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Inflammatory Cell Types in Normal Human Epidermis—An Immunohistochemical and Morphometric Study

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Inflammatory cells in 15 specimens of normal human epidermis were selectively stained by a monoclonal antibody immunoperoxidase technique. The quantitative assessment using an interactive image analysis system revealed OKT 11 positive cells (T lymphocytes) and Leu 2a positive cells (suppressor/cytotoxic cells). As there was no significant difference in the distribution of these markers, helper/inducer cells obviously are not present in considerable amounts. OKM 5 positive cells outnumbered OKM 1 positive cells, indicating the presence of a OKM 5+, OKM 1- macrophage subset. The epidermal dendritic cells clearly showed a striking heterogeneity regarding the expression of HLA-DR (62% of OKT 6-positive cells) and Leu 3a (47%), suggesting the existence of immunologically distinct subsets of human epidermal dendritic cells. *Key words: T-lymphocytes; Langerhans' cell subsets, Immunoperoxidase method; Quantification.* (Received May 29, 1985.)

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The epidermis is considered to play a crucial role in immunological defense of the organism (1) and is known to harbour immunologically competent cells. Monoclonal antibody techniques are available to stain well defined surface markers of lymphocytes and 'accessory' cells. Thus different cell populations can be distinguished in situ by immunohistochemical methods.

There are several reports concerning the quantity of Langerhans' cells in normal human epidermis (2, 3). However, the distribution of other inflammatory cells has not been considered up to now.

We investigated the relative proportions of Langerhans' cells—regarding different Langerhans' cell markers—lymphocyte subsets, and macrophages in normal human epidermis by immunohistochemical and morphometric methods.

MATERIAL AND METHODS

Specimens

Fifteen specimens of clinically normal appearing skin were obtained during surgery for neoplastic skin lesions. The biopsies were taken under local anesthesia from various sites (trunk, 6 cases; face and neck, 6; extremities, 4). The specimens were snap frozen in liquid nitrogen and stored ad −70°C.

Immunoperoxidase procedure

Five μm cryostat sections were air dried, fixed in acetone for 10 min and mounted with the primary mouse antibody. The monoclonal antibody panel is listed in Table I. After careful rinsing rabbit-anti-mouse peroxidase conjugate and swine-anti-rabbit peroxidase conjugate were applied as second and third step reagents. The staining reaction was achieved by aminoethylcarbazole and hydrogene peroxide. The sections were counterstained with hematoxylin and coverslipped using glycerine jelly. For further details see Huber et al. (4).

Table I. *Inflammatory cell types as defined by monoclonal antibodies in normal human epidermis*

15 cases; 5 measurements per case

Monoclonal antibody	Specificity	Mean (cells/mm^2 epidermal section)	Standard error
OKT 11[a]	Pan T-cells	46	±11
Leu 2a[b]	Suppressor/cytotoxic T-cells	25	±4
Leu 3a[b]	Helper/inducer T-cells, some Langerhans cells, macrophages	178	±10
Leu 12[b]	B-lymphocytes	–	–
OKT 6[a]	Langerhans cells, thymocytes	377	±20
OKIa 1[a]	HLA-DR-antigen; some Langerhans cells, macrophages, B-lymphocytes, activated T-lymphocytes	235	±12
OKM 1[a]	Macrophages, some T-suppressor/ cytotoxic cells, granulocytes	17	±3
OKM 5[a]	Macrophages	84	±14

[a] Ortho Pharmaceutical Corporation.
[b] Becton Dickinson.

Morphometric evaluation

Immunohistochemically labelled cells in the epidermis were counted at a magnification of 250 using an interactive image analysis system (IBAS 1, Zeiss). From each case and each antibody, five consecutive microscopic fields were evaluated and the results of each measuremenet espressed in cell number per mm^2 epidermal section. Only positive cells showing the nucleus within the section plain were counted; single dendrites were omitted. The results obtained with various antibodies were compared to each other by a 2-sided Student's *t*-test and U-test, for OKT 11 and Leu 2a the *t*-test for paired samples was additionally applied. A *p*-value of less than 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

All antibodies stained various amounts of cells within the epidermis, only Leu 12-positive cells were never observed. The most common cell type was the OKT 6-positive cell followed by HLA-DR and Leu 3a. OKM 5-, OKT 11-, Leu 2a and OKM 1-positive cells were less frequently found (Table I).

OKT 6-positive cells were significantly more numerous than HLA-DR-positive cells ($p \leq 0.0001$), which comprised 62% of the OKT 6-positive dendritic cells. As OKT 6 is present on Langerhans cells and indeterminate cells (5, 6), which are commonly considered to express HLA-DR antigen (7), these results indicate that there also exists a subset of OKT 6+, HLA-DR−dendritic epidermal cells. This is in agreement with Claudy & Rouchouse 1984 (3), Harrist et al. (8) and McKie & Turbitt 1983 (9), whereas Czernielewsky et al. (10) suggested almost all Langerhans' cells to be HLA-DR positive.

Leu 3a monoclonal antibody only labelled 47% compared with OKT 6 positive cells. The difference of the cell densities is highly statistically significant ($p \leq 0.0001$) (Fig. 1). Leu 3a has originally been designed against helper/inducer T-cells (11), but has subsequently also been found on macrophages and Langerhans' cell lines (12). As T-lymphocytes and macrophages were only found in small numbers, the major portion of the Leu 3a positive cells within the epidermis most likely belonged to the Langerhans' cell/indeterminate cell series. Furthermore, almost all Leu 3a positive cells revealed a dendritic outline and were preferentially located in the suprabasal layer. However, it is obvious

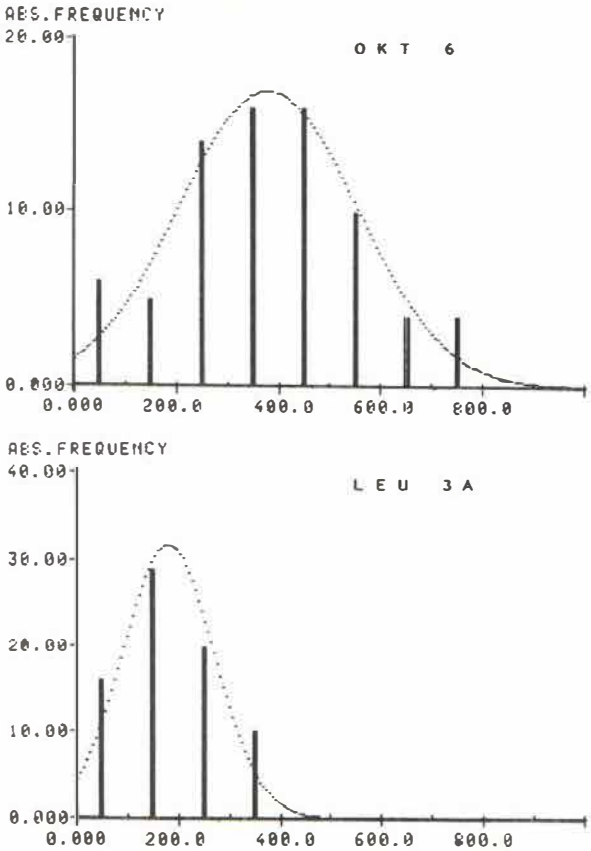

Fig. 1. OKT 6 positive cells/mm^2 epidermal section (top) compared with Leu 3a positive cells/mm^2 epidermal section (bottom). Note the difference between both distributions, indicating that Leu 3a positive cells are less common than OKT 6 positive cells. 15 cases, 5 measurements per case. Students *t*-test: $p \leq 0.0001$.

from our data, that in normal epidermis only a subset of Langerhans' cells/indeterminate cells expresses this antigen in considerable amounts.

The distribution of Leu 3a is not identical to HLA-DR, as there is a significant difference between these markers ($p = < 0.001$). However, as they represent 62% and 47% of OKT 6 positive cells, there must be at least 9% of epidermal dendritic cells, which are stained by both antibodies. These findings reflect the immunohistochemical heterogeneity of Langerhans/indeterminate cells in normal human skin in situ.

There is evidence for the presence of at least three subsets: OKT 6+, HLA-DR−, Leu 3a−; OKT 6+, HLA-DR+, Leu 3a−; and OKT 6+, HLA-DR+, Leu 3a+. Whether these phenotypes represent transitional developmental stages or mediate different immunological functions like dendritic epidermal cells in the mouse system (13, 14), remains to be determined.

Regarding different body regions, OKT 6 positive cells and HLA-DR positive cells were significantly less frequently found on the extremities (OKT 6: 255±30; HLA-DR: 161±18) than in biopsies from face and neck (OKT 6: 464±33; HLA-DR: 261±19) or trunk (OKT 6: 387±23; HLA-DR: 269±18). Leu 3a positive cells were more numerous on face and neck (214±19) than on other regions (trunk: 150±13; extremities: 143±20).

Though OKT 11 positive cells (pan T-cells) were slightly more numerous than Leu 2a positive cells (suppressor/cytotoxic cells), the difference was not statistically significant (U-test, students *t*-test and *t*-test for paired samples: $p > 0.05$). These findings indicate, that Leu 2a positive cells represent the major portion of intraepidermal lymphocytes in normal

human skin. The presence of intraepidermal T-helper cells cannot be deduced from our data. The immunohistological detection of lymphocytes in normal human epidermis is in agreement with the ultrastructural demonstration of lymphocytes crossing the basement membrane in normal skin (15).

OKM 1 only stained cells at a proportion of 1 : 22 compared to OKT 6 positive cells, and were even less numerous than Leu 2a positive cells ($p=<0.05$). The OKM 1 positive cells may represent either epidermal macrophages or the OKM1+, Leu 2a + positive lymphocyte subset previously described (16). A granulocytic nature of the OKM 1-positive cells could be ruled out on nuclear morphology.

OKM 5 positive cells were significantly more numerous than OKM 1 positive cells ($p\leq0.01$) indicating the presence of OKM 1−, OKM 5+ macrophages. This subset had previously been described in the peripheral blood and had been found to trigger the autologous mixed lymphocyte reaction (17). Both OKM 1 positive and OKM 5 positive cells were almost exclusively found in the basal layer.

For T-lymphocytes and macrophages, no significant regional variations were evident.

Our study demonstrates, that inflammatory cell subsets can be selectively quantified in normal human epidermis by an immunohistological and morphometric approach, which might be useful for comparison with pathological conditions.

We conclude, that normal human epidermis consistently harbours T-suppressor/cytotoxic cells and macrophages. T-helper/inducer cells and B-cells do not occur in considerable amounts. For Langerhans' cells/indeterminate cells, a striking heterogeneity regarding immunohistochemical staining properties is evident.

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REFERENCES

1. Streilein JW. Skin-associated lymphoid tissues (SALT): Origins and functions. J Invest Dermatol 1983; 80/Suppl: 12–16.
2. Berman B, Chu VL, France DS, Dotz WI, Petroni G. Anatomical mapping of epidermal Langerhans' cell densities in adults. Br J Dermatol 1983; 109: 533–558.
3. Claudy AL, Rouchouse B. Langerhans' cell and vitiligo: quantitative study of T 6 and HLA-DR antigen-expressing cells. Acta Derm Venereol (Stockh) 1984; 64: 334–336.
4. Huber H, Pastner D, Gabl F. Laboratoriumsdiagnose hämatologischer Erkrankungen. 1. Hämatologie und Immunhämatologie. Berlin, Heidelberg, New York, Tokyo: Springer, 1983; 464-467.
5. De Panfilis G. ATPase, antigens detected by monoclonal antibodies, ultrastructural characters: which is the best marker for Langerhans' cells? Arch Dermatol Res 1983; 275: 407-411.
6. Fithian E, Kung P, Goldstein G, Rubenfeld M, Fenoglio C, Edelson R. Reactivity of Langerhans' cells with hybridoma antibody. Proc Natl Acad Sci USA 1981; 78: 2541–2544.
7. Wolff K, Stingl G. The Langerhans' cells. J Invest Dermatol 1983; 80: 017–021.
8. Harrist TJ, Muhlbauer JE, Murphy GF, Mihm MC, Bhan AK. T 6 is superior to Ia (HLA-DR) as a marker for Langerhans' cells and indeterminate cells in normal epidermis: a monoclonal antibody study. J Invest Dermatol 1983; 80: 100–103.
9. MacKie RM, Turbitt ML. Quantitation of dendritic cells in normal and abnormal human epidermis using monoclonal antibodies directed against Ia and HTA antigens. J Invest Dermatol 1983; 81: 216-220.
10. Czernielewski JM, Schmitt D, Faure MR, Thivolet J. Functional and phenotypic analysis of isolated human Langerhans' cells and indeterminate cells. Br J Dermatol 1983; 108: 129–138.
11. Evans RL, Wall SW, Platsoucas CO, Siegal FP, Fikrig SM, Testa CM, Good RA. Thymus-dependent membrane antigens in man: inhibition of cell mediated lympholysis by monoclonal antibodies against TH 2 antigen. Proc Natl Acad Sci USA 1981; 78: 544–548.

12. Wood GS, Warner NL, Warnke RA. Anti Leu 3/T4 antibodies react with cells of monocyte/macrophage and Langerhans' lineage. J Immunol 1983; 131: 212–216.
13. Bergstresser PR, Tigelaar RE, Dees JH, Streilein JW. Thy-1 antigen bearing dendritic cells populate murine epidermis. J Invest Dermatol 1983; 81: 286–288.
14. Tschachler E, Schuler G, Hutterer J, Leibl H, Wolff K, Stingl G. Expression of Thy-1 antigen by murine epidermal cells. J Invest Dermatol 1983; 81: 282–285.
15. Warfel KA, Hull MT. Migration of lymphocytes through the cutaneous basal lamina in normal skin: An ultrastructural study. Anat Rec 1984; 208: 329–355.
16. Ceuppens JL, Gualde N, Goodwin JS. Phenotypic heterogeneity of the OKM 1-positive lymphocyte population: Reactivity of OKM 1 monoclonal antibody with a subset of the suppressor/cytotoxic T-cell population. Cell Immunol 1982; 69: 150–165.
17. Knowles DM, Tolidjian B, Marboe C, D'Agati V, Grimes M, Chess L. Monoclonal anti-human monocyte antibodies OKM 1 and OKM 5 possess distinctive tissue distributions including differential reactivity with vascular endothelium. J Immunol 1984; 132: 2170–2173.