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The Mixed Epidermal Cell-Lymphocyte Reaction. II. Epidermal Langerhans Cells Are Responsible for the Enhanced Allogeneic Lymphocyte-Stimulating Capacity of Normal Human Epidermal Cell Suspensions*

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Earlier studies carried out in our laboratory which demonstrated that disaggregated human epidermal cells isolated from normal flexor forearm skin produced a greater degree of primary allogeneic lymphocyte blastogenic response than did autologous peripheral blood mononuclear cells have since been confirmed by others. We have now completed a series of additional studies designed to determine the basis for this difference. Blocking studies with anti-HLA-DR antibodies revealed that the allogeneic response triggered by epidermal cells was completely dependent on the presence of unbound HLA-DR molecules eliminating the possibility that nonspecific mitogenic effects produced by the epidermal cell suspension might be responsible for the difference. In addition we were unable to demonstrate that epidermal keratinocytes were supplying a nonspecific helper or growth-promoting effect to the interaction between stimulating HLA-DR bearing Langerhans cells and responding T lymphocytes. Since it has recently been suggested that the entire alloantigen-presenting capacity of unfractionated peripheral blood mononuclear cells can be attributed to a small population of HLA-DR antigen-bearing dendritic cells, we have considered the possibility that the greater degree of allogeneic lymphocyte response produced by epidermal cells could be due to the presence of a greater percentage of HLA-DR positive dendritic cells present in epidermal cell suspensions (i.e., Langerhans cells). Peripheral blood dendritic cell-enriched fractions and epidermal cell suspensions that contained similar percentages of dendritic cells produced equivalent amounts of allogeneic lymphocyte stimulation, whereas peripheral blood dendritic cell-enriched fractions that contained greater percentages of dendritic cells than were present in epidermal cell suspensions produced greater amounts of allogeneic stimulation. We therefore conclude that the enhanced mixed lymphocyte reaction stimulating capacity of human epidermal cell suspensions could be explained by the fact that epidermal cell suspensions contain a greater percentage of HLA-DR bearing alloantigen-presenting dendritic cells than do unfractionated peripheral blood mononuclear cell suspensions.

In an earlier study we presented data which demonstrated that epidermal cells isolated from flexor forearm skin of normal adult humans consistently produced greater degrees of allogeneic lymphocyte blastogenesis than did autologous peripheral blood mononuclear cells (PBMC) [1]. It has been assumed that the Ia (DR) antigen-bearing dendritic Langerhans cell (LC) is the epidermal cell type that functions as the stimulator cell in the primary allogeneic mixed epidermal cell-lymphocyte reaction (MECLR). However, LC comprise only about 4% of human epidermal cells. The vast majority of the remaining cells in this population are keratinocytes. Epidermal keratinocytes have recently been shown to be capable of secreting Interleukin-1 (IL-1)-like [2–4] and thymopoietin-like [5] substances. In addition, keratinocytes can express Ia (DR) antigens in vivo under certain conditions [6–7]. These observations would suggest the possibility that epidermal keratinocytes might also be involved in the MECLR, perhaps by augmenting the response initially triggered by LC. Another possible explanation for the enhanced response seen in the MECLR might be that epidermal LC are inherently more efficient at alloantigen presentation than are those cells in the peripheral blood that serve as allogeneic stimulators in the mixed lymphocyte reaction. It might also be possible that LC represent a greater percentage of epidermal cell suspensions than do the alloantigen-presenting cell type present in PBMC suspensions. An examination of these possibilities has revealed that this final one could explain the MECLR–mixed lymphocyte reaction (MLR) differences that we have previously observed.

MATERIALS AND METHODS

Human Volunteers

Healthy young adult male and female volunteers, consisting predominantly of medical students and laboratory personnel, were paid to donate specimens of epidermis and venous blood simultaneously that was used to prepare the stimulator cell populations. Other unrelated healthy male and female volunteers were randomly selected and paid to donate venous blood that was used to prepare the responder PBMC.

Reagents

A murine monoclonal antibody (#203) specific for a nonpolymorphic determinant present on all HLA-DR molecules [8] was a generous gift from Dr. Peter Stastny. Indomethacin was purchased from Merck-Sharp-Dohme.

Isolation of Epidermal Cells

Fresh epidermal cell suspensions were prepared from full-thickness epidermal biopsies taken from the flexor aspect of the forearm with a suction blister technique that has previously been described in detail [1]. Each blister top, consisting entirely of epidermis, was removed

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Abbreviations:
BSA: bovine serum albumin
CPM: counts per minute
FCS: fetal calf serum
HBSS: Hanks' balanced salt solution
IL-1: interleukin-1
LC: Langerhans cell(s)
MECLR: mixed epidermal cell-lymphocyte reaction
MLR: mixed lymphocyte reaction
PBDC: peripheral blood dendritic cells
PBMC: peripheral blood mononuclear cells
[^{3}H]TdR: tritiated thymidine

with sterile iris scissors and forceps without anesthesia and washed in Hanks' balanced salt solution (HBSS). All five blister top epidermal sheets were then incubated in a 0.3% solution of hog pancreas trypsin (1-250; ICN Nutritional Biochemicals, Cleveland, Ohio) for 30 min at 37°C and then were gently agitated at 5-min intervals over the next 30 min to give a single cell suspension. After 3 washings in cold Ca^{+2} and Mg^{+2}-free HBSS and a 5-min exposure at 23°C to 0.025% deoxyribonuclease (Sigma, St. Louis, Missouri) to prevent mucoid reaggregation, the epidermal cells were filtered through sterile 100-μm pore size nylon mesh (Nitex HC3-100; Tetco, Inc., Elmsford, New York) and then were resuspended in RPMI 1640 medium supplemented with 2 mM fresh L-glutamine, 15% heat-inactivated pooled human AB serum, penicillin (400 U/ml), streptomycin (400 μg/ml), and fungizone (1 μg/ml) (GIBCO, Grand Island, New York). Cell yield was then determined in a hemacytometer and the cell suspension was exposed to 4000 rad gamma-irradiation from a ^{137}Cesium source. Viability was then determined with a fluorescein diacetate ethidium bromide assay [9].

Primary Allogeneic Mixed Epidermal Cell-Lymphocyte Cultures

PBMC were prepared from venous blood by Isolymph (Gallard-Schlesinger Chemical Co., Carle Place, New York) density gradient centrifugation, washed 3× in HBSS and suspended in the same complete medium as described above. Mixed cultures of epidermal cells and PBMC were then set up in round-bottomed microtiter wells (Dynatech, Alexandria, Virginia). The cultures consisting of 5×10^4 viable stimulator epidermal cells and 1×10^5 responder PBMC in a final volume of 0.2 ml were incubated for 144 h in a humidified 5% CO_2 atmosphere at 37°C. Eighteen hours before harvesting, each culture was pulsed with 1 μCi of tritiated thymidine ([^{3}H]TdR) (New England Nuclear, Boston, Massachusetts). After washing, cells were collected with a MASH II automated cell harvester onto glass fiber filters and the amount of incorporated [^{3}H]TdR was determined by liquid scintillation spectroscopy. All cultures were carried out in triplicate and the results were expressed as mean counts per minute (CPM) ± 1 SD. Data were expressed as the Δ CPM (CPM of cultures containing both stimulator and responder cells minus the sum of the CPM of the stimulator and responder cell populations cultured separately.)

Primary Allogeneic Mixed Lymphocyte Cultures

Mixed lymphocyte cultures were set up in a manner similar to that described for the mixed epidermal cell-lymphocyte cultures with the following exception. The stimulator cell population consisted of gamma-irradiated PBMC that had been exposed to the same trypsin and DNAase conditions that were necessary for epidermal cell isolation.

Epidermal Keratinocyte Culture Technique

The technique of Liu and colleagues [10] was used to establish confluent primary cultures of epidermal keratinocytes on collagen gels. Epidermal sheets derived from either suction blister biopsies of adult normal flexor forearm skin or trypsin separated 0.3 mm-keratomed slices of normal adult trunkal skin were incubated in 0.3% trypsin in GNK solution (0.1% glucose, 0.15 M NaCl and 0.04% KCl) at pH 7.6 in a 37°C water bath. The resulting epidermal cell suspension was passed through a coarse mesh filter to remove debris. A 15 ml volume of Ca^{2+}- and Mg^{2+}-free HBSS was added and the cells were centrifuged at 1800 rpm for 6 min. The supernatant was discarded and the cells were washed 2 additional times. The cell pellet was resuspended in Minimal Essential Medium with 10% fetal calf serum (FCS) containing antibiotics (penicillin 400 U/ml, streptomycin 400 μg/ml and fungizone 1 μg/ml) and hydrocortisone (0.4 μg/ml). After counting and viability determination the cells were plated onto plastic dishes covered with a thin layer of bovine collagen gel (Vitrogen-Collagen Corporation) at a concentration of $1.0–1.5 \times 10^5$ viable cells/cm^2. The cells were then incubated at 37°C in a humidified 5% CO_2–95% air atmosphere. At 18 h and then approximately every 3 days thereafter the cells were fed with McCoy's 5A medium containing 10% FCS, antibiotics, and hydrocortisone at the concentrations listed above.

Peripheral Blood Dendritic Cell (PBDC) Enrichment Technique

Peripheral blood dendritic cells (PBDC) were enriched by the technique of Van Voorhis et al [11]. PBMC were isolated from 50 ml of whole heparinized blood using Ficoll 400/sodium diatrizoate (Histopaque 1.077, Sigma Chemical Co.). The PBMC's were suspended in RPMI 1640 containing 5% FCS, antibiotics (100 U/ml penicillin, 100 U/ml streptomycin, and 50 μg/ml gentamicin), 1 mM L-glutamine, and 5×10^{-5} M 2-mercaptoethanol. The cells were then plated onto a 100-mm plastic culture plate for 1 h at 37°C following which the non-adherent cells were removed and discarded. Fresh media was added to the adherent cells, after which they were reincubated for 16 h at 37°C. The cells that were not attached after this overnight incubation were collected, plated in fresh medium onto another 100 mm plate and incubated for 1 additional h at 37°C. The cells that had remained attached after overnight incubation were collected with a rubber policeman and used as the "adherent macrophage" population. The cells that had not attached for the second time (the "released 2×" population) were resuspended in 4 ml of bovine serum albumin (BSA) (d = 1.082) and carefully overlayed with 2 ml of BSA (d = 1.047). The tube was centrifuged for 10,000 *g* for 10 min at 4°C. The interface cell layer (the "low density released 2×" population) was removed and washed 3× in RPMI 1640. This layer contained approximately 10–30% dendritic cells. The cells at the bottom of the tube were collected, washed 3× in RPMI 1640 and used as the "high density released 2×" population.

To examine the cells by phase contrast microscopy, 2×10^5 cells were centrifuged onto 15-mm circular coverslips (which had been precoated with 75 μg/ml poly-L-lysine) for 10 min at 400 *g*. The cells were incubated for at least 20 min in RPMI 1640 at 37°C to allow spreading of the dendritic cells. Prior to examination the cells were fixed in 1.25% glutaraldehyde in phosphate buffered saline for 15–30 min.

RESULTS

The Allogeneic Lymphocyte Response Induced by Epidermal Cells Requires Expression of Unbound HLA-DR Molecules

Fig 1 shows the results of a study which examined the blocking effect of a monoclonal HLA-DR antibody on the MECLR and MLR response. As can be seen both responses were blocked in a similar dose-dependent fashion across a broad range of antibody concentrations. At a 1:10 antibody dilution both the MECLR and MLR response was inhibited by approximately 80%. Both responses were still significantly depressed at 1:160 antibody concentration. No blocking effect was produced by an irrelevant monoclonal antibody (anti-arsonate) in a similar study (data not shown).

The kinetics of this HLA-DR antibody blocking effect were examined in an experiment shown in Fig 2. The maximum blocking effect was seen when the antibody was added at the time of culture initiation. Addition of the antibody after 48 h of culture did not produce significant blocking of the MECLR or MLR response. The failure of the antibody to block either response when added after 48 h argues against the possibility

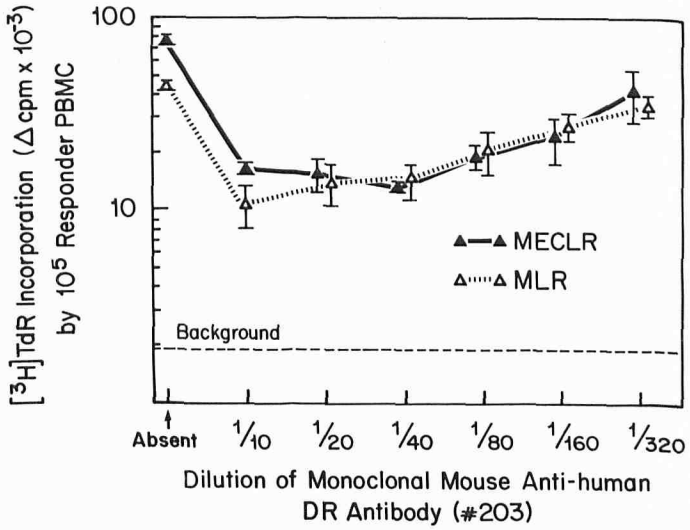

FIG 1. Effect of anti-HLA-DR antibody on primary allogeneic MECLR and MLR responses. Varying dilutions of anti-HLA-DR antibody (v/v) were added at the time of initiation of MECLR and MLR cultures. The amount of [^{3}H]TdR incorporation was determined after 6 days (144 h) in culture. Note that the [^{3}H]TdR incorporation on the ordinate is expressed on a logarithmic scale. The *horizontal broken line* represents the background [^{3}H]TdR incorporation by 10^5 responder PBMC cultured alone.

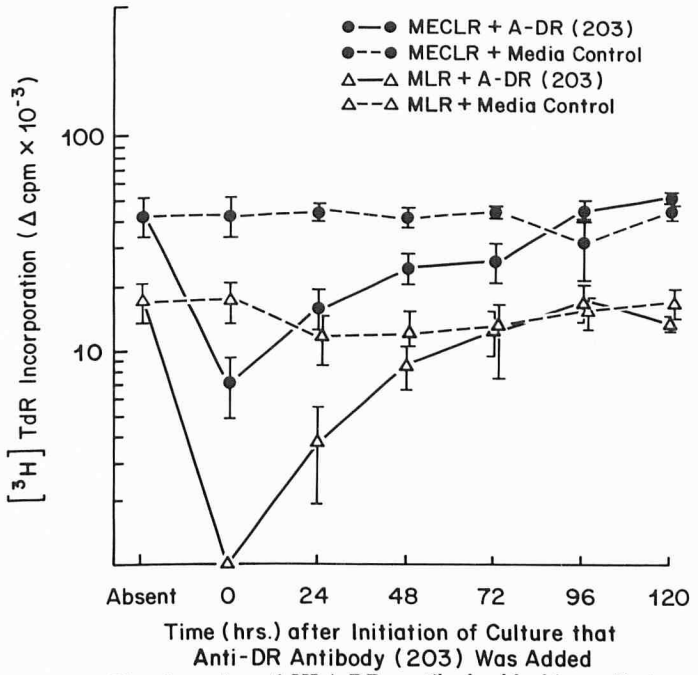

FIG 2. Kinetics of anti-HLA-DR antibody blocking effect on MECLR and MLR responses. A 1:10 dilution of anti-HLA-DR antibody was added to MECLR and MLR cultures at the time of culture initiation or at various time intervals thereafter. Equal volumes of fresh media were added to control MECLR and MLR cultures at the same timepoints. The cultures were harvested and [^{3}H]TdR incorporation assayed at 144 h.

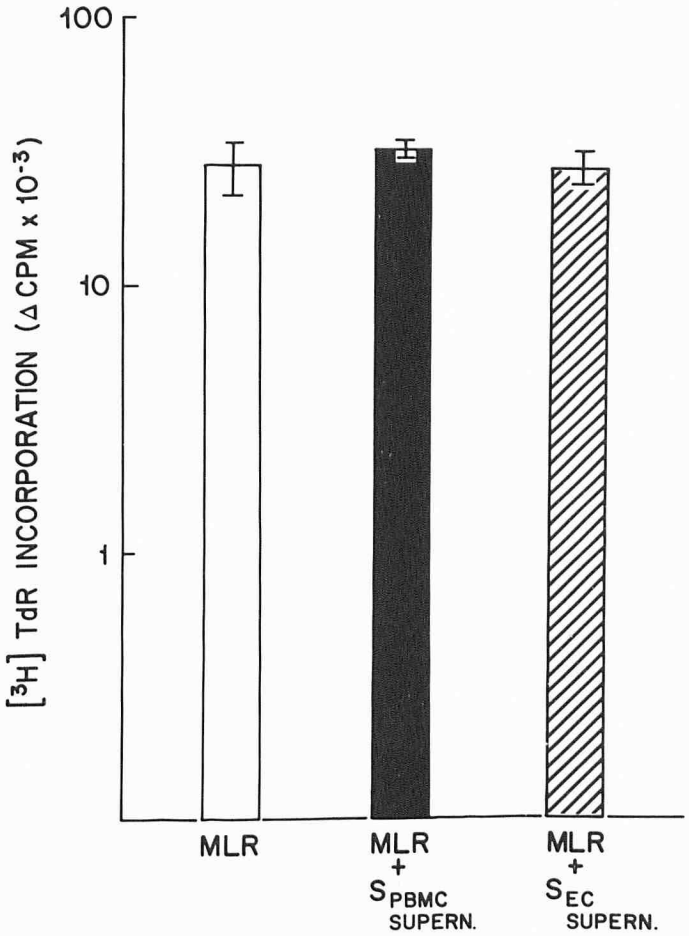

FIG 3. Effect of epidermal cell culture supernatants on the MLR response. 10^5-irradiated epidermal cells in suspension were cultured alone in microtiter wells under the same culture conditions previously described for MECLR cultures. At the end of 6 days the supernatant from the epidermal cell culture was collected and added to fresh MLR cultures at a 25% (v/v) concentration. As controls, MLR cultures were carried out in the presence of 6-day culture supernatants from PBMC or cultured alone in the absence of any added culture supernatant.

that the blocking effect seen when antibody was added at earlier time points was the trivial result of nonspecific toxic effects of the antibody preparation. It is likely that the HLA-DR antibody blocking effect results from binding of the antibody to the DR antigens present on the stimulating cells (i.e., Langerhans cells). In support of this are the findings of Hefton et al [12] who showed that pretreatment of human epidermal cell suspensions with anti-HLA-DR antibody and complement completely abrogated the MECLR response. It is likely that the activated responder T cells in a MECLR reaction also become HLA-DR positive since helper T cells activated in different ways have been shown to express these antigens. The kinetics of the MECLR blocking effect suggest that the DR antibody blocking effect is not due to reaction with DR antigens on activated T cells. If this were the case then one would expect that addition of the antibody near the end of the culture period would be as effective in blocking the response. Our studies showed however that addition of the antibody after 48 h in culture resulted in no significant blocking.

The results of these 2 experiments strongly suggest that, as has been shown previously for the MLR, the MECLR response is very much dependent on the expression by a viable stimulating cell population of cell surface HLA-DR molecules. These results also argue against the remote possibility that the enhanced allogeneic lymphocyte stimulating capacity of epidermal cells that we had observed was due to nonspecific mitogenic effects produced by something in the epidermal cell suspension.

Keratinocytes Present in Epidermal Cell Suspensions Are not Responsible for the Enhanced MECLR Response

We have taken several approaches to examining the possibility that keratinocytes present in the epidermal cell suspensions might be supplying some growth promoting effect (e.g., IL-1) that could be producing the enhanced MECLR response.

Fig 3 shows the results of an experiment which examined the effects on the MLR response produced by the addition of culture supernatants from stimulator epidermal cells or PBMC cultured alone. The addition of epidermal cell culture supernatants did not augment the MLR response above that seen with standard MLR cultures or MLR cultures containing PBMC culture supernatants. Similar results were seen with MLR cultures carried out in the presence of 50% epidermal cell culture supernatants (data not shown).

The effect on the MLR response produced by the addition of epidermal cells that were autologous to the responding PBMC cell population is shown in Fig 4. The addition of epidermal cells isolated from the same individual whose PBMC were being used as responder cells did not augment the MLR response. In fact the MLR response was somewhat suppressed by the addition of such responder-derived epidermal cells. However, this probably resulted from competition for nutrients in the culture medium in view of the fact that these cultures contained 5×10^4 more viable cells than did the standard MLR cultures since the same effect was seen on the MECLR cultures.

Our final attempt to demonstrate a MLR-augmenting effect by keratinocyte-derived products is shown in Fig 5. No MLR response enhancing effect was seen with the addition of keratinocyte culture supernatants taken at any time point. In fact in both experiments the supernatants collected on and after day 3 appeared to significantly suppress the MLR response.

These studies taken together virtually eliminate the possibility that products produced by the keratinocytes in our MECLR culture system are responsible for augmenting the allogeneic lymphocyte stimulating response.

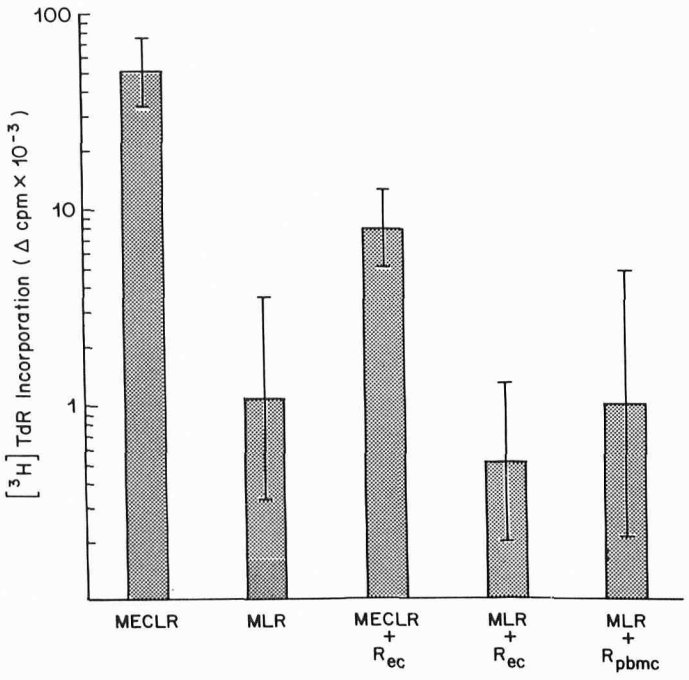

FIG 4. Effect on MLR response produced by the presence of epidermal cells that were autologous to the responder PBMC. MECLR and MLR cultures were set up as previously described using 5×10^4-irradiated stimulator- and 10^5-responder cells. 5×10^4-irradiated epidermal cells taken from the same individual whose PBMC were being used as the responder population were added to the MECLR and MLR cultures at the time of culture initiation.

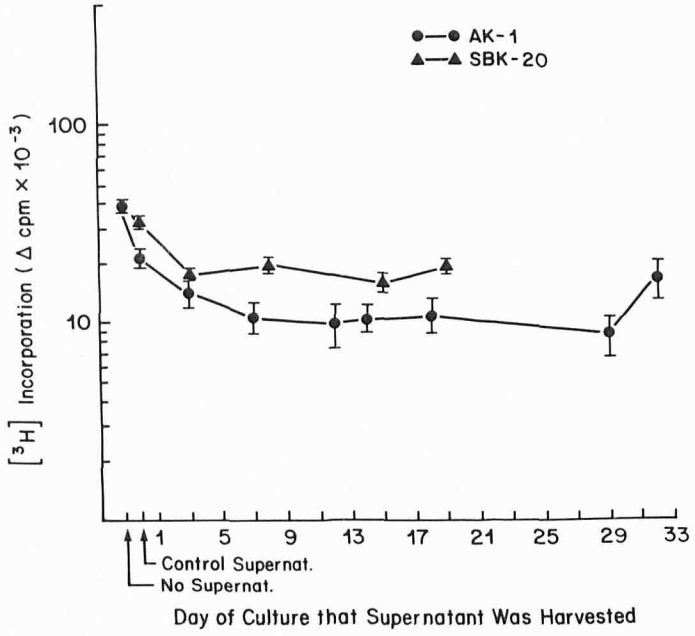

FIG 5. Effect on MLR response of supernatants from primary cultures of keratinocytes cultivated on collagen gels. Primary cultures of epidermal keratinocytes were initiated on hydrated collagen gels with cells obtained from either forearm suction blister tops (*SBK-20*) or adult trunkal skin obtained at autospy (*AK-1*) (see *Materials and Methods* section for details of culture conditions). Culture supernatants were harvested at various time intervals up to 33 days after initiation of culture and kept frozen at −70°C. These supernatants were later thawed and aliquots from each time point were added in a 25% final concentration (v/v) to MLR cultures at the time of culture initiation. In this system confluent keratinocyte growth is usually seen between days 5 and 7.

Comparison of Epidermal Cell and Peripheral Blood Dendritic Cell (PBDC) Allogeneic Lymphocyte-Stimulating Capacities

We attempted to enrich for PBDC by the differential adherence technique described by Van Voorhis and coworkers [11]. We were successful in producing cell fractions that contained small percentages of cells which assumed a unipolar or bipolar dendritic appearance on poly-L-lysine (see Fig 6). However our reproduction of this technique yielded lower percentages of dendritic cells than those reported by Van Voorhis et al (see Table I).

Fig 7 shows the results of 5 separate experiments in which the MLR stimulating capacity of PBMC was compared to that of autologous firmly adherent macrophages and cell fractions containing different percentages of autologous PBDC. The firmly adherent macrophages elicited virtually no MLR response, whereas those cell fractions which were enriched for PBDC produced greater responses than did unfractionated PBMC. The cell fraction which contained the greatest percentage of PBDC (the "low density released 2×" population) elicited a response that was 3-fold greater than that produced by unfractionated PBMC. These results are in agreement with the findings of Van Voorhis et al and others [11,13–14].

Fig 8 shows the result of 3 separate experiments in which the MLR stimulating capacities of epidermal cells and autologous PBDC-enriched fractions were compared. As previously demonstrated unfractionated PBMC produced a lower response than that of epidermal cells. The PBDC-enriched fraction which contained a percentage of dendritic cells that was roughly

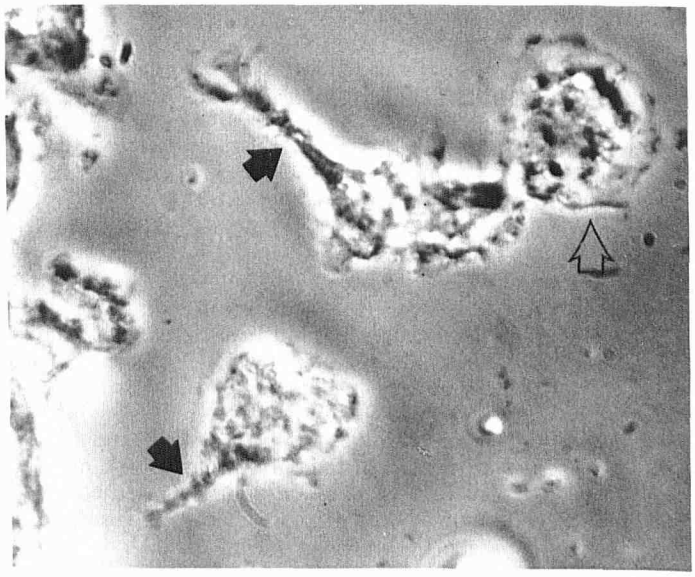

FIG 6. Phase contrast microscopic appearance of peripheral blood dendritic cells on a poly-L-lysine-coated coverslip. Note the unipolar dendritic morphology (*closed arrowheads*). A fried egg-shaped, ruffled-edged macrophage can be seen in the upper right-hand corner (*open arrowhead*). Original magnification × 630.

TABLE I. *Yields of peripheral blood dendritic cell enrichment procedures*

Cell fraction	% Dendritic cells	
	Van Voorhis et al [11]	Our technique
Released 2X	3–6%	1–5%
Low density Released 2X	10–30%	8–15%
High density Released 2X	1–4%	0–4%

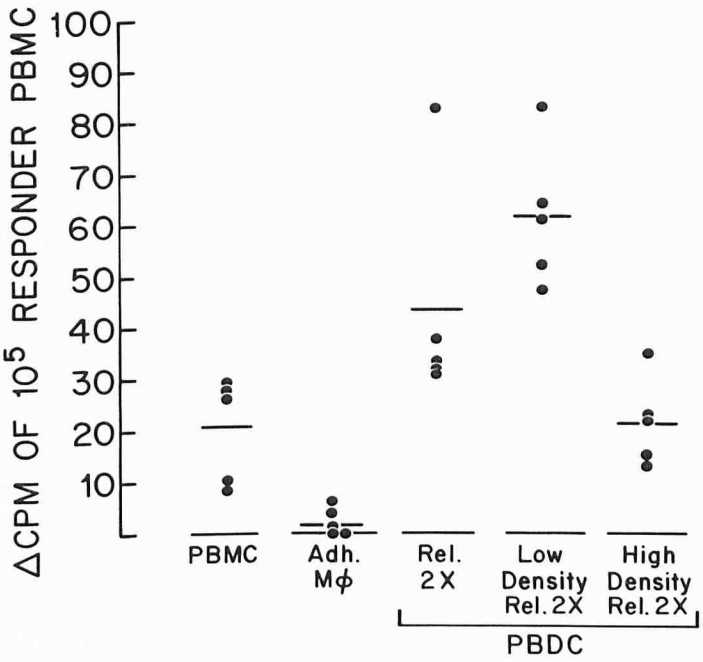

FIG 7. Comparison of MLR-stimulating capacities of different cell fractions derived from PBMC. Each of the 5 experiments shown here examined the MLR response of 10^5-responder cells produced by 5×10^4-irradiated stimulator cells present in the adherent macrophage (*Adh. MØ*), released 2× (*Rel.* 2×), low density released 2×, and high density released 2× populations isolated from the same individual.

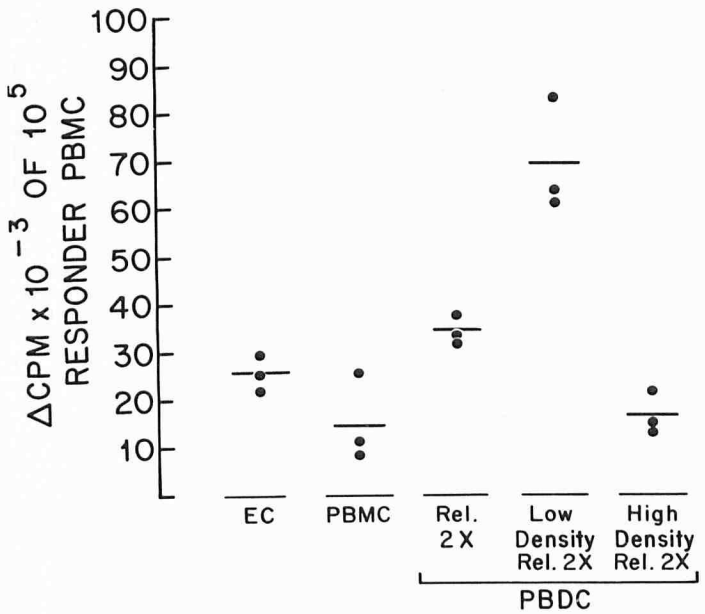

FIG 8. Comparison of MLR-stimulating capacities of epidermal cells (*EC*) and *PBDC*-enriched fractions. In each of the 3 experiments the epidermal cells and PBDC-enriched fractions were derived from the same individual.

equivalent to the percentage of epidermal LC present in our epidermal cell suspensions (the "released 2×" fraction) produced an MLR response that was similar to the response elicited by epidermal cells. The PBDC-enriched fraction which contained a higher percentage of dendritic cells than were present in epidermal cell suspensions (the "low density released 2×" population) produced a significantly higher MLR response.

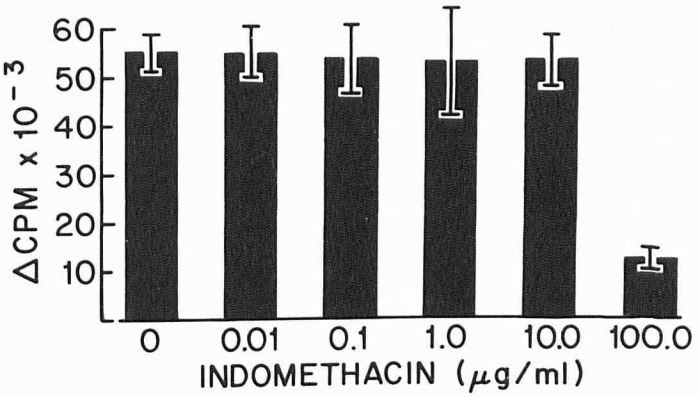

FIG 9. Effect on MLR response of inhibiting prostaglandin production. Different concentrations of indomethacin were added at the time of initiation of MLR cultures.

Effect of Indomethacin on MLR

It is possible that a radioresistant suppressor cell is lost during the PBDC enrichment procedure. The loss of such a cell might result in a higher MLR response. Since one form of suppression is prostaglandin mediated, we have examined the effect on the MLR of blocking prostaglandin production. Fig 9 shows the effect that a wide range of indomethacin concentrations had on a MLR response. None of the indomethacin concentrations studied augmented the response, as would be predicted if a prostaglandin-mediated suppressor mechanism was responsible for down regulating the MLR response. Thus it seems unlikely that the high MLR response induced by PBDC-enriched fractions was due to loss of a prostaglandin-mediated suppressor cell function.

DISCUSSION

A number of earlier studies have documented that human epidermal cells can substitute for lymphoid cells as stimulators in the primary allogeneic MLR [15–18]. These studies have found for the most part that the response produced by epidermal cells was equal to or less than that produced by autologous PBMC. Our more recent studies, however, have clearly demonstrated that freshly prepared flexor forearm epidermal cells are considerably more efficient alloantigen-presenting cells, on a cell for cell basis, than are PBMC [1]. This observation has now been confirmed by other investigators [19]. It is likely that differences between the various techniques used in these studies to obtain epidermal cell suspension might account for the discrepancy between our finding and those of the earlier investigators.

The current series of studies were carried out to determine the basis for the enhanced alloantigen-presenting capacity possessed by epidermal cell suspensions. Even though epidermal keratinocytes are capable of secreting immunologically active cytokines and expressing HLA-D region products, our studies suggest that these qualities do not confer upon this cell type the capacity to augment the primary T-cell response to alloantigens. Our studies however have not excluded the possibility that the T-cell response is being augmented by organ specific alloantigens that might be present on epidermal cells. The presence of such antigens has never been documented on human epidermal cells; however, there is some data which suggest their presence on murine epidermal cells [20,21]. We have begun to examine this possibility by looking for MECLR reactivity between HLA identical siblings. We have found no significant MECLR reactivity between 2 separate HLA identical sibling pairs. A third sibling pair did demonstrate MECLR reactivity, however a question was raised as to their true HLA identity by the fact that they were also MLR reactive (unpub-

lished personal observations). Pittelkow and Steinmueller [19] have found no significant primary allogeneic MECLR reactivity between a larger number of HLA identical sibling pairs. Therefore, it seems unlikely that epidermal cell-specific alloantigens are responsible for the augmented MECLR response that we have observed.

The demonstration by Van Voorhis et al [11] that PBMC contain a small percentage (0.1%–0.5%) of dendritic cells which can be enriched by differential adherence and density separation techniques, has allowed us to directly compare the alloantigen-presenting capacities of the dendritic cell populations present is epidermis and venous blood. These studies have revealed that the alloantigen-presenting capacities of these 2 populations of dendritic cells are very similar and have confirmed Van Voorhis et al's observations which suggest that the dendritic cells present in peripheral blood is a very potent alloantigen-presenting cell type. This functional similarity between epidermal LC and PBDC raises the possibility that the PBDC might be the circulating precursor of the epidermal LC. Human PBDC and LC differ in that PBDC lack certain membrane constituents and cytoplasmic organelles possessed by LC (OKT-6 antigen, Fc receptors and Birbeck granules) [11]. Perhaps these characteristics are acquired by the PBDC as this cell differentiates and matures on its way to, or while in, its final resting place in the suprabasalar layer of the epidermis.

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This work is dedicated to the memory of Dr. James N. Gilliam who died while the manuscript was being prepared. His advice concerning the design, execution, and interpretation of the results of these studies was vital to the completion of this project. His support, guidance, and friendship will be greatly missed.

REFERENCES

1. Sontheimer RD: The mixed epidermal cell-lymphocyte reaction. I. Human epidermal cells elicit a greater allogeneic lymphocyte response than do autologous peripheral blood lymphoid cells. J Immunol 130:2612–2614, 1983
2. Luger TA, Stadler BM, Katz SI, Oppenheim JJ: Epidermal cell-(keratinocyte)derived thymocyte activating factor. J Immunol 127:1493–1498, 1981
3. Luger TA, Stadler BM: Murine epidermal cell derived thymocyte activating factor resembles murine interleukin-1. J Immunol 128:2147–2152, 1982
4. Sauder DN, Carter CS, Katz SI, Oppenheim JJ: Epidermal cell production of thymocyte activating factor (ETAF). J Invest Dermatol 79:34–39, 1982
5. Chu AC, Patterson JAK, Goldstein G, Berger CL, Takezaki S, Edelson RL: Thymopoietin-like substance in human skin. J Invest Dermatol 81:194–197, 1983
6. Breathnach SM, Katz SI: Keratinocytes synthesize Ia antigen in acute graft-versus-host disease. J Immunol 131:2741–2745, 1983
7. Volc Platzer B, Majdic O, Knapp W, Wolff K, Hinterberger W, Lechner K, Stingl G: Evidence of HLA-DR antigen biosynthesis by human keratinocytes in disease. J Exp Med 159:1784–1789, 1984
8. Lampson LA and Levy R: Two populations of Ia-like molecules on a human B cell line. J Immunol 125:293–299, 1980
9. Takasugi M: An improved fluorochromatic cytotoxicity test. Transplantation 12:148–151, 1971
10. Liu SC, Eaton MJ, Karasek MA: Growth characteristics of human epidermal keratinocytes from newborn foreskin in primary and serial cultures. In Vitro 15:813–822, 1979
11. Van Voorhis WC, Hair LS, Steinman RM, Kaplan G: Human dendritic cells: enrichment and characterization from peripheral blood. J Exp Med 155:1172–1187, 1982
12. Hefton JM, Amberson JB, Biozes DG, Weksler ME: Loss of HLA-DR expression by human epidermal cells after growth in culture. J Invest Dermatol 83:48–50, 1984
13. Van Voorhis WC, Valinsky J, Hoffman E, Luban J, Hair LS, Steinman RM: Relative efficacy of human monocytes and dendritic cells as accessory cells for T cell replication. J Exp Med 158:174–191, 1983
14. Santiago-Schwarz F, Woodward JG, Dixon JFP, Parker JW, Frelinger JA, O'Brien RL: Low density mononuclear cells. Potent stimulators of the human MLR. Transplantation 35:463–469, 1983
15. Cochrum KC, Main RK, Kountz SL: A new matching technique: the mixed skin cell-leukocyte reaction. Surgery 70:97–102, 1971
16. Levis WR, Miller AE: Leukocyte/skin cultures as a measure of histocompatibility in man. Lancet 2:357–359, 1972
17. Shochat S, Abulata N, Knight S, Hardy DA, Ling NR, Wallin J: Activation of peripheral lymphocytes by human epidermal cells. Cytobios 6:21–26, 1972
18. Hirschberg H, Thorsby E: Lymphocyte-activating alloantigens on human epidermal cells. tissue Antigens 6:183–194, 1975
19. Pittelkow MR, Zinsmeister AR, Steinmuller D: Donor-recipient matching with the mixed lymphocyte-epidermal cell reaction, Recent Advances in Bone Marrow Transplantation. Edited by RP Gale. UCLA Symposia on Molecular Biology, New Series. New York, 1983, pp 227–241
20. Boyse EA, Lance EM, Carswell BA, Cooper S, Old LJ: Rejection of skin allografts by radiation chimeras: selective gene action in the specification of cell surface structure. Nature 227:901–903, 1970
21. Steinmuller D, Tyler JD, David CS: Cell-mediated cytotoxicity to non-MHC alloantigens on mouse epidermal cells. I. H-2 restricted reactions among strains sharing the $H\text{-}2^k$ haplotype. J Immunol 126:1747–1753, 1981