0022-202X/85/8504-0299$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 85:299–303, 1985

Vol. 85, No. 4
Printed in U.S.A.

Purification and In Vitro Growth of Human Epidermal Basal Keratinocytes Using a Monoclonal Antibody

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We have made a new monoclonal antibody, EL-2, and used it with an immunorosetting procedure combined with Ficoll-Hypaque gradient centrifugation to purify and culture basal keratinocytes. Immunofluorescence of cell suspensions and immunoperoxidase staining of tissue sections demonstrate that EL-2 reacts with malignant cell lines, activated lymphocytes and monocytes, and basal keratinocytes. Sequential immunoprecipitation studies demonstrate that monoclonal antibodies EL-2 and 4F2 detect the same membrane protein. However, we have extended previous studies by making the new observation that both EL-2 and 4F2 react with cultured melanocytes.

Basal keratinocytes were purified from single-cell epidermal suspensions by incubation with EL-2 followed by rosetting with rabbit antimouse IgG antibodies covalently linked to bovine red blood cells. Rosetting (basal) keratinocytes were separated from EL-2 negative cells by Ficoll gradient centrifugation. We obtained basal keratinocyte populations of >90% purity as assessed by reactivity with EL-2 and another basal keratinocyte-specific monoclonal antibody, HCl. Langerhans cell, fibroblast, and melanocyte contamination was negligible. Cultures of basal keratinocytes were enriched in EL-2-reactive cells throughout the entire 19 days of culture studied.

EL-2 is being used to characterize disorders of keratinocyte proliferation; EL-2 reacted with both squamous and basal cell carcinomas. EL-2 stained only the basal layer of lesional skin from patients with psoriasis, pityriasis rubra pilaris, and Darier's disease.

Purification of basal keratinocytes will be important in biochemical and functional studies of normal skin and in establishing long-term keratinocyte lines from normal cells.

Studies of epidermal basal keratinocytes would be enhanced by the availability of suitable antibodies that could facilitate basal cell separation. Morhenn et al [1] have reported a panning technique for isolating purified basal keratinocytes with two basal cell-specific monoclonal antibodies, VM-1 and VM-2. However, panning with VM-1 inhibited subsequent in vitro culture of these cells and, although the authors demonstrated that VM-2-enriched cells could be grown in vitro, only 48-h cultures were studied. Other studies have used Percoll gradient centrifugation [2,3] velocity sedimentation [4], and flow cytometry technology [5] to isolate keratinocyte subpopulations. We and others [6–8] have described monoclonal antibodies which detect surface membrane antigens shared by subpopulations of lymphocytes and epidermal basal keratinocytes. In this report, one such monoclonal antibody, specific for basal keratinocytes, is used to separate pure populations of basal cells from human epidermis using a rosetting technique. Our subsequent experience in successfully culturing these cells will also be described.

MATERIALS AND METHODS

Cell Lines

Human cell lines were grown in RPM1-10% fetal calf serum (FCS) plus penicillin-streptomycin (100 U/ml) and glutamine (2 mM) in 5% CO_2 at 37°C. BOL4 was a human T-T hybridoma obtained by fusion of 6-day human tetanus toxoid blasts [9] with the hypoxanthine guanine phosphoribosyl transferase-deficient T-cell line KE37 [10]. Human melanocytes were cultured from neonatal foreskin using the method of Eisinger and Marko [11] and Horikoshi et al [12].

Generation of Monoclonal Antibodies

Production of monoclonal antibodies was performed as follows [13]: Briefly, 20 million BOL4 cells in alum were injected i.p. into BALB/c mice (The Jackson Laboratories, Bar Harbor, Maine) at days 1, 21, and 29. Three days later, mice were sacrificed, spleen cells harvested and fused with 10 million SP2/0 mouse myeloma cells/spleen using the technique of Kennett et al [14]. Hybridomas of interest were cloned in soft agar.

Purification of Monoclonal Antibody

BALB/c mice were primed by i.p. injection of 0.5 cc pristane (Aldrich Chemical Company, Inc., Milwaukee, Wisconsin) 10–21 days prior to i.p. injection of 10^6 growing murine hybridoma cells. Purified monoclonal antibody was isolated from ascitic fluid by 45% ammonium sulfate precipitation. Subclass determination was performed by Ouchterlony analysis, using rabbit antimouse immunoglobulin subclass-specific antisera purchased from Miles Biochemicals (Elkhart, Indiana).

Screening of Hybridoma Supernatants

Normal hematologic cells, malignant cell lines, cultured keratinocytes, melanocytes, and fibroblasts were screened by indirect immunofluorescence [15]. Tetra-methyl-rhodamine isothiocyanate-conjugated Fab'_2 fragments of a goat antimouse IgG antiserum were purchased from Tago Inc. (Burlingame, California). For each data point, at least 200 cells were counted using a Leitz Ortholux II fluorescence microscope (Leitz, Wetzler, Germany). Keratinocytes, melanocytes, and fibroblasts were cultured on glass coverslips and fixed in phosphate-buffered formalin pH 7.0 prior to staining. Immunopreoxidase staining of 6 μm-thick acetone-fixed frozen tissue sections was performed using 3-amino-9 ethylcarbazole (Sigma Chemical Co., St. Louis, Missouri) [6,7]. Skin biopsies were taken after informed consent was obtained in accordance with procedures approved by the Rockefeller University Institutional Review Board.

Manuscript received January 16, 1985; accepted for publication May 7, 1985.

PHS Grant No. AI-19080 (ABG). Dr. Gottlieb is a recipient of a John A. and George L. Hartford Fellowship Award. The work was also partly supported by a grant from the National Institutes of Health to The Rockefeller University General Clinical Research Center.

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Abbreviations:
bRBC: bovine red blood cells
E_N: neuraminidase-treated sheep red blood cells
FCS: fetal calf serum
KCM: keratinocyte culture medium
PBMC: peripheral blood mononuclear cells

Isolation of Cell Populations

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll-Hypaque gradient centrifugation. T cells were isolated by rosetting with neuraminidase-treated sheep red blood cells (E_N). In certain experiments, T cells were depleted by rosetting with E_N followed by depletion of monocytes with carbonyl iron [15]. Monocytes were isolated by adherence of PBMC onto glass coverslips for 45 min at 37°C. Granulocytes were purified by sedimentation in Dextran T500 (Pharmacia Fine Chemicals Inc., Piscataway, New Jersey) [7].

For certain experiments, T cell-depleted PBMC were incubated in plastic flasks at 2×10^6 cells/ml in RPMI 1640–10% FCS at 37°C for 16 h. The nonadherent fraction was then further depleted of monocytes with carbonyl iron. This population was further fractionated into high- and low-density fractions as described by Crow and Kunkel [16] as follows: 20 million cells were suspended in 100% Percoll (Pharmacia Fine Chemicals). Two milliliters of 50% Percoll followed by 2 ml of 30% Percoll were layered onto the 100% Percoll. The tube was centrifuged at 2300 rpm for 12 min. The high-density cells, enriched in resting B lymphocytes, were those obtained from the 50%/100% Percoll interphase, and the low-density cells, enriched in dendritic cells and in vivo-activated B lymphocytes, were obtained from the 30%/50% Percoll interphase [16].

Isolation of Keratinocyte Subpopulations

Single-cell suspensions of human keratinocytes were obtained from cadaver skin in cooperation with Dr. Daniel Alonso and Dr. C. Richard Minick of The New York Hospital, Department of Pathology as follows. Briefly, epidermis was separated from dermis using a Goulian keratome (Edward Weck and Co. Inc., Research Triangle Park, North Carolina) followed by incubation with 0.5% trypsin 1:250 (Difco, Detroit, Michigan) for 90 min at 37°C. Epidermal sheets were lifted off the dermis. Single-cell suspensions were obtained by agitation with a glass rod in a solution of deoxyribonuclease I from bovine pancreas (Sigma Chemical Co.) at a concentration of 25 μg/ml and separated from other tissue material by filtration through gauze. Cell populations were greater than 95% viable by trypan blue dye exclusion and typically contained less than 1% Langerhans cells, melanocytes, and fibroblasts as determined by OKT6 reactivity, dopa, and leucine aminopeptidase histochemical staining, respectively [17].

Isolation of basal keratinocytes was performed using a modified rosetting technique. Single-cell suspensions of keratinocytes were incubated overnight at 4°C in keratinocyte culture medium (KCM), which consisted of minimum essential medium with Earle's salts plus L-glutamine and 20% FCS plus hydrocortisone 0.5 μg/ml, penicillin (100 U/ml), streptomycin (100 μg/ml), and Fungizone (0.25 μg/ml) (Gibco Laboratories, Grand Island, New York). EL-2 reactivity was diminished by 50% immediately after trypsinization. Therefore, 20 million unseparated keratinocytes were preincubated overnight at 4°C prior to incubation with 0.2 ml undiluted cloned hybridoma EL-2 supernatant for 1 h at 4°C followed by 4 washes and resuspension in 0.5 ml KCM. Affinity-purified rabbit antimouse IgG antibodies were covalently attached to bovine red blood cells (bRBC) as previously described [15]. A 2% suspension of antibody-coated bRBC (0.5 ml) was added to the keratinocytes and the mixture pelleted for 10 min at 4°C and 1000 rpm followed by incubation for 1 h on ice. The pellet was gently resuspended and subjected to Ficoll-Hypaque gradient centrifugation. The interphase was enriched for antibody-nonreactive keratinocytes, and the pellet was enriched for EL-2-reactive (basal) keratinocytes. bRBC were lysed with 0.83% NH_4Cl, and the remaining keratinocytes were washed in phosphate-buffered saline. In order to assess purity, isolated subpopulations were tested for reactivity with the basal keratinocyte-specific monoclonal antibodies, EL-2 and HC-1 [7], by indirect immunofluorescence.

Cell Cultures

For certain experiments, lymphocytes were cultured with a 1/200 dilution of pokeweed mitogen (Gibco Laboratories) [18] or tetanus toxoid 40 μg/ml [9]. Keratinocytes were cultured in KCM at 37°C in a 5% CO_2 atmosphere [17]. 2×10^6 keratinocytes were seeded per 9.6 cm^2 flask (Linbro flat-bottomed wells, Cat no. 76-058-06, McLean, Virginia). Medium was changed 3 times per week. At various times during culture, keratinocytes were trypsinized with 0.5% trypsin, counted, and incubated for 1 h on ice prior to testing for EL-2 reactivity by indirect immunofluorescence.

Membrane Immunoprecipitation Analysis

For immunoprecipitation the CEMT cell line was used. These cells are strongly positive by immunofluorescence with the monoclonal antibodies EL-2 and 4F2. Cells (50×10^6) were radioiodinated with lactoperoxidase as described elsewhere [19], and lysed in 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 0.5% NP 40, 0.02% Na azide, phenylmethylsulfonyl fluoride 18 μg/ml (Sigma Chemical Company), and iodoacetamide 100 mM at 4°C. Lysate (50 μl) was absorbed with 25 μl Protein A Sepharose CL 4B (Pharmacia Fine Chemicals) and 15 μg rabbit antimurine IgG (sham-absorption) or with Protein A Sepharose CL 4B, rabbit anti-murine IgG and 50 μg monoclonal antibody, EL-2 or 4F2. The lysate was absorbed sequentially 3 times for 3 h each at 4°C. Thereafter 50 μl lysate was used for each immunoprecipitation with 25 μl Protein A Sepharose CL 4B, 15 μg rabbit antimurine IgG and 1 of 4 murine monoclonal antibodies [EL-2, 4F2, 9A4-a monoclonal antibody reactive with β_2-microglobulin kindly provided by Dr. B. Pernis, and FFA85-a monoclonal antibody reactive with T lymphocytes, which immunoprecipitates a membrane protein of M_r 35,000 (Posnett, unpublished observations)]. The immunoprecipitation protocol used has been described [19,20]. Immunoprecipitates were analyzed on a 12.5% polyacrylamide gel under reducing conditions (dithiothreitol 0.1 M).

RESULTS

Monoclonal Antibodies EL-2 and 4F2 Detect the Same Membrane Protein

An IgG_{2a} K monoclonal antibody, EL-2, was raised by immunization with a human T-T hybridoma. Sequential immunoprecipitation and immunohistologic studies demonstrated that EL-2 detected the same membrane protein as did monoclonal antibody 4F2, described by Haynes and his colleagues [8,21]. As shown in Table I, EL-2 reacted with a ubiquitous antigen on lymphoid and nonlymphoid malignant cell lines, monocytes, and antigen- and mitogen-activated lymphocytes. In order to determine whether resting or activated B cells accounted for the staining seen in the T cell- and monocyte-depleted PBMC population, Percoll gradient centrifugation was performed. In 2 experiments, the low-density (30%/50% inter-

TABLE I. *Reactivity of EL-2 with hematologic and nonhematologic cells*

Cell type	Immunofluorescence (% positive)[a]
Malignant cell lines	
Lymphoblastoid:	
T(7)[b,c]	100
B (8)[d]	100
Nonlymphoid:	
HL60, U937, 1604, K562, A431	100
Normal	
Peripheral blood T (4)	0.5
T cell- and monocyte-depleted PBMC (3)	5
Low-density T cell- and monocyte-depleted PBMC (2)[e]	51
High-density T cell- and monocyte-depleted PBMC (2)[f]	8
Monocytes (2)	89
Granulocytes (2)	0
Red blood cells (2)	0
Five-day PWM blasts (2)	57
Six-day tetanus toxoid blasts (1)	76

[a] By indirect immunofluorescence using tetra-methyl-rhodamine-isothiocyanate-conjugated Fab'_2 fragments of a goat antimouse IgG antiserum. Numbers in this column represent the average % positive.

[b] Numbers in parentheses denote numbers of separate specimens tested.

[c] The T-cell lines tested were BOL-4, KE37, Jurkat, MOLT4, CEMT, SKW3, HUT78.

[d] The B-cell lines tested were 1899, GM4154A, CESS, 0467, 2p68, EB3, 8866, 32A1.

[e] 30–50% Percoll interphase from T cell- and monocyte-depleted PBMC.

[f] 50–100% Percoll interphase from T cell- and monocyte-depleted PBMC.

phase) fraction, enriched in activated B cells [16], was 51% reactive with EL-2. In contrast, only 8% of the high-density (50%/100% interphase) fraction, enriched in resting B cells [16], stained positively. Thus, activated B cells accounted for the staining observed in the T cell- and monocyte-depleted PBMC population.

In order to establish the identity of the antigen recognized by EL-2 to that defined by 4F2, sequential immunoprecipitation analysis was performed using lactoperoxidase-catalyzed, radioiodinated membrane extracts of the T lymphoblastoid cell line CEMT. The results are shown in Fig 1. In previous experiments both EL-2 and 4F2 immunoprecipitated a 120 kD heterodimer under nonreducing conditions from CEMT cells. Comigrating 80 and 40 kD bands were seen under reducing conditions as also shown here in lanes 3 and 4. The identity of the antigen precipitated by these two monoclonal antibodies was further demonstrated by sequential immunoabsorptions with 4F2 (lanes 5–8), EL-2 (not shown), and sham absorptions (lanes 1–4) as described in *Materials and Methods.* The control immunoprecipitates with FFA85 and anti-β_2-microglobulin (lanes 1, 2, 5, 6) are not affected by 4F2 absorption. In contrast, the antigen immunoprecipitated by EL-2 (lane 3) is completely removed by 4F2 absorption (lane 7), as is the 4F2 antigen (lane 8). In the reverse situation, the 4F2 antigen was also removed by EL-2 absorption (data not shown). Faintly visible bands were present in all lanes at molecular M_r 45,000 and 12,000. These bands comigrated with HLA heavy chain and β_2-microglobulin (lanes 2, 6) and appeared to be nonspecific. While it is clear that the protein recognized by EL-2 and 4F2 is identical, the possibility that these two antibodies may react with different epitopes of this protein cannot be ruled out by these studies.

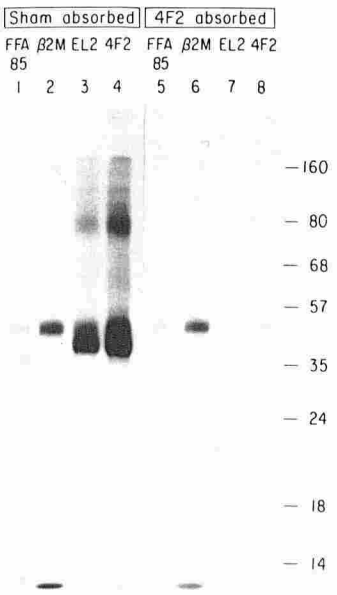

FIG 1. Monoclonal antibodies EL-2 and 4F2 immunoprecipitate the same molecule. Sodium dodecyl sulfate 12.5% polyacrylamide gel pattern of the immunoprecipitates obtained from ^{125}I-labeled CEMT cells by monoclonal antibodies. All lanes were run under reducing conditions with 0.1 M dithiothreitol: The identity of the antigen precipitated by monoclonal antibodies EL-2 and 4F2 was demonstrated by sequential immunoabsorptions with 4F2 (*lanes 5–8*) and sham absorptions (*lanes 1–4*) as described in detail in *Materials and Methods.* The control immunoprecipitates with monoclonal antibody FFA85 (*lanes 1, 5*) and a monoclonal anti-β_2-microglobulin antibody (*lanes 2, 6*) are unaffected by 4F2 absorption. In contrast, the antigen immunoprecipitated by EL-2 (*lane 3*) is completely removed by 4F2 absorption (*lane 7*) as is the 4F2 antigen (*lane 8*). Faintly visible bands are present in all lanes at M_r 45 and 12 kD. These bands comigrated with HLA heavy chain and β_2-microglobulin (*lanes 2, 6*) and appear to be nonspecific.

EL-2 Reacts with Basal Keratinocytes and Cultured Melanocytes

The reactivity of antibody EL-2 with normal human skin was tested by immunoperoxidase staining, and the results are shown in Fig 2*a*. Only the basal epidermal layer stained positively. In other experiments, this antibody reacted with basal epithelial cells in eccrine sweat glands, sebaceous glands, and hair follicles. These appendages are developmentally derived from basal epidermal cells. The membrane pattern of reactivity is clearly shown in Fig 2*b*, which illustrates the reactivity of EL-2 with an invasive squamous cell carcinoma of the squirrel monkey oral cavity. EL-2 was also reactive with tissue sections from 5 human basal cell carcinomas and the epidermoid carcinoma line, A431. This antibody is being used to characterize disorders of keratinocyte differentiation whose etiologies are as yet unknown. Lesional skin from a patient with psoriasis was tested for reactivity with EL-2, and the results are shown in Fig 2*c*. As has been reported with monoclonal antibody 4F2 [8], only the basal layer stained with EL-2. The same results (i.e., staining of the basal layer only) were obtained with lesional skin from 4 other psoriasis patients and from patients with two other disorders of keratinization, pityriasis rubra pilaris and Darier's disease (data not shown). It was impossible to determine in tissue sections whether melanocytes, which account for 1 out of every 10–12 cells in the basal layer, also were reactive. Therefore, cultured neonatal foreskin melanocytes were stained with antibody EL-2 and another basal keratinocyte-specific monoclonal antibody, EL-1. EL-1 was produced by the same fusion as EL-2. It recognizes a 105 kD membrane protein expressed by a subset of immature lymphocytes and epidermal basal keratinocytes [6]. The results are shown in Fig 3. EL-2 showed clear membrane reactivity with formalin-fixed melanocytes (Fig 3*b*). One hundred percent of the cells scored were reactive. In other experiments, the same results were obtained with antibody 4F2. In contrast, EL-1, which also reacts with basal keratinocytes, did not stain cultured melanocytes (Fig 3*d*). These results were confirmed in 2 additional experiments. In all 3 cases, rapidly growing melanocytes were tested. Both pigmented and nonpigmented melanocytes were reactive with EL-2 and 4F2.

Purification and Culture of Basal Keratinocytes

Since EL-2 reacted with a membrane antigen expressed only on basal keratinocytes, it was selected to separate epidermal basal keratinocytes, using a modified rosetting technique, from

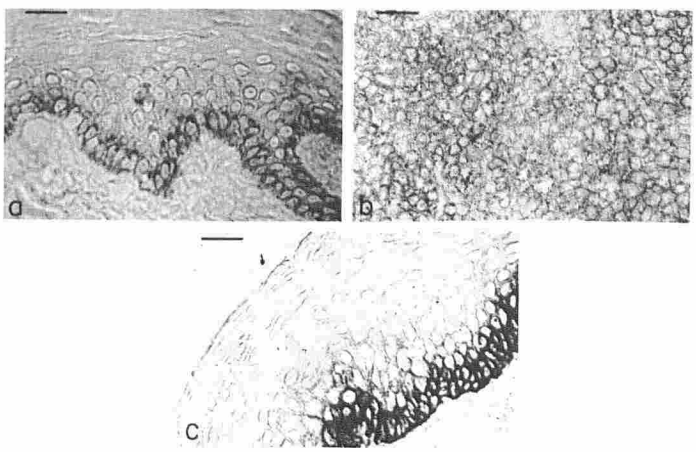

FIG 2. Immunoperoxidase staining of skin with monoclonal antibody EL-2. Three sections are shown. *Scale bars* = 10 μm. *a*, Normal human skin; only epidermal basal keratinocytes stain positively. *b*, Squirrel monkey invasive squamous cell carcinoma of the oral cavity; EL-2 demonstrates a membrane reactivity pattern. *c*, Lesional human psoriasis skin; only epidermal basal keratinocytes stain positively.

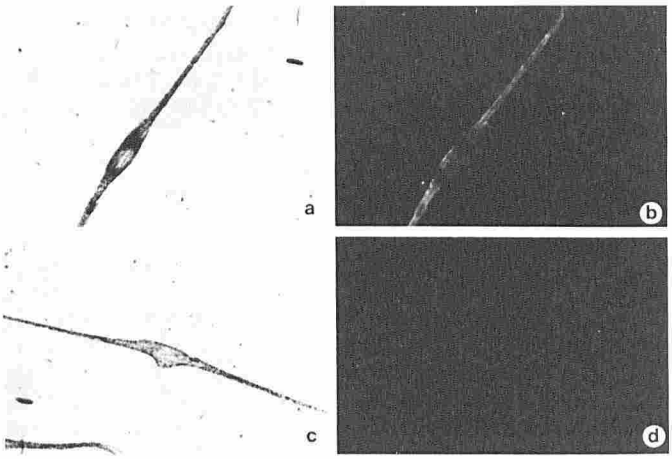

FIG 3. Fluorescent staining of cultured melanocytes by monoclonal antibodies. × 630. EL-2, *b*; EL-1, *d*; *a* and *c* show phase contrast.

TABLE II. *Purification of epidermal basal keratinocytes with EL-2*

	Keratinocyte subpopulation	Immunofluorescence (% positive)[a]		
		EL-2	HC-1	GAM[b] alone
Exp 1	Unseparated	44	42	0
	Pellet (basal cell-enriched)	96	93	0
	Interphase (basal cell-depleted)	12	6	0
Exp 2	Unseparated	34	46	0
	Pellet	91	98	0
	Interphase	0.5	11	0

[a] By indirect immunofluorescence as described in Table I.
[b] GAM = tetra-methyl-rhodamine-isothiocyanate-conjugated Fab'$_2$ fragments of a goat antimouse IgG antiserum.

single-cell suspensions of epidermal cells derived from neonatal cadaver skin. Purity of the basal cell population was assessed by reactivity of epidermal subpopulations with monoclonal antibodies EL-2 and HC-1. HC-1 detects an antigen expressed on hairy cell leukemia cells, endothelial cells, and basal keratinocytes that is clearly different from that defined by EL-2 in both its staining and biochemical characteristics. It was therefore chosen as second reagent to quantitate the number of basal cells in each cell fraction because staining with HC-1 would not be affected by the isolation procedure itself [7]. The results are shown in Table II. The average yield of basal keratinocytes was 71% of the cells subjected to rosetting, and the average viability was 81%. In both experiments, the basal keratinocyte-enriched pellet was >90% positive with the monoclonal antibodies EL-2 and HC-1. In contrast, the basal keratinocyte-depleted interphase contained <12% cells reactive with these two antibodies. The positive staining in both the pellet and interphase populations was not due to residual monoclonal antibody carried over from the rosetting procedure. No staining was observed with tetra-methyl-rhodamine-isothiocyanate-conjugated Fab'$_2$ fragments of a goat antimouse IgG antiserum alone. In addition, another IgG_{2a} K monoclonal antibody, EL-4, with specificity for an antigen found in the stratum corneum (Gottlieb, unpublished observation), did not stain. The percentage of contaminating Langerhans cells in the basal keratinocyte-enriched pellet was 3% as compared with 0.4% and none in the unseparated and interphase keratinocyte populations, respectively. There was <1% melanocyte and fibroblast contamination in all subpopulations.

In a representative experiment, an equal number of isolated basal and unseparated keratinocytes were cultured in 9.6 cm^2 wells. At days 5, 12, and 19 of culture, cells were trypsinized, counted, and tested for EL-2 reactivity by indirect immunofluorescence. The viability of these cells was 80–99% by trypan blue dye exclusion. The results are shown in Fig 4. Cultures of isolated basal cells contained a higher proportion of basal cells than did those from unseparated keratinocytes throughout the entire culture period. For both basal and unseparated keratinocytes the proportion of EL-2-positive cells peaked at day 12. These results confirm previous observations using frequency distribution histograms of the DNA content of individual cells and [^{3}H]thymidine incorporation [17]. When these same cells were stained with toluidine blue, the proportion of EL-2-positive cells correlated well with the estimated proportion of cells with high nuclear/cytoplasmic ratios. This is consistent with the fact that EL-2 stains basal keratinocytes. Surprisingly, the total number of cells obtained by trypsinization of basal and unseparated keratinocyte cultures did not differ significantly. It may be that purified basal keratinocytes have optimal culture conditions which differ from those that have been developed for unseparated keratinocytes. Studies are in progress to determine whether this is indeed the case.

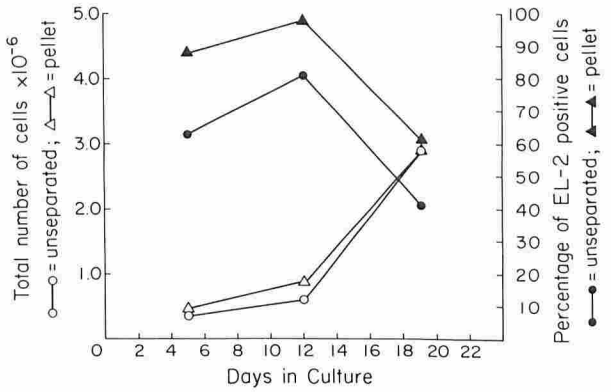

FIG 4. In vitro growth of purified basal cells as compared with unseparated keratinocytes. *Left ordinate* represents the total number of cells per culture (○——○ = unseparated cells; △——△ = basal cells); *right ordinate* represents the proportion (%) of EL-2-reactive cells as determined by indirect immunofluorescence (●——● = unseparated cells; ▲——▲ = basal cells).

DISCUSSION

In this report we have described a monoclonal antibody, EL-2, which detects the same antigen as does antibody 4F2. However, the possibility that both antibodies detect different epitopes of the same glycoprotein cannot be ruled out. We have extended previous observations by demonstrating that the reactivity of both these antibodies with the basal epidermal layer is with melanocytes in addition to keratinocytes. We are currently in the process of testing dysplastic nevi, melanomas, and melanoma cell lines for reactivity with EL-2. The demonstration of melanocyte staining by EL-2 and 4F2 is an important observation, because previous investigators had reported the epidermal reactivity of 4F2 solely with basal keratinocytes and did not investigate melanocyte reactivity [8]. As a result of our experiments, any future hypothesis for the function of the antigen defined by EL-2 (or 4F2) will have to take into account the fact that both basal keratinocytes and melanocytes are reactive.

EL-2 is being applied to the study of disorders of keratinocyte differentiation whose etiologies are as yet unknown. Basal and squamous cell carcinomas were reactive with EL-2. Tissue sections from lesional skin from patients with psoriasis, pityriasis rubra pilaris, and Darier's disease showed reactivity with only the basal layer. This is despite the fact that suprabasilar keratinocytes are labeled with [^{3}H]thymidine in lesional psoriasic skin [22]. We speculate that the antigen recognized by EL-2 may be important in maintaining basal keratinocyte and melanocyte attachment to the basement membrane as opposed to stem cell proliferation and differentiation.

The function of the antigen detected by EL-2 and 4F2 is incompletely understood. Certain observations suggest that it is an activation antigen: lymphocytes activated by mitogens and antigens, and lymphoid and nonlymphoid malignant cell lines were reactive with EL-2. Resting lymphocytes were negative. This antigen is clearly not solely stem cell-associated, since Lavker and Sun have demonstrated that epidermal stem cells are concentrated in the rete ridges and not uniformly distributed throughout the basal layer [23]. However, stem cells were enumerated by autoradiographic techniques which measured only [^{3}H]thymidine uptake. One can speculate that although most basal keratinocytes do not demonstrate significant labeling with [^{3}H]thymidine, they may be "activated," possibly by factors secreted by the stem cells themselves. This would account for the reactivity of all basal keratinocytes with EL-2 and 4F2. Studies of lymphocyte activation have been enhanced by the availability of monoclonal antibody-defined activation antigens [24]. Similarly, antibodies EL-2 (and 4F2) may be useful in establishing the concept of keratinocyte activation.

Using EL-2 and a modified rosetting technique, basal keratinocytes were isolated to greater than 90% purity and successfully cultured in vitro. These results represent an improvement over previous studies: Brysk et al [2] used discontinuous isokinetic gradients of PERCOLL to separate newborn rat epidermal cells and demonstrated that their basal cell-enriched fraction was 98% viable, but they did not specify the exact purity of this fraction. Morhenn et al [1] achieved basal cell-enriched populations of 90% purity with monoclonal antibody VM1 but were unable to subsequently culture these cells successfully. In our experiments, significant melanocyte contamination did not occur despite the fact that EL-2 reacted with both melanocytes and basal keratinocytes. The reason for this observation is that the conditions used to dissociate epidermal cells from skin, and the relatively melanocyte-poor areas of skin selected for study (extremities and back) resulted in <1% melanocyte contamination. In addition, the culture conditions used were not favorable for melanocyte growth [11,17].

This is the first application of rosetting technology to the isolation of keratinocyte subpopulations. It has been demonstrated to be efficacious in both the enrichment and depletion of epidermal basal keratinocytes. Cultures of purified basal cells maintained higher proportions of EL-2-reactive cells (i.e., basal cells) throughout the entire culture period as compared with those of unseparated keratinocytes.

Recently a number of investigators have reported the use of autologous cultured epidermal cells as grafts to replace skin lost by burn injuries [25,26] and as grafts to cover nonhealing wounds [27,28]. These grafts were made by culturing unpurified epidermal keratinocytes. The use of cultures of purified basal keratinocytes should be considered, since such populations may be enriched in proliferating cells. Patients with epidermolysis bullosa and individuals with nonhealing wounds represent candidates for autologous grafts with such purified basal keratinocytes. The efficacy of such grafts could be compared with cultures established from autologous unpurified epidermal keratinocytes. Purification of basal keratinocytes will also be important in biochemical and functional studies of normal skin and in establishing long-term keratinocyte lines from normal keratinocytes.

The authors would like to thank Ms. Ann Hoy and Ms. Jeannine Acocella for their excellent technical assistance.

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