIMSF.

**Preparation of Skin Extract**

The discs of guinea pig skin biopsied by keratome, mainly consisting of epidermis, were homogenized in a 5-fold volume of ice-cold phosphate buffer, pH 7.4, containing 1 mM phenylmethlysulfonyl fluoride using a Polytron homogenizer chilled with ice. After removing unbroken tissues by centrifugation at 800 ¥ g for 10 min, the supernatant fraction was subjected to further centrifugation at 105,000 ¥ g for 60 min to obtain skin extract for further biochemical assay.

**FIG. 1. Gel chromotographic purification (TSK 2000SW) of cytotoxic factors from skin extracts following PAN allergy treatment.** Melanogenic activities (melanin synthesis and tyrosinase activity) of each fraction were measured by ¹⁴C H₂O release into medium and incorporation of [¹⁴C]thiouracil into melanin. Mitogenic activity (DNA synthesis) was measured by incorporation of [³H]thymidine into cells. Melanocytes were incubated for 24 h with fractions at 100 μl/ml and assayed as detailed under “Experimental Procedures.” Skin extract at day 28 postchallenge was used for this experiment.
Purification of PIMSF

All procedures were carried out at 4 °C unless otherwise indicated.

TSK 2000SW Column Chromatography—1 ml of skin extract was applied to a TSK 2000SW column (21.5 × 600 mm, Toyo Soda, Tokyo, Japan) equilibrated with PBS. Elution was performed with the same buffer at a flow rate of 5 ml/min, and 1.0-ml fractions were collected under the control of an HRLC Bio-Dimension System (Bio-Rad Laboratories). Melanogenic and proliferative activities of each fraction were evaluated for a 24-h incubation period at a 100 ml/ml concentration according to the methods described above, and active fractions were pooled.

Mono Q Ion Exchange Column Chromatography—The pooled active fractions were applied to a Mono Q ion exchange column (HR 10/10, Pharmacia Biotech Inc.) equilibrated with 160 mM phosphate buffer, pH 7.4. The proteins were eluted at a flow rate of 3.0 ml/min with an increasing NaCl gradient from 0 to 1.0M over 30 min, using the HRLC Bio-Dimension System. Fractions of 1.0 ml were collected, assayed as above, and active fractions were pooled.

Octadecyl-NPR Column Chromatography—The active fractions obtained from the Mono Q column were applied to an octadecyl-NPR column (HR, Toyo Soda). The proteins were eluted at a flow rate of 1.0 ml/min with an increasing acetonitrile (containing 0.03% trifluoroacetic acid) gradient of 0–100% over 30 min. Fractions of 0.5 ml were collected and assayed as above. The active fractions were pooled and were dialyzed for 20 h against 2 liters of 160 mM phosphate buffer, pH 7.4. The dialyzed active samples were concentrated to about 0.5–0.8 ml in Centricon 3 (Amicon, Millipore Corp., Bedford, MA).

Mono Q Ion Exchange Column Rechromatography—The concentrated active fractions were applied to a Mono Q ion exchange column (HR 5/5, Pharmacia) equilibrated with 160 mM phosphate buffer, pH 7.4. The proteins were eluted at a flow rate of 1.0 ml/min with an increasing NaCl gradient of 0–1.0M over 30 min, using the HRLC Bio-Dimension System. Fractions of 0.5 ml were assayed as above, and active fractions were pooled.

TSK 2000SW Column Rechromatography—The active fraction obtained from the Mono Q column was applied to a TSK 2000SW column (7.5 × 600 mm) equilibrated with PBS. Elution was performed with PBS at a flow rate of 0.6 ml/min, and 0.3-ml fractions were collected and assayed. Active fractions were pooled.

Measurement of PKC Activity and Translocation

The cultured melanocytes were incubated in MEM (without fetal calf serum) with purified PIMSF at 37 °C for the times indicated, washed twice with cold PBS, and scraped from the culture dishes. The cell content was harvested and used for PKC activity assays.

**TABLE I**

| Steps in purification procedure | Total activity | Total protein | Specific activity | Yield | Purification |
|---------------------------------|----------------|--------------|------------------|--------|-------------|
| 1. Homogenate                   | 12,501.90      | 28,280.50    | 0.44             | 100.00 | 1.00        |
| 2. 105,000 × g supernatant      | 9,021.21       | 1,550.20     | 5.82             | 72.10  | 13.23       |
| 3. TSK 2000SW                   | 2,626.07       | 77.33        | 33.96            | 21.01  | 77.18       |
| 4. Mono Q                       | 2,147.05       | 13.21        | 162.53           | 17.17  | 369.39      |
| 5. Octadecyl-NPR                | 1,493.01       | 2.19         | 681.74           | 11.94  | 1,549.41    |
| 6. Mono Q (rechromatography)    | 1,059.09       | 0.64         | 1,654.83         | 8.47   | 3,700.98    |
| 7. TSK 2000SW                   | 740.64         | 0.324        | 2,285.93         | 5.92   | 5,195.30    |

**Figure 2.** Elution profiles of Mono Q column rechromatography (at purification step 6). For details of the purification and assays, see “Experimental Procedures.” For assay of DNA synthesis, 20 ml of 10-fold diluted samples of each fraction was applied to cultured guinea pig melanocytes (to a total volume of 500 μl).
suspensions were centrifuged for 5 min at 20,33 g and resuspended in 25 mM Tris/HCl buffer, pH 7.5, containing 2 mM EGTA, 5 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. Cytosol and membrane fractions were prepared as described previously (14). PKC activity was assayed by measuring the incorporation of 32P from [γ-32P]ATP into histone H-1 (type III-S) as described (14).

Other Methods

Analytical sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (16) in 5–20% gradient polyacrylamide gels. For Western immunoblotting analysis, purified proteins as detailed before were separated on 5–20% gradient SDS gels and then transferred to nitrocellulose membrane sheets or polyvinylidene difluoride membranes (Immobilon-P, Millipore Corp.) and incubated with primary antibodies (1/1,000 dilution). Subsequent visualization of antibody binding was carried out with an ABC kit (Funakoshi, Tokyo, Japan) according to the manufacturer’s instructions. Protein concentrations were determined using Bio-Rad protein assay kits with bovine serum albumin as the standard.

RESULTS

Purification of Melanogenic Soluble Factors—In previous studies we reported that DNA synthesis (as measured by [3H]thymidine incorporation) and melanogenesis (as measured by tyrosinase assay and melanin formation) of cultured guinea pig melanocytes were stimulated by the addition of skin extracts obtained on days 7, 16, 19, and especially on day 28 postchallenge with PAN (2). About 85% of the total melanocyte-stimulating activity in the brownish guinea pig skin homogenate was found in the 105,000 g supernatant (cytosolic) fraction at day 28 postchallenge, and the specific activity was the highest in this fraction (2). Therefore, we have now purified the melanogenic factors from the cytosolic fraction at day 28 postchallenge. When the cytosolic fraction was applied directly to a TSK 2000SW column, the elution profile exhibited three active fractions (fractions I, II, and III) with different molecular weights.

Other Methods

Analytical sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (16) in 5–20% gradient polyacrylamide gels. For Western immunoblotting analysis, purified proteins as detailed before were separated on 5–20% gradient SDS gels and then transferred to nitrocellulose membrane sheets or polyvinylidene difluoride membranes (Immobilon-P, Millipore Corp.) and incubated with primary antibodies (1/1,000 dilution). Subsequent visualization of antibody binding was carried out with an ABC kit (Funakoshi, Tokyo, Japan) according to the manufacturer’s instructions. Protein concentrations were determined using Bio-Rad protein assay kits with bovine serum albumin as the standard.
masses, as shown by the stimulation of DNA synthesis and melanogenesis by guinea pig melanocytes (Fig. 1). By contrast, similar gel chromatographic analyses of skin extracts (cytosolic fraction) obtained at day 0 postchallenge lack the 8–9-kDa fraction (fraction III) (data not shown), suggesting that PIMSF has a molecular mass of approximately 8–9 kDa. For this reason, we attempted to purify further the PIMSF from fraction III. Furthermore, because the biological activity of the stimulating factor was destroyed completely by heat (100 °C for 5 min) or by trypsin (37 °C for 30 min) (data not shown), it is considered to be a polypeptidic component. TSK 2000SW column chromatography was efficient in separating and purifying this PIMSF species, demonstrating about a 6-fold increase in its specific activity (step 3 in Table I). This PIMSF fraction was purified further by successive chromatographies on Mono Q (step 4), octadecyl-NPR (step 5), Mono Q (step 6, rechromatography), and TSK 2000SW (step 7, rechromatography) columns. Figs. 2 and 3 show the elution profiles of the Mono Q column rechromatography (step 6) and the TSK 2000SW column rechromatography (step 7), respectively. In the latter chromatography where various melanogenic factors were used as molecular marker and compared, the peak fraction with bioactivity is very similar in elution position to rat GRO-α (Fig. 3).

At this final purification step (step 7), the specific bioactivity of PIMSF had been increased by 5,195-fold over the original skin homogenate (Table I). The purified active fraction from TSK 2000SW rechromatography gave a single band with an apparent molecular mass of 7.9 kDa when examined by SDS-PAGE (Fig. 4). Furthermore, the PIMSF is similar in molecular size to rat GRO-α (molecular mass of 7.9 kDa) upon SDS-PAGE.

We next determined whether the purified PIMSF has the potential to activate intracellular signal transduction systems such as Ins(1,4,5)P₃ formation and intracellular calcium mobilization in guinea pig melanocytes through ligand-receptor interaction. The purified PIMSF elicited a quick increase in

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**FIG. 6.** Pseudocolor images of Fura-2 fluorescence intensity ratio imaging in guinea pig melanocytes at various times after stimulation with purified PIMSF. Melanocytes were loaded with the calcium indicator Fura-2/AM (2 μM) for 30 min at 37 °C in the MCM excluding PMA and cholera toxin. Fura-2/AM-loaded melanocytes were stimulated by purified PIMSF at a 1 μg/ml concentration. Fluorescence images were obtained at alternating excitation wavelengths of 340 and 380 nm through an SIT vidicon camera and were processed using an Argus-200 image analyzer.

**FIG. 7.** Purified PIMSF induces a marked stimulation of intracellular calcium mobilization in cultured guinea pig melanocytes (GPMC) (panel A) which can be inhibited by pretreatment with pertussis toxin (PT) (panel B). Melanocytes were loaded with the calcium indicator Fura-2/AM (2 μM) for 30 min at 37 °C in the MCM excluding PMA and cholera toxin. The assay for intracellular calcium mobilization was carried out in MEM without fetal calf serum. Pertussis toxin was added at a concentration of 100 ng/ml, 15 h before incubation with purified PIMSF. The purified PIMSF was added at a 1 μg/ml concentration. The arrow indicates when the purified PIMSF was added. The results are the ratio of the fluorescence emitted at 340 nm to that at 380 nm and represent the average curve from seven cells.

**FIG. 8.** Purified PIMSF induces a rapid translocation of PKC from the cytosol to the membrane when added to cultured guinea pig melanocytes. Melanocytes were incubated with purified PIMSF (1 μg/ml) at 37 °C for the indicated periods. The cytosolic and membrane fractions were prepared as described previously (14). PKC activity was assayed by measuring the incorporation of 32P from [γ-32P]ATP into histone H-1 (type III-S) as described elsewhere (14). Bar, S.D. from three experiments.
Ins(1,4,5)IP$_3$ levels within 40 s postincubation (Fig. 5). Although epidermal growth factor, transforming growth factor α, bFGF, and GM-CSF showed little or no significant effect on calcium mobilization (Table II), the purified PIMSF, ET-1, and rat GRO-a stimulated a marked increase in intracellular calcium mobilization in cultured guinea pig melanocytes (Fig. 6 and Table II). The calcium mobilization induced by purified PIMSF was perturbed by the prior addition of pertussis toxin (Fig. 7, A and B). In many cells, the production of Ins(1,4,5)IP$_3$ is intrinsically accompanied by the generation of 1,2-diacylglycerol, which activates PKC through translocation modulation (6). Because membrane association of PKC is thought to represent an initial step in PKC activation, we have examined whether PIMSF has any effect on the redistribution of PKC. When purified PIMSF was added to cultured guinea pig melanocytes, a rapid translocation of PKC from the cytosol to the membrane was observed (Fig. 8). When guinea pig melanocytes were exposed to phorbol 12,13-dibutyrate for 20 h to downregulate PKC activity, the stimulation of DNA synthesis and melanogenesis induced by PIMSF were suppressed markedly (Fig. 9, A–C). These findings suggested the involvement of PKC activation in PIMSF-mediated stimulation of melanogenesis by melanocytes even though the signal pathway via phosphorylation after PKC activation which leads to the melanogenic acti-
The applied concentrations were 10 ng/100 ml purified PIMSF (lane 1) and 10 ng/100 ml rat GRO-α (lane 2). Anti-rat GRO-α antibody was used at a concentration of 400 μg/ml. Molecular mass markers (in kDa): 9.7, collagen peptide A; 6.6, aprotinin; 4.5, sauvagine; 3.1, secretin; 2.1, atriopeptin; 1.4, bacitracin.

**FIG. 10.** Immunoblotting of purified PIMSF shows cross-reactivity with anti-rat GRO-α antibody. The applied concentrations were 10 ng/100 ml purified PIMSF (lane 1) and 10 ng/100 ml rat GRO-α (lane 2). Anti-rat GRO-α antibody was used at a concentration of 400 μg/ml. Molecular mass markers (in kDa): 9.7, collagen peptide A; 6.6, aprotinin; 4.5, sauvagine; 3.1, secretin; 2.1, atriopeptin; 1.4, bacitracin.

**FIG. 11.** Mitogenic activities of purified PIMSF were abolished markedly by the addition of anti-GRO-α antibody but not by anti-bFGF or by anti-ET antibodies. Purified PIMSF or GRO-α and antibodies were mixed and added to the guinea pig melanocyte cultures. The applied concentrations were as follows (in μg/ml): PIMSF, 1; GRO-α, 1; anti-GRO-α, 100; anti-bFGF, 120; anti-ET-1, 90. Mitogenic activity (DNA synthesis) was measured by incorporation of [3H]thymidine into cells. Melanocytes were incubated for 24 h with purified PIMSF and assayed as detailed under “Experimental Procedures.” Bar, S.D. from three experiments.
Purification and Characterization of an Allergy-induced MSF

Epidermal pigmentation. It has been reported that GRO-α mRNA is expressed in cultured human keratinocytes and that its expression is stimulated in response to UVB exposure (24) or to IL-1α treatment (25). In inflammatory and hyperproliferative skin diseases such as psoriasis, GRO-α mRNA is overexpressed selectively in the epidermis as a keratinocyte response to activated T cells (25). On the other hand, human foreskin fibroblasts express a 10-fold elevation in their steady-state levels of GRO mRNA in response to serum or PMA stimulation (26). It is interesting to note that IL-1 induces at least a 100-fold elevation of GRO mRNA expression without changing in c-myc or c-fos gene expression (26).

ET, which we have recently reported as a novel keratinocyte-derived mitogen and melanogen for human melanocytes in UVB-induced epidermal pigmentation (6–8), is very similar in its mode of action to purified PIMSF. ET-1 is stimulated in production and secretion from keratinocytes by the action of IL-1 in an autocrine fashion and activates the signal transduction pathway, including Ins(1,4,5)P3 formation, intracellular Ca2+ mobilization, and PKC in human melanocytes. In UVB-exposed keratinocytes, ET-1 is secreted with a lag time of a few days following IL-1 secretion (7), demonstrating a similar time course between ET secretion in culture and the onset of in vivo pigmentation following UVB irradiation. Significant differences between ET and GRO-α other than their molecular masses include the time course of their secretion in culture or in epidermal tissue. The production and secretion of ET-1 in cultured human keratinocytes require autocrine stimulation by IL-1α, which is released following UVB irradiation and which takes approximately 2–3 days to maximize (7). This time course of secretion is corroborated by the profile of ET-1 mRNA expression in culture (7) and in UVB-exposed human skin (8). On the other hand, the production or secretion of the PIMSF in allergy-induced epidermis is triggered by the release of IL-1α. However, it remains unclear why a similar autocrine mechanism initiated by IL-1α secretion which leads to the secretion of GRO-α and ET-1 occurs in a different time course. Taken together, it seems likely that PAN allergy provides a new mechanism of hyperpigmentation in which bio-physical factors, such as the GRO-α superfamily, synthesized within the epidermis, stimulate melanocytes through the activation of the PKC-related signal transduction system.

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