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# Reactivity of Langerhans cells with hybridoma antibody

(delayed hypersensitivity/monoclonal antibody/epidermis/antigen presentation/thymocyte antigen)

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**ABSTRACT** **Reactivity of a monoclonal antibody with human Langerhans cells was demonstrated by a double-labeling immunofluorescence technique. Ia-bearing cells of the epidermis (Langerhans cells) were reactive with this antibody both in frozen sections and in cell suspensions prepared from human epidermis. This monoclonal antibody was unreactive with non-Ia-bearing epidermal cells and with peripheral blood B cells, T cells, and monocytes but did bind to 70% of intrathymic lymphocytes. These observations further distinguish Langerhans cells from classical monocytes. Furthermore, this monoclonal antibody is a highly specific marker for the *in vivo* identification and *in vitro* isolation of Langerhans cells.**

Substantial interest has been focused recently on populations of dendritic mononuclear cells found in thymus, lymph node, and spleen because of the ability of these cells to perform important immunologic functions (1). Of these cells, epidermal Langerhans cells are the most accessible for study. They localize in a suprabasalar position in the epidermis of all mammalian species studied (2), and cumulative evidence now suggests that they are the primary immunocompetent cells of the epidermis.

The demonstration that Langerhans cells are the epidermal cells that express Ia (immune response-associated) antigens (3–7) and that stimulate allogeneic T cells in mixed lymphocyte cultures (8) implicated them as possible targets of the cellular reaction directed against histoincompatible skin allografts. This suggestion has recently received strong support from the experimental observation that corneal allografts, epidermal tissues naturally devoid of Langerhans cells, are not rejected by a host differing from the tissue donor only with respect to Ia antigens (7).

The capacity of Langerhans cells to present exogenous antigens *in vitro* to syngeneic T cells (8) and their apposition to mononuclear cells at sites of contact allergic dermatitis suggested an important role for them in contact delayed hypersensitivity (reviewed in ref. 9). The finding that skin deficient in Langerhans cells is incapable of initiating a normal delayed hypersensitivity reaction after application of an appropriate antigen supports this impression (10).

Most of the evidence has suggested that these dendritic epidermal cells are related to monocytes. First, the above described functional properties are generally attributed to monocytes. Second, they appear to be derived from bone marrow precursors which migrate to epidermis and localize in a suprabasalar position (11, 12). Third, they have membrane receptors for the split product of the third component of complement and for the Fc fragment of IgG. Finally, they are clearly distinguishable from B cells or T cells in that they lack endogenously produced surface immunoglobulin and the T-cell membrane receptor for sheep erythrocytes (13). However, Langerhans cells differ from typical monocytes by the presence of a distinctive cytoplasmic "granule" (14) and by being only moderately phagocytic (reviewed in ref. 15).

In this paper, we present evidence that human Langerhans cells have a membrane-associated antigen which further distinguishes them from classical monocytes. The monoclonal antibody with specificity for this membrane antigen provides a highly specific marker for the isolation and further characterization of this important cell.

## MATERIALS AND METHODS

**Monoclonal Antibodies.** Seven monoclonal antibodies were tested for reactivity with epidermal cells in frozen sections. Production of these antibodies, the OK series, has been reported. Briefly, they include antibodies that were generated in a mouse hybridoma system by immunization with human erythrocyte-rosette purified peripheral lymphocytes (OKT1, OKT3, OKT4) (16–19) or thymocytes (OKT5, OKT6, and OKT8) (20, 21) and that react selectively with differentiation antigens on these human cells. OKI1 was also generated in a hybridoma system and reacts with a public determinant of human Ia-like antigen (22). The protein content of the antibody solutions undiluted was in the range 2–5 mg/ml in all cases.

**Tissue Specimens.** Skin specimens were obtained surgically in accordance with the regulations of the Columbia University Investigational Review Board. They were then snap frozen in liquid $N_2$ and maintained in a liquid $N_2$ freezer until they were sectioned.

**Indirect Immunofluorescence of Tissue Sections.** Frozen sections (4 $\mu$m) of skin were allowed to air dry for 30 min. They were then overlaid with 100 $\mu$l of the primary antibody at a dilution of 1:100 and placed in a moist chamber at room temperature for 30 min. After two 10-min rinses in phosphate-buffered saline (GIBCO), excess liquid was removed from the slide and the sections were overlaid with 100 $\mu$l of the second antibody (fluorescein-conjugated goat anti-mouse IgG, Meloy Laboratories, Springfield, VA, lot 92438) at a dilution of 1:20. The slides were again incubated in a moist chamber at room temperature for 30 min. Sections were then rinsed twice in phosphate-buffered saline and placed in a slide rack before immersion in 1 liter of buffered saline in the dark with constant stirring for 90 min. Finally, sections were mounted on coverslips with 10% phosphate-buffered saline in glycerin and examined under a Leitz Ortholux II fluorescence microscope.

**Double-Staining Technique.** Indirect immunofluorescence was performed with OKI1 at a dilution of 1:100 as the primary antibody and rhodamine-conjugated goat anti-mouse immu-

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noglobulin (donated by James Halper, Columbia University) at a dilution of 1:20 as described above. This was followed by two 10-min rinses in phosphate-buffered saline and subsequent overlaying of tissue sections with 100 μl of fluorescein-labeled OKT6 (fluorescein/protein ratio, 4:1, 10 μg/ml) at a dilution of 1:4. Finally, the sections were immersed in buffered saline for 90 min, mounted, and examined as described above.

**Epidermal Cell Suspension.** Epidermal cell suspensions were prepared from normal epidermis obtained from a mastectomy specimen (approximately 3 by 3 cm) by the method of Eisinger *et al.* (23).

**Indirect Immunofluorescence of Epidermal Cell Suspension.** The epidermal cell suspension was adjusted to a concentration of $1.0 \times 10^7$ cells per ml in RPMI-1640 (GIBCO). For each assay, 100 μl of this suspension was placed in a microtiter well. Cell suspensions were centrifuged at 600 × *g* for 3 min, and the supernatant was discarded. Then 50 μl of the primary antibody at a dilution of 1:100 was added to each well, and the cells were then resuspended by pipetting. They were then incubated at 4°C for 30 min, after which they were recentrifuged and the supernatant was discarded. They were then washed three times with 50 μl of RPMI-1640. 50 μl of fluorescein-conjugated goat anti-mouse antibody was then added at a dilution of 1:20 and the cells were reincubated at 4°C for another 30 min. Following this, they were washed three times in RPMI-1640 and resuspended in one drop of 50% glycerin in phosphate-buffered saline. One drop of this suspension was placed on a slide with a coverslip and examined under the Leitz Ortholux II microscope.

**Double Staining of Epidermal Cell Suspension.** The technique of indirect immunofluorescence using OKI1 and the rhodamine label followed by direct immunofluorescence with fluorescein-conjugated OKT6 was also used for cells in suspension. The only modifications were those described above for the staining of cell suspensions rather than tissue sections.

**Preparation of $F(ab')_2$ Fragment of OKT6.** A $F(ab')_2$ fragment of OKT6 was prepared by overnight digestion of the antibody with pepsin (24).

## RESULTS

**Reactivity of Epidermis with Monoclonal Antibodies.** In the first set of experiments, we investigated reactivity of human epidermis with a battery of monoclonal anti-human mononuclear cell antibodies. Frozen sections of human skin (four normal, two with deep dermal cutaneous T-cell lymphoma not involving the epidermis, and one with benign dermal lymphocytoma cutis) were studied by indirect immunofluorescence with the individual OK antibodies as a first reagent and the fluorescein-labeled goat anti-mouse IgG as the second antibody. OKT1 and OKT3 react with human peripheral blood T cells; OKT4 reacts with the inducer (helper) T-cell subset; OKT5 and OKT8 react with suppressor/cytotoxic T cells; and OKT6 reacts with the majority of thymocytes (reviewed in ref. 25). OKI1 reacts with human Ia-like antigens (22).

Of these antibodies, only OKT6 and OKI1 were reactive with human epidermis (Table 1). Both antibodies reproducibly stained a suprabasalar dendritic population of cells (see Fig. 1*c*). The reactivity of OKI1 confirmed previous reports that Langerhans cells display Ia antigens (3–7). However, the staining of a similar population of suprabasalar dendritic cells with OKT6 was unexpected because the only known reactivity of this antibody was with human thymocytes. Specifically, OKT6 is completely unreactive with mononuclear cells separated from human peripheral blood by Ficoll/Hypaque and with human bone marrow cells. It is reactive with 70% of human thymocytes (21). These thymocytes have been shown to be largely located in the cortex and to represent a subset of cells at an early stage of intrathymic differentiation (26).

**Double Labeling of Epidermal Cells with OKI1 and OKT6.** Four skin sections (one from a leg amputation, two mastectomy specimens with normal epidermis, and one reduction mammoplasty specimen) were sequentially stained to determine whether the OKI1-positive and OKT6-positive cell populations were identical—i.e., whether the OKT6-positive cells were also Langerhans cells. Indirect immunofluorescence with OKI1 and rhodamine-conjugated goat anti-mouse Ig was followed by direct immunofluorescence with fluorescein-labeled OKT6. OKI1 and OKT6 stained identical cells in tissue sections. (Fig. 1 *a* and *b*). In control experiments in which mouse serum was substituted for OKI1, fluorescein-stained cells did not exhibit rhodamine staining: washing apparently had been effective, leaving no excess rhodamine-labeled goat anti-mouse Ig to stain with OKT6.

**Double Labeling of Epidermal Cells in Suspension.** The identity of OKI1- and OKT6-positive cells in skin was further confirmed by parallel double-labeling experiments with epidermal cell suspensions prepared from mastectomy skin in which the epidermis was normal. OKT6-positive cells consistently comprised between 1% and 3% of the total epidermal cells (400 cells counted per assay). Of 50 OKT6-positive cells, 45 were scored as also being OKI1 positive and 39 of 40 OKI1-positive cells were also OKT6 positive.

**Additional Controls.** Several other experiments were performed to establish the specificity of the system. Preincubation with OKT6 abolished staining with fluorescein-labeled OKT6 but preincubation with OKI1 did not. Thus, distinct antigens are being recognized by these antibodies. Next, a purified $F(ab')_2$ preparation of OKT6 gave a staining pattern identical

Table 1. Reactivity of monoclonal antibodies with epidermal cells in frozen sections

| Source of skin* | OKT6 IgG1 (anti-thymocyte) | OKI1 IgG2 (anti-Ia) | OKT1 IgG1 (anti-pan T cell) | OKT3 IgG2 (anti-pan T cell) | OKT4 IgG2 (anti-inducer T cell) | OKT5 IgG1 (anti-cytotoxic/ suppressor T cell) | OKT8 IgG2 (anti-cytotoxic/ suppressor T cell) |
|---|---|---|---|---|---|---|---|
| Mastectomy 1 | + | + | − | − | − | − | − |
| Mastectomy 2 | + | + | − | − | − | − | − |
| Reduction mammoplasty | + | + | − | − | − | − | − |
| Leg amputation | + | + | − | − | − | − | − |
| CTCL 1 | + | + | − | − | − | − | − |
| CTCL 2 | + | ND† | − | − | − | − | − |
| Lymphocytoma cutis | + | ND† | − | − | − | − | − |

The column headings show primary antibody (e.g., OKT6) and Ig class.
* CTCL, cutaneous T-cell lymphoma.
† ND, not done.

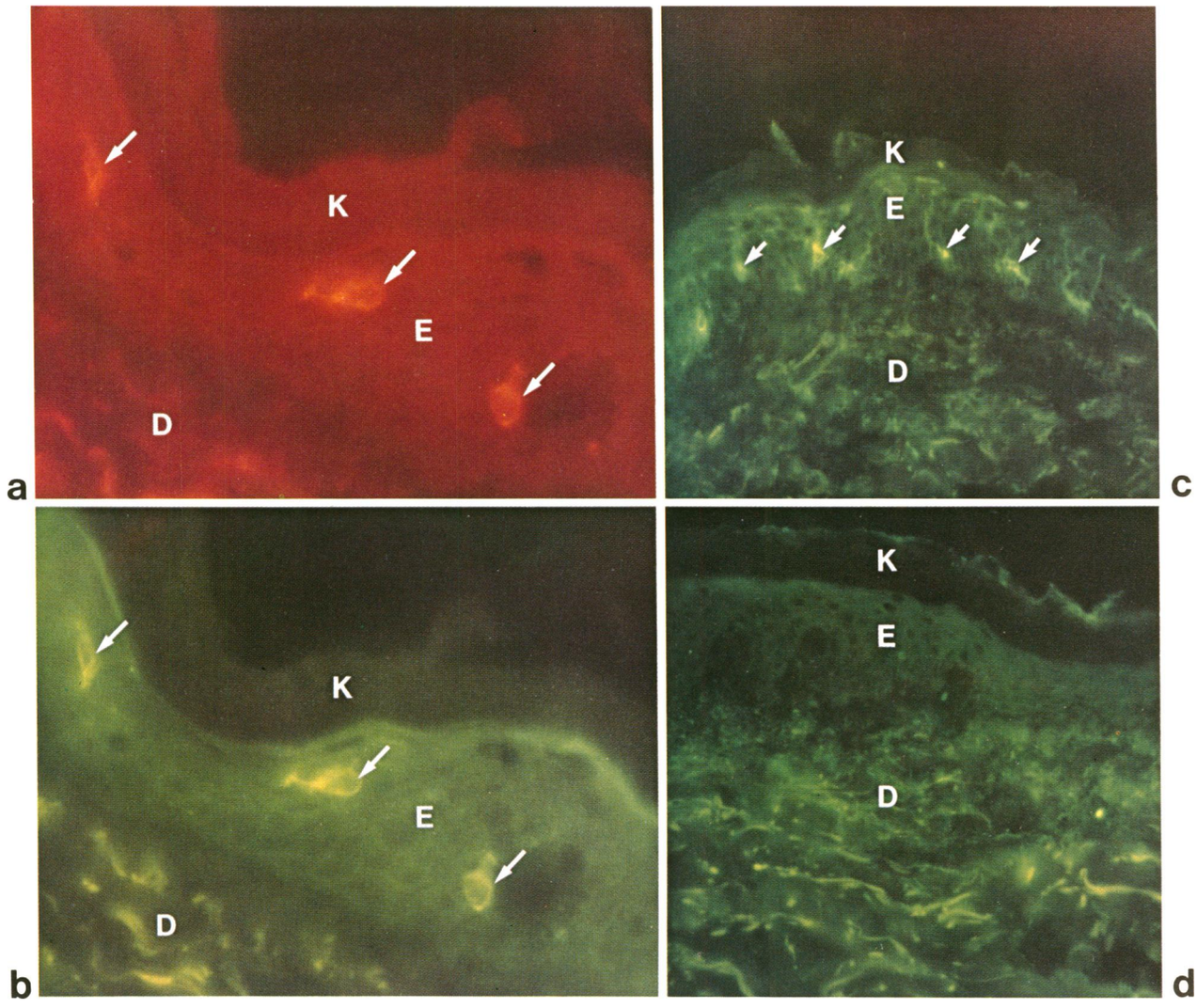


FIG. 1. (*a*) Histologically normal skin from amputated leg, showing three Ia-positive cells in the epidermis labeled with rhodamine. (×440.) (*b*) The same section viewed through a fluorescein filter, showing that the rhodamine-labeled (Ia positive) cells are also fluorescein labeled (OKT6 positive). (×440.) (*c*) Histologically normal skin from amputated leg, showing OKT6-positive cells in the suprabasal layer of the epidermis. (×175.) (*d*) Completely negative epidermis after use of OKT1 as primary antibody. (×175.) The specificity of the reaction is limited to the epidermis (E). No cellular reactivity is noted in the keratin layer (K). Nonspecific autofluorescence of collagen is present in the dermis (D) as is invariably seen in fluorescence studies of skin sections.

to that of intact OKT6, establishing that staining was due to specific antibody binding and not to Fc receptor binding. Finally, all anti-T cell antibodies exhibited bright staining of interfollicular zones in control lymph node sections in indirect immunofluorescence assays conducted in parallel with the skin sections.

## DISCUSSION

These studies demonstrate that dendritic epidermal cells express a distinctive surface differentiation antigen identified by OKT6 which coexists with, but is different from, Ia on their cytoplasmic membranes. Because Langerhans cells comprise at least the majority of Ia-positive epidermal cells (3–7), it is now clear that they are also OKT6 positive. The absence of OKT6 reactivity with peripheral blood monocytes of normal adult humans (21) raises two intriguing possibilities. First, Langerhans cells may represent a cell lineage separate from that of classical monocytes. Alternatively, a subset of circulating monocytes may localize in the epidermis and first express the differentiation antigen in that microenvironment. The reactivity of intrathymic lymphocytes with OKT6 does not necessarily imply a common derivation of Langerhans cells and thymocytes because cells of disparate lineage may express a common differentiation antigen. For example, murine T cells and brain neurons both express the Thy-1 antigen (27).

Steinman *et al.* (28) have described a population of dendritic cells in murine spleen, lymph nodes, thymus, and liver whose relationship to Langerhans cells is not clear. Although these cells resemble Langerhans cells by being Ia positive, dendritic, poorly phagocytic, and potent stimulators of the mixed leukocyte reaction, they lack the Fc receptors, ATPase activity, and Birbeck granules characteristic of Langerhans cells. Preliminary studies in our laboratory suggest that human lymph nodes contain a population of OKT6-positive dendritic cells, and it will be important to determine whether they exhibit other features characteristic of epidermal Langerhans cells or Steinman cells.

Langerhans cells have been implicated in several types of T cell–epidermis interactions. The epidermotropic variant of cutaneous T-cell lymphoma, the only lymphoreticular malignancy of adult humans regularly demonstrated to be of T-cell origin

(29, 30), characteristically contains intraepidermal collections of neoplastic T cells which may congregate around Langerhans cells (31). These neoplastic T cells frequently have functional (32, 33) and phenotypic (34) features of helper T cells and, in at least one patient (35), have receptors for Ia antigens.

The concept that there is an important relationship between the helper T cell and the Langerhans cell in the skin is further supported by two recent studies of delayed type hypersensitivity. In the first set of experiments, the capacity of the host to become contact sensitized correlated with the presence of epidermal Langerhans cells. Indeed, attempts to contact-sensitize at skin sites devoid of Langerhans cells resulted in a state of specific unresponsiveness to subsequent challenge (10). A separate report indicated that, when injected intravenously into a guinea pig, hapten-conjugated epidermal cells enriched for Langerhans cells induced a state of immunity to the hapten (36). This was in distinction to the state of tolerance induced by intravenous injection of hapten-conjugated peritoneal macrophages. Thus, the Langerhans cell may represent a unique type of cell, similar to the macrophage in function but primed to react preferentially with cells of the helper T-cell lineage.

Studies of Langerhans cells have been severely hampered by the relative difficulty of isolating them from skin. They comprise 5% of the cells in normal epidermis and, with current technology, epidermal cell suspensions rarely can be enriched for Langerhans cells beyond 35% (8). The availability of a monoclonal antibody that reacts with Langerhans cells, but not with other epidermal cells, normal blood T cells, B cells, or monocytes, should greatly simplify the investigation of this important cell population. Fluorescence-activated cell sorting using OKT6 should permit isolation of Langerhans cells from epidermis in nearly pure form, and *in situ* identification of these cells in tissue section can be expected to facilitate their characterization in disease states.

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