Differential Expression of Calgranulin A and B in Various Epithelial Cell Lines and Reconstructed Epidermis

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The monoclonal antibody F12, raised against epidermal cells from a psoriatic lesion, decorated antigens highly expressed in psoriatic epidermis and in cultured normal human keratinocytes. In normal human skin, F12 reacted only with follicular keratinocytes. Characterization of the immunoprecipitated antigens by two-dimensional gel electrophoresis revealed their identity with calgranulin A and B. A semi-quantitative study with various established epithelial cell lines demonstrated that the expression of calgranulin A and B in hyperproliferative keratinocytes correlates with their potential to undergo terminal differentiation. In epidermis reconstructed in vitro, the antigen expression was stimulated by retinoids and suppressed under vitamin A starvation. J Invest Dermatol 99:639–644, 1992

Psoriasis is a skin disorder affecting about 2% of the world population. This multifactorial disease is characterized by epidermal hyperplasia due to a greatly accelerated rate of epidermal turnover. To better understand the pathophysiology of psoriasis and to identify molecular markers specific for this disease, monoclonal antibodies (MoAb) were raised against psoriatic epidermal cells [1]. One of these MoAb, F12, has previously been shown by immunofluorescence to react specifically with psoriatic epidermis but not with interfollicular normal epidermis [1]. Here we report the characterization of the antigens recognized by F12 and their expression in cultured normal human keratinocytes, epidermis reconstructed at the air-liquid interface in vitro, and various established epithelial cell lines with a distinct potential to differentiate.

MATERIALS AND METHODS

Chemicals  The culture media and fetal calf serum used were from Flow Laboratories and Gibco. [35S]-methionine and ENHANCE were from New England Nuclear. Insulin, epidermal growth factor, and retinoic acid were from Sigma. The synthetic retinoid CD 271 (6-[3-(1-adamantyl)-4-methoxyphenyl]-2-
naphthoic acid) was synthesized in our laboratories. Acrylamide and other products for gel electrophoresis were obtained from Bio-Rad. Protein A Sepharose was from Pharmacia LKB Biotechnology Inc. The goat anti-mouse immunoglobulins and the fluorescein conjugated rabbit anti-mouse IgG were purchased from Cappel.

F12 Monoclonal Antibody  Monoclonal antibodies were produced by immunizing mice with dispersed epidermal cells from a psoriatic plaque. Among the 128 growing hybridoma clones, F12 was selected, because this antibody reacted specifically with psoriatic epidermis and did not react with interfollicular normal epidermis [1].

Cells and Culture Conditions  Human skin obtained from plastic surgery was cut with a keratome set at 0.4 mm. Split-thickness skin was incubated overnight at 4°C in a 0.25% (w/v) trypsin solution and the epidermis was separated from the dermis in a 0.05% (w/v) trypsin solution containing 0.02% (w/v) ethylene-diamine tetraacetic acid (EDTA). After the addition of FCS to a final concentration of 50% (v/v) the cell suspension was gauze filtered to eliminate the stratum corneum. The keratinocytes were recovered by centrifugation at 1000 X g for 5 min and were grown with the 3T3 cell cultivation technique of Rheinwald and Green [2].

The SCC-13, SCC – 12-F2, and SCC-4 human squamous carcinoma keratinocyte lines were a kind gift of Dr. J. Rheinwald (Dana-Farber Cancer Institute, Boston). The TR146 and TR131 squamous carcinoma cell lines were from Dr. T. Rupniak (Imperial Cancer Research Fund, London). NM1 and HaCat keratinocytes were kindly provided by Dr. H. Baden (Massachusetts General Hospital, Boston) and Dr. N. Fucenig (German Cancer Research Center, Heidelberg), respectively.

The above cell lines were grown as described for normal human keratinocytes (NHK). The SV-40 transformed human foreskin keratinocyte line SV-K14 (a kind gift of Drs. B. Lane and J. Taylor-Papadimitriou, Imperial Cancer Research Fund, London) was used after about 20 passages. SV-K14 cells as well as Hela cells were grown in Dulbecco’s modified Eagle’s medium (DME) and F12 (1:1) medium supplemented with 5% (v/v) fetal calf serum (FCS) and containing 100,000 units penicillin, 100 mg streptomycin, and 250 μg amphotericin B per liter of medium.

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Abbreviations:
BSA: bovine serum albumin
DMEM: Dulbecco’s modification of Eagle’s medium containing 5.5 mM glucose and 4 mM glutamine
EDTA: ethylenediamine tetraacetic acid
FCS: fetal calf serum
F12: Ham’s medium F12
GAG: glycosaminoglycan
MoAb: monoclonal antibody
NHF: normal human fibroblasts
NHK: normal human keratinocytes
PBS: phosphate-buffered saline
SDS: sodium dodecylsulfate
Tris: Tris (hydroxymethyl) aminomethane
Reconstructed Skin on a Collagen-GAG Lattice  Biopolymer substrates prepared from bovine skin collagen and chondroitin-6-sulfate (GAG) were obtained from Dr. S. Boyce, University of Cincinnati. Reconstruction of the epidermis on the lattice colonized with normal human fibroblasts (NHF) was performed as described [3]. The NHK—normal human fibroblasts (NHF) collagen—glycosaminoglycan (GAG) skin substitutes were kept air exposed in a humidified incubator for 14 d. The medium [DMEM and Ham’s medium F12 (1 : 1) containing 10% FCS, 100,000 units penicillin, 100 mg streptomycin, and 250 μg amphotericin B per liter] was changed every second day.

Reconstructed Epidermis on Dead De-Epidermized Epidermis  Epidermis reconstructed on dead de-epidermized dermis was prepared as described by Regnier et al [4]. One day after seeding the NHK, the cultures were air-exposed. The medium, DMEM:F12 (3:1), 10% FCS, containing 10 ng/ml epidermal growth factor and 5 μg/ml insulin was changed each second day. After 10 d the above medium was supplemented, except for the controls, with either retinoic acid (100 nM) or CD 271 (100 nM). In some cultures, FCS was replaced by 10% delipidized FCS. After an additional 6 d of air exposure and two medium changes, the cultures were used for indirect immunofluorescence staining.

Indirect Immunofluorescence Staining  Frozen sections were air-dried and immunolabeled at room temperature by the indirect method [5]. All sections were incubated for 30 min with the same undiluted supernatant from F12 cultures. Control sections were incubated with the supernatant of the hybridoma cell line SP2/0. After washing with phosphate-buffered saline (PBS), the sections were incubated with fluorescein-conjugated goat anti-mouse IgG (diluted 1:100 in PBS). They were then washed in PBS and mounted in 90% glycerol (v/v) in PBS containing 5 mM p-phenylenediamine.

Immunoprecipitation and Electrophoresis  For immunoprecipitation studies, the cell proteins were metabolically labeled by an overnight incubation with 100 μCi/ml of [35S]-methionine (1000 Ci/mmol). After scraping, the cells were homogenized in 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet-P40 containing 10 mg/ml BSA, 0.1 mM phenyl methyl sulfonyl fluoride, and 1 μg/ml of aprotinin and chymostatin each. After centrifugation (10,000 × g, 10 min) the supernatant was incubated for 30 min at 4°C with the same volume of undiluted supernatant from F12 cultures, followed by an incubation with a preformed complex of Protein A Sepharose and goat anti-mouse-immunoglobulins. (Control immunoprecipitations were performed with the supernatant of SP2/0 cultures). After 1 h at 4°C the sample was centrifuged.

Figure 1. Indirect immunofluorescence staining of sections obtained from involved psoriatic skin (A), normal skin (B), hair follicle (C), NHK sheet detached from the Petri dish with dispase (D), and epidermis reconstructed in vitro on a collagen-GAG substrate (E). e, epidermis; d, dermis; re, reconstructed epidermis; ad, artificial dermis exhibiting autofluorescence. (The biopsy was taken from a stable psoriatic plaque of a patient who had not received any treatment for 1 month prior to sampling.)
(1000 x g, 5 min). The pellet was washed three times with 20 mM Tris-HCl, pH 7.5, 140 mM NaCl, 1% Nonidet-P40, 1% sodium deoxycholate, and 0.1% SDS. After solubilization in electrophoresis sample buffer the immunoprecipitates were analyzed by sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis under reducing conditions [6]. A 15% acrylamide gel was used and immunoprecipitates obtained from about one tenth of the cells in a 10-cm culture dish were applied per slot. Fluorography was performed by treating the gels with ENPHANCE. The dried gels were exposed to Kodak XAR-5 films.

**Two-Dimensional Gel Electrophoresis** Immunoprecipitates of NHK were prepared as described above and mixed with unlabeled, non-fractionated psoriatic keratinocyte extracts and subjected to two-dimensional gel electrophoresis as described in detail by Celis et al [7]. The labeled immunoprecipitated proteins were revealed by fluorography and the unlabeled proteins by Coomassie Blue staining.

**RESULTS**

As revealed by immunofluorescence, MoAb F12 decorated intracellularly all layers of involved psoriatic epidermis (Fig 1A), but did not react with interfollicular normal epidermis (Fig 1B). However, hair follicles were heavily stained (Fig 1C). As shown in Fig 1D, F12 reacted also with NHK cultivated on plastic under submerged conditions as well as with those of a reconstructed epidermis cultured on a collagen-GAG substrate at the air-liquid interface (Fig 1E).

The antigens recognized by F12 were immunoprecipitated from a protein extract of cultured NHK. One-dimensional electrophoretic separation of the immunoprecipitate revealed two protein bands with molecular weight of 11 and 14 kd, respectively (Fig 2, lane C). The same result was obtained with proteins immunoprecipitated from extracts of reconstructed epidermis cultured on a collagen-GAG substrate at the air-liquid interface (Fig 2, lane E).

A more detailed analysis by two-dimensional gel electrophoresis of labeled, immunoprecipitated NHK proteins mixed with a non-fractionated extract of psoriatic keratinocyte revealed the presence of three immunoprecipitated proteins in the NHK extract (Fig 3B).

The same proteins were highly expressed in psoriatic epidermis (Fig 3A). According to the very recently established NHK protein database by Celis et al [7], the immunoprecipitated proteins are identical to proteins classified under the sample spot numbers 1003, 5007, and 6017, proteins already identified and named as calgranulin A or MRP 8 for spot number 1003 and calgranulin B, MRP 14, LI, or calprotectin for spots 5007 and 6017.

Applying the immunoprecipitation procedure to a number of established epithelial cell lines with a distinct potential to differentiate (Table I) revealed that some of these cell lines do not at all express the calgranulins, whereas others express them in various amounts.
Expression of the antigens in epidermis reconstructed on de-epi-
dermized dermis is considerably diminished compared to epidermis
on the collagen-GAG lattice (Fig 4A to be compared with Fig 1E).
Calgranulins are induced by retinoids and suppressed under vitamin
A deficiency. Figure 4B shows that after 6 d of culture in the ab-

ence of vitamin A (delipidized serum), expression of the antigens
in the living epidermis is suppressed. The addition of retinoic acid
strongly increases the expression of calgranulins (Fig 4D). The
synthetic retinoid CD 271 stimulates expression to a lesser extent
and the antigens are detected preferentially at the cell periphery
(Fig 4C).

**DISCUSSION**

Among the MoAb produced by using a psoriatic epidermal cell
suspension as antigenic material, one of them, named F12, reacted
strongly with psoriatic epidermis and cultured NHK, but not with
interfollicular uninvolved epidermis.

In a first step to characterize the antigens recognized by F12 they
were immunoprecipitated from a protein extract prepared from
cultured NHK. The proteins thus obtained migrated as two dis-
tinct bands when subjected to one-dimensional gel electrophoresis
under reducing condition, exhibiting molecular weights of 11 and
14 kd.

The fact that these antigens are highly expressed in psoriatic
epidermis, absent in interfollicular epidermis, as well as their mole-
cular weights of 11 and 14 kd, was taken as an indication that the
antigens recognized by F12 might be calgranulin A and B [8]. These
two proteins, identical to the two macrophage proteins, MRP-8 and
MRP-14, the leukocyte L1 light and heavy chains, and the cystic
fibrosis antigen [9] have been discovered independently. Calgranu-
lins are intracellular calcium-binding proteins; their genes have
been cloned and sequenced and are located on chromosome 1 [8,10].
Expression of these antigens has been described in various inflam-

matory dermatoses [8,11 – 13] skin tumours [14] and in cultured epithe-

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* −, not at all; +, weak; ++, intermediate; +++, strong.

**Figure 4.** Indirect immunofluorescence staining of sections obtained from reconstructed epidermis on dead de-epidermized dermis. Cultures were main-
tained under identical conditions for 10 d before exposing them for 6 d to the same culture medium as before (control A), to medium containing 10% delipidized FCS instead of FCS (B), and to the original medium containing either 100 nM CD 271 (C) or 100 nM retinoic acid (D). SC, stratum corneum; LE, living epidermis.
that the dense structure of the de-epidermized dermis with its basement membrane constitutes a much more important diffusion barrier for retinoids that are normally bound to serum albumins.

The fact that calgranulins are calcium-binding proteins could explain the above-described observations. By interfering with the intracellular calcium sequestration they could affect the control of keratinocyte proliferation and differentiation that is calcium dependent. Another possibility could be the described effect of calcium-binding proteins on tyrosine-specific kinases [23], because tyrosine phosphorylation plays a major role in keratinocyte proliferation and differentiation [24].

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REFERENCES


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