Neonatal Murine Epidermal Cells Express a Functional Multidrug-Resistant Pump

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Phospho-glycoproteins are members of the ABC transporter family encoded by the multidrug-resistant genes. These proteins are highly expressed in many tumor cells derived from patients undergoing treatment with anti-cancer drugs. Phospho-glycoproteins are large 12 transmembrane spanning molecules of 170 kDa, involved in adenosine-5′-triphosphate-dependent efflux of molecules out of the cell, known currently as multidrug-resistant pumps. Expression analysis of phospho-glycoproteins in mice and humans indicates widespread distribution in a number of organs, such as brain and testis. We have analyzed skin, and more particularly keratinocytes, to determine whether they express phospho-glycoproteins and express the multidrug-resistant phenotype. Immunofluorescent staining of skin showed that keratinocytes located in the basal layer of the epidermis preferentially expressed phospho-glycoproteins, as did the outer root sheath cells of hair follicles. Phospho-glycoprotein expression on the basal cells was restricted to the cell surface. Polymerase chain reaction analysis of first strand cDNA from keratinocytes identified the phospho-glycoproteins to be *mdr*1b. Using β1 integrin expression and density gradient centrifugation we were able to enrich and identify the basal cell compartment by flow cytometric analysis and assay this subset of cells for phospho-glycoprotein activity. Basal cells loaded with rhodamine 123, a substrate for multidrug-resistant pumps, effluxed the molecule from the cells in a time-dependent manner. This study shows that basal layer keratinocytes express functional phospho-glycoproteins. We speculate that phospho-glycoproteins may play a role in regulating the level of environmental toxins and differentiation factors, as has been suggested for other progenitor cell compartments.

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A major cause of failure in cancer chemotherapy is the development of drug-resistant tumor cells during treatment. Tumor cells may be resistant or become resistant to a broad range of chemotherapeutic agents. This phenomenon is known as multidrug resistance (mdr) and the mechanisms that underlie it are now beginning to be understood. A comparative study of drug-resistant and drug-sensitive cell lines revealed that drug-resistant cell lines expressed elevated levels of phospho-glycoproteins (p-gps) in the cell membrane (Juliano and Ling, 1976; Riordan and Ling, 1979). These p-gps have subsequently been shown to reduce the intracellular accumulation of a number of diverse agents, ranging from drugs and ions to peptides, by acting as an efflux pump (reviewed by Gottesman and Pastan, 1993). The main gene responsible for drug resistance in man encodes for a 170 kDa p-gp and is known as *MDR*1. Subsequent research has shown that in both rodents and humans there is a family of *MDR* genes that are members of the ATP-binding cassette superfamily. In the mouse they comprise *mdr*1a, *mdr*1b, and *mdr*2;[1] however, only *mdr*1a and *mdr*1b gene products have the mdr properties (Devault and Gros, 1990).

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Abbreviations: mdr, multidrug resistance; p-gp, phospho-glycoprotein; Rh123, rhodamine 123.

[1]Nomenclature of Ruetz and Gros (1994).

Although a majority of the research has focused on clinical aspects of p-gps, they are also expressed in noncancerous tissues. Expression has been identified in adrenal cortical cells, the brush border of renal proximal tubule epithelium, secretory epithelia of the uterus, biliary hepatocytes, intestinal mucosal cells, pancreatic ductules, endothelia of the brain and testis, placenta, peripheral lymphocytes, and CD34 positive hematopoietic progenitor cells (Fojo *et al*, 1987; Thiebaut *et al*, 1987, 1989; Arceci *et al*, 1988; Sugawara *et al*, 1988; Cordon-Cardo *et al*, 1989; Croop *et al*, 1989; Bradley *et al*, 1990; Chaudhary and Roninson, 1991; Chaudhary *et al*, 1992). Their role in these tissues is unclear, especially as single and double knockout (*mdr*1a-/–, and *mdr*1a and 1b-/–) mice have what appears to be a normal phenotype (Schinkel *et al*, 1994, 1997). Nevertheless, it has been suggested that they protect cells from potentially lethal or mutagenic toxins entering from the environment or formed as metabolic breakdown products (Baldini, 1997). Therefore it is not surprising that organs containing progenitor cells such as testis and intestine, and progenitor cells themselves such as the CD34 positive hemopoietic stem cells, are rich in these proteins, as these are the most important cells within the body to protect from a carcinogenic insult. Based on the tissue distribution it is apparent that epithelial tissues are well represented, leading us to investigate p-gp expression in skin. Like the intestine, the skin is constantly replenished by a population of stem cells. Skin stem cells are located in the basal layer of the epidermis and are thought to account for between 1% and 10% of basal keratinocytes (Withers, 1967; Potten and Hendry, 1973). Stem cells give rise to a population of keratinocytes known as transit amplifiers, which

undergo rapid proliferation before differentiating and migrating into the suprabasal layers of the skin. One of the best means of identifying the basal layer is to stain for β1 integrin expression. From immunohistochemical analysis it has been shown that as keratinocytes leave the basal layer they downregulate the cell surface expression of β1 integrin (reviewed by Watt and Hertle, 1994). As the basal layer contains the skin's progenitor cells, we investigated this region to determine whether it contains a functional multidrug-resistant pump.

In this paper we demonstrate that basal keratinocytes express multidrug-resistant p-gps, the *mdr*1b gene, and have the ability to expel the mdr substrate rhodamine 123 (Rh123). Furthermore, we show that mdr-driven efflux of Rh123 can be blocked in these cells by verapamil, a known mdr antagonist (Cornwell *et al*, 1986, 1987). We suggest that this novel keratinocyte phenotype may play a role in protecting the epidermis from environmental toxins.

MATERIALS AND METHODS

Animal husbandry 1–2-d-old neonatal BALB/cJ mice used for all experiments were bred by timed matings from a colony at Genesis Research and Development Corporation, Auckland, New Zealand.

Immunohistochemistry BALB/cJ neonatal mouse pelts were embedded in OCT (Tissue Tek) and frozen over liquid nitrogen. Five micron sections were cut and stored at –70°C. Prior to staining, sections were thawed and air dried for 30 min. Sections were fixed in acetone for 30 s. Staining of tissue was as follows: acetone fixed sections were washed in phosphate-buffered saline (PBS) and then incubated in 1% rabbit sera for 15 min at room temperature. After PBS washing, the sections were incubated with 10 μg per ml goat anti-mdr (C-19, Santa Cruz Biotechnology) or 10 μg per ml goat IgG (Southern Biotechnology) as negative control for 1 h at room temperature. Sections were washed in PBS and incubated with 10 μg per ml rabbit anti-goat IgG-fluorescein isothiocyanate (FITC) (Sigma) as above. Sections were then washed in PBS, stained for 10 min in 100 nM Hoescht 33342 dye, and mounted with Citifluor (Agar Scientific). Photomicrography was performed on a Zeiss Axioscop under epifluorescent illumination and images were prepared using Adobe Photoshop.

Isolation of epidermal cells Epidermal cells were collected from pelts of 1–2 d postpartum BALB/cJ mice. Pelts were washed in PBS before incubation in 0.125% trypsin, 0.02% ethylenediamine tetraacetic acid (Life Technologies) overnight at 4°C. The epidermis was separated from the dermis and the trypsin was blocked with 2% fetal bovine serum and Dulbecco's minimal essential medium (D-MEM) supplemented with 2 mM l-glutamine (Sigma), 1 mM sodium pyruvate (Life Technologies), 0.77 mM L-asparagine (Sigma), 0.2 mM L-arginine (Sigma), 160 mM penicillin G (Sigma), and 70 mM dihydrostreptomycin sulfate (Boehringer Mannheim). The epidermal sheets were then briefly vortexed to generate a single-cell suspension, sieved through a 70 μm nylon mesh, and pelleted at 200 × *g* for 5 min. The cells were resuspended in medium, applied to a 1.05 g per ml Percoll density gradient (Pharmacia), and centrifuged at 4°C for 60 min at 400 × *g* with no brake. Cells with a density greater than 1.05 g per ml were collected, washed, resuspended in medium, counted, and used for analysis.

Polymerase chain reaction (PCR) with first strand cDNA from isolated epidermal cells Total RNA was isolated from approximately 1×10^7 epidermal cells using Trizol Reagent (Life Technologies) as described in the manufacturer's protocol. Epidermal cell total RNA (1 μg) was then converted to first strand cDNA using 5 U Superscript II reverse transcriptase (Life Technologies) with 10 mM dNTPs, 100 mM dithiothreitol, 1 × reverse transcriptase buffer, and 1 μM oligo dT primer to a final volume of 20 μl. Reactions were mixed and incubated for 60 min at 42°C. The final volume was made up to 100 μl and a single microliter was used per PCR. First round PCRs were performed with epidermal cDNA using 1 unit of *Taq* polymerase (Qiagen), 0.1 mM dNTPs (Life Technology), 10 × PCR buffer (Qiagen), 1.5 mM $MgCl_2$, and the following oligonucleotides: *mdr*1a forward primer 5′ CCAGCA-GTCAGTGTGCTTAC 3′, *mdr*1a reverse primer 5′ GTTAGCTTC-CAGCCACGGG 3′, *mdr*1b forward primer 5′GGCTGGACAAGCT-GTGCATG 3′, *mdr*1b reverse primer 5′GACAAGGGTTAGCTTC-CAACC 3′, *mdr*2 forward primer 5′CATGGATCAGGTCTTCCC-CTC 3′, and *mdr*2 reverse primer 5′GAGAGCCCCAGGATGG-GGCTG 3′, at a concentration of 1 μM, in a final reaction volume of 20 μl. The PCR cycling parameters for first round and nested reactions were 94°C (2 min) for one cycle; 94°C (1 min), 64°C (30 s), 72°C (30 s) for 10 cycles; 94°C (1 min), 62°C (30 s), 72°C (30 s) for two cycles; 94°C (1 min), 60°C (30 s), 72°C (30 s) for two cycles; 94°C (1 min), 58°C (30 s), 72°C (30 s) for two cycles; 94°C (1 min), 56°C (30 s), 72°C (30 s) for two cycles; 94°C (1 min), 54°C (30 s), 72°C (30 s) for two cycles; 94°C (1 min), 52°C (30 s), 72°C (30 s) for two cycles; 94°C (1 min), 50°C (30 s), 72°C (30 s) for two cycles; 94°C (1 min), 48°C (30 s), 72°C (30 s) for two cycles; followed by 94°C (1 min), 46°C (30 s), 72°C (30 s) for 15 cycles. PCR products were resolved routinely on a 1.2% agarose gel. First round PCR products were diluted 1:50 and a microliter was used as a template in a second round PCR as previously described using the following nested 5′ oligonucleotides: *mdr*1a nested primer 5′ GAGCCATGTTTGCC-AAACTGG 3′, *mdr*1b nested primer 5′GTTGCCTACATCCAG-GTTT-C 3′, and *mdr*2 nested primer 5′CTGGACTTTGGCAG-CTGGCCG 3′, with their respective reverse primers from the first round reaction. The degenerate actin primers 5′ TAGAAGC-$A^{C}/_{T}{}^{C}/_{T}$TCC$^{G}/_{T}$-GTG$^{G}/_{c}$AC$^{A}/_{G}$A$^{G}/_{T}$G 3′ and 5′ TGACGGGGTCA-CCCACACTGT-GCCCATCTA 3′ were used as positive controls for the quality of first strand cDNA. The PCR cycling for the actin primers was as follows: 94°C (2 min) for one cycle, 94°C (1 min), 58°C (1 min), 72°C (1 min) for 25 cycles.

Subcloning and sequencing products from PCRs Second round PCR products were desalted using PCR purification columns (Qiagen) and quantified on a 1.2% agarose/1 × TAE gel with 4 μl of low mass ladder (Life Technologies). Approximately 200 ng of second round product was ligated into 50 ng pGEM-T-tailed vector (Promega) using 3 units of T4 DNA ligase (Promega), 2 μl of 5 × ligase buffer to a final reaction volume of 10 μl and incubated overnight at 16°C. Ligation reaction was transformed into XL1 Blue MRF′ competent cells (Stratagene) plated on LB agar plates supplemented with 100 μg per ml ampicillin spread with IPTG and X-Gal. Plates were grown overnight at 37°C. Positive colonies were used to inoculate 3 ml of LB with 100 μg per ml ampicillin and grown overnight at 37°C at 220 rpm. Plasmids were isolated by alkali lysis PEG precipitation and sequenced in both directions using BIG dye terminator chemistry (Perkin Elmer). Sequences were analyzed using BLASTX searches (Altschul *et al*, 1997) of the Swissprot-TrEMBL database (Version 37).

Flow cytometric analysis of murine epidermal cells with anti-β1 integrin Isolated murine epidermal cells were stained with an antibody to β1 integrin (Pharmingen, clone 9EG7) as described. Epidermal cell suspensions were washed in staining buffer [2% fetal bovine serum (FBS), 0.2% sodium azide in PBS], pelleted, and labeled with 10 μg per ml anti-β1 integrin for 30 min on ice. Cells were then given a second wash, pelleted, and stained with 10 μg per ml of secondary antibody, goat anti-rat IgG_{2a} FITC. To determine viability cells were then labeled with 10 μg per ml propidium iodide (Sigma). As a negative control cells were labeled with the isotype rat IgG_{2a}. Positive β1 integrin fluorescence was measured on a log scale using an FITC filter arrangement with peak transmittance at 530 nm and a bandwidth of 10 nm on an Elite cell sorter (Coulter Cytometry).

Rh123 staining and verapamil blocking of isolated epidermal cells Isolated epidermal cells were stained with Rh123 as described by Bertoncello *et al* (1985). Briefly, a single-cell suspension was stained with 0.1 μg per ml Rh123 (Sigma) per 1×10^6 cells per ml for 30 min at 37°C in the dark in 2% FBS and D-MEM or 2% chelex-treated FBS and D-MEM. Cells were then washed twice in 2% FBS and D-MEM or the chelex-treated equivalent, resuspended in the same medium to a concentration of 1×10^6 cells per ml, and incubated at 37°C for 0–120 min to allow the p-gp positive cells to expel the dye. Cells were stained with 10 μg per ml propidium iodide (Sigma) in order to exclude any dead cells. The cells were then analyzed for Rh123 staining every 30 min. Rh123 fluorescence was measured on an integral scale using an FITC filter arrangement with peak transmittance at 530 nm and a bandwidth of 10 nM on an Elite cell sorter (Coulter Cytometry). To assess the effect of verapamil (Sigma) on the levels of Rh123 retention, cells were stained for 30 min at 37°C in the presence of a range of verapamil concentrations from 50 μM to 200 μM, washed as before, and then allowed to incubate for a further 120 min again in the presence of verapamil.

RESULTS

Immunohistochemistry with anti-p-gp To determine whether p-gps were expressed in the epidermis we stained neonatal mouse skin with a polyclonal antibody raised to an intracytoplasmic epitope shared by all three p-gps. These

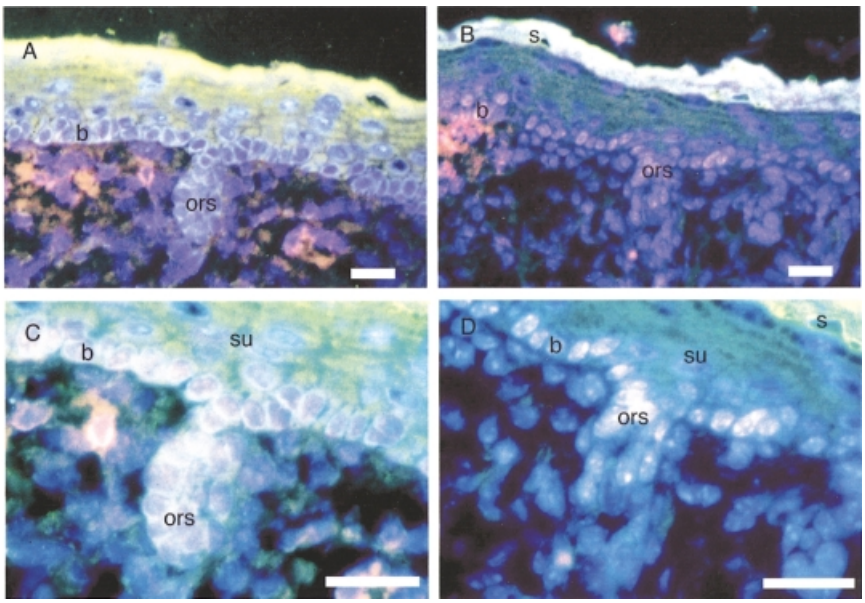

Figure 1. P-gps in neonatal murine skin are present on the surface of basal keratinocytes and outer root sheath cells of hair follicles. Cryostat sections were stained by standard immunohistochemical techniques. (*A*) Expression occurs throughout the epidermis and the outer root sheath cells of hair follicles, with (*B*) the corresponding negative control. At higher magnification (*C*), p-gp expression occurs on the cell membranes whereas in the differentiated layers label is within the cytoplasm. Nonspecific labeling occurs in the squamous layer (*B*, *D*). Sections were counterstained with Hoescht 33342 dye: basal layer (b), outer root sheath (ors), suprabasal layer (su), and squamous layer (s). (*Scale bar* = 40 μm.)

experiments revealed that p-gps were expressed by basal keratinocytes and the outer root sheath cells of the pelage hair follicles (**Fig 1*A***, ***C***). Positive label could also be detected in all layers of the epidermis. Expression was continuous throughout the basal layer with no obvious differences in expression levels between cells. At higher magnification, p-gp was uniformly expressed on all surfaces of the basal cell membranes (**Fig 1*C***). In the epidermis, however, above the basal layer, p-gp was also detected within the cytoplasm of the differentiating cells. Nonspecific labeling, with the control goat IgG, was only evident in the squamous layer (**Fig 1*B***, ***D***).

Mdr gene expression on isolated epidermal cells Immunohistochemistry showed that epidermal cells expressed a p-gp. To determine which protein was being expressed first strand cDNA was prepared from freshly isolated epidermal cells and used in a PCR with specific primers to murine *mdr*1a, *mdr*2, and *mdr*1b. Positive bands were identified for each primer pair in the first round, with only *mdr*1b generating a band of the predicted size of 518 bp (data not shown). To confirm specificity a nested PCR was performed on the products from the first round of PCR. From the nested PCR a single band of 270 bp was obtained for *mdr*1b (**Fig 2*A***) with a nonspecific smear for *mdr*1a and no product for *mdr*2. The nested *mdr*1b product was subcloned and sequenced. Sequence results confirmed that the nested product was murine *mdr*1b (**Fig 2*B***).

β1 integrin expression on isolated epidermal cells Immunohistochemistry and PCR demonstrated that the epidermis expresses the p-gp encoded by *mdr*1b. Immunohistochemistry also showed that p-gp was preferentially expressed on the surface of basal keratinocytes and the outer root sheath cells. We enriched for basal keratinocytes by centrifugation over a density gradient as previously described (Brysk *et al*, 1981; Goldenhersh *et al*, 1982; Sasai *et al*, 1984; Gross *et al*, 1987; Morris *et al*, 1990) and assessed their viability by propidium iodide staining. The purity of the basal keratinocytes was determined by staining with an antibody to β_1 integrin as it has been shown that basal keratinocytes preferentially express this adhesion molecule (Watt and Hertle, 1994; Jones *et al*, 1995). Analysis of cell size and granularity was determined using the forward scatter *versus* side scatter histogram. The majority of cells resided in the region R1 (**Fig 3*A***), which accounted for 80% of the viable cells. Cells gated on region R1 on the forward-side scatter histogram revealed that all viable cells were β1 integrin positive (**Fig 3*B***), demonstrating that this region contained the basal cell population. Rat IgG_{2a}, used as a negative control, demonstrated no non-specific binding (**Fig 3*B***). The cells in region R1 were analyzed in all subsequent flow cytometric analysis.

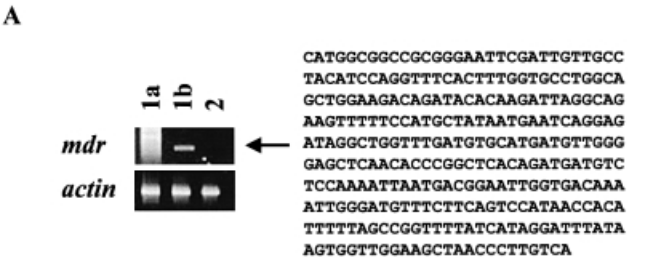

Figure 2. P-gps in neonatal murine skin are encoded by the multidrug-resistant gene *mdr*1b. (*A*) Agarose gel showing the nested PCRs with mdr specific primers. Nested PCRs were used to confirm the identity of first round products. A single product, with the expected size of 270 bp, was amplified using *mdr*1b nested primers (*lane 1b*) but not for *mdr*1a (*lane 1a*) and *mdr*2 (*lane 2*) primers. Sequence results from the *mdr*1b specific band are as shown. Actin PCRs were used as controls to confirm the quality of the first round cDNA in each reaction. (*B*) Analysis of two sequence alignment with the sequence from the nested product (*lane 1b*) with mouse *mdr*1b confirms that neonatal murine epidermal cells express *mdr*1b.

Efflux of Rh123 by murine epidermal basal cells We tested whether the p-gp detected on the surface of basal epidermal cells was active by monitoring levels of the mdr substrate, Rh123, retained within basal cells over a 120 min period. **Figure 4**(***A***) illustrates the typical staining profile of murine epidermal cells loaded with Rh123 and allowed to "pump" for 30 min at 37°C in the dark. **Figure 4**(***B***) shows that the isolated epidermal cells stained with Rh123 expel the dye progressively over the period analyzed. Samples were run in duplicate with 20,000 events collected and the mean channel fluorescence was calculated. The mean channel

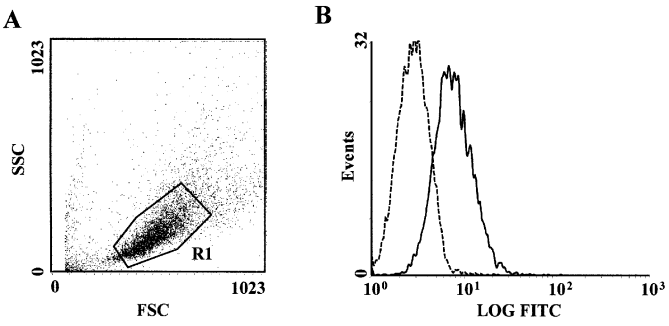

Figure 3. Murine epidermal basal cells reside in region R1 as determined by staining with β1 integrin. (*A*) Dual parameter histogram showing the forward and side scatter properties of neonatal murine epidermal cells after centrifugation over a 1.05 g per ml density gradient. (*B*) Analysis of epidermal cells gated on region R1 shows that the cells are β1 integrin positive. Rat IgG_{2a} negative control (- - -), β1 integrin (—).

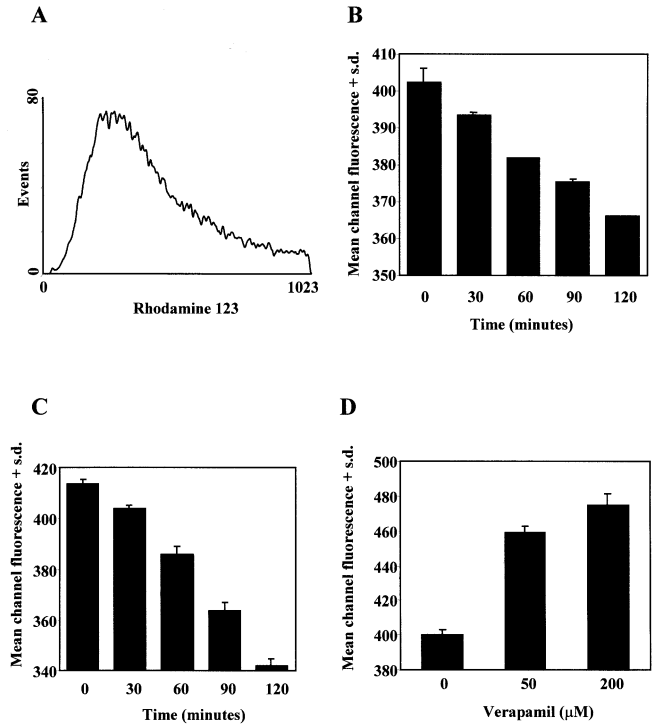

Figure 4. Murine epidermal cells loaded with Rh123, an mdr substrate, pump out the dye with time, whereas verapamil, the p-gp antagonist, inhibits Rh123 pumping in murine epidermal basal cells. (*A*) Staining profile of epidermal basal cells incubated with the p-gp substrate Rh123 and incubated for 30 min in Rh123-free medium. (*B*) Cells stained with Rh123 in the presence of serum and then incubated for up to 120 min in Rh123-free medium at 37°C had progressively decreasing Rh123 levels. (*C*) Cells stained with Rh123 in the presence of chelex-treated serum and then incubated for up to 120 min in Rh123-free medium at 37°C demonstrated lower levels of Rh123 retention compared with normal serum. (*D*) Murine epidermal basal cells were loaded simultaneously with Rh123 and verapamil. Rh123-stained cells incubated in increasing concentrations of verapamil showed a marked retention of Rh123. Fluorescence of Rh123 retention was measured in arbitary units on an integral scale at 530 nm from 20,000 gated events and the mean channel fluorescence was calculated. Propidium iodide was used to identify and gate out dead cells. Assay was repeated in duplicate and is representative of three different experiments.

fluorescence dropped linearly by 37 channels over 120 min from 403 to 366. As serum causes differentiation of murine keratinocytes in suspension (Li *et al*, 1996) we repeated the "pumping" experiment in the presence of chelex-treated serum. **Figure 4(*C*)** demonstrates that isolated epidermal cells pump out Rh123 over the period analyzed as described above. The degree of pumping increased with the mean channel fluorescence, however, dropping by 71 channels over the same time period, from 413 to 342. We confirmed that the efflux of Rh123 was due to the actions of an mdr pump by using verapamil, a known mdr antagonist. Murine basal cells loaded with Rh123 were incubated with increasing concentrations of verapamil. **Figure 4(*D*)** shows that the efflux of Rh123 was effectively inhibited by 50 μM verapamil. Furthermore, inhibition by verapamil increased with increasing concentration from 50 μM to 200 μM.

DISCUSSION

In this study, several observations lead to the first demonstration of p-gp expression and activity in skin epidermal cells. Immunofluorescent staining of skin sections revealed, first, that keratinocytes in the basal layer express mdr proteins on their cell surface, and second, that freshly isolated epidermal cells express the *mdr*1b gene. Third, when murine epidermal cells were incubated with the mdr substrate Rh123, the cells were shown to efflux or "pump" out the Rh123 over time. Finally, this efflux was inhibited by the mdr antagonist verapamil.

From the immunohistochemical data we showed that p-gp is preferentially, but not exclusively, expressed by the basal layer keratinocytes. The p-gp appears to be predominantly expressed on the cell surface of the basal keratinocytes, whereas the majority of the p-gp is located in the cytoplasm of the suprabasal keratinocytes. The reason for this change in the expression profile of these differentiating cells is unclear. It is interesting, however, that we could enhance the biologic activity of Rh123 extrusion in the suspension cells if the assay was carried out in the presence of chelex-treated serum rather than non-treated serum. Serum is a known potentiator of differentiation in suspension keratinocytes (Li *et al*, 1996); furthermore this differentiation could be inhibited if cells were grown in chelex-treated serum (Hennings, 1994). Therefore, although we have not shown a direct link between differentiation and mdr levels, these data suggest a potential link between p-gp function and differentiation. Due to the cross-reactivity of the antibody used in the immunohistochemistry, we could not identify which of the three p-gps is expressed. The PCR analysis, however, revealed that epidermal cells express *mdr*1b. Devault and Gros (1990) showed that only *mdr*1a and *mdr*1b genes, and not *mdr*2, conferred mdr in transfected cells. Our Rh123 and verapamil blocking experiments (**Fig 4**) provide functional evidence that the drug-pumping *mdr*1b is expressed in skin.

In this paper we demonstrate for the first time that multidrug-resistant properties are associated with the epidermis. It has been shown, however, that other epithelial tissues such as the small intestine express p-gp (Fojo *et al*, 1987; Thiebaut *et al*, 1987). The function of p-gp in these tissues is unclear, however. P-gps are members of the ATP-binding cassette family that are expressed in a wide range of eukaryotes from mammals to insects (Wu *et al*, 1991) and plants (Dudler and Hertig, 1992). In *Drosophila*, the gene has been implicated in conferring resistance to colchicine in larval development, whereas in mice the disruption of the *mdr*1a p-gp leads to an increase in the permeability of mdr-expressing tissues to drugs such as vinblastine (Schinkel *et al*, 1994). It has been suggested that one of the roles of p-gp is to expel toxic substances, such as drugs, from cells, and that this function has been conserved throughout evolution (Gottesman and Pastan, 1993). As keratinocytes provide a barrier to a variety of environmental toxins, it is likely that p-gps expressed in skin play a similar role.

Mdr has also been defined as a marker of hematopoietic progenitor cells. A number of groups (Bertoncello *et al*, 1985; Mulder and Visser, 1987; Visser and de Vries, 1988; Spangrude and Johnson, 1990; Udomskdi *et al*, 1991; Baum *et al*, 1992; Li and Johnson, 1992; Srour *et al*, 1993; Uchida *et al*, 1996) have shown that $Rh123^{dull}$ cells, in both mouse and human bone marrow, contain hematopoietic stem cells that have the ability to reconstitute the immune system. The function of high levels of

p-gp on these hematopoietic stem cells is unclear but has been implicated as a protection mechanism for these progenitor cells, preventing accumulation of toxins or as means of reducing the levels of differentiation factors in the cell (Chaudhary and Roninson, 1991). P-gps may play a similar role in basal keratinocytes as the epidermal progenitors reside within this population. Furthermore, as epidermal cells are prone to differentiate under the influence of a number of exogenous molecules, such as calcium (Li *et al*, 1996), a means of controlling this would be of critical importance. The concept of p-gp having an important role for the epidermis is supported by the observation that epidermal growth factor can increase p-gp expression in hepatocytes (Hirsch-Ernst *et al*, 1995) and that epidermal growth factor receptor is expressed predominantly in the basal layer (Nanney *et al*, 1984). Direct co-expression of epidermal growth factor receptor and p-gp has previously been demonstrated in breast epithelial tissue (Scala *et al*, 1995). This might be suggestive of epidermal growth factor and p-gp working in synergy to maintain and protect the proliferative basal layer prior to initiation of differentiation.

In this study we have clearly demonstrated that basal epidermal cells from murine neonatal skin express p-gps with mdr activity. The identification of this phenotype in skin adds another important biologic property to this complex organ.

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