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The simultaneous presence of Langerhan's cell and interdigitating cell antigenic markers on inflammatory dendritic cells

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SUMMARY

Immunohistological studies have been performed on tissues from various dermatological conditions using two monoclonal antibodies, RFD1 and NA1/34. These reagents were used to determine whether antigen expression restricted to interdigitating cells ($RFD1^+$) and Langerhan's cells ($NA1/34^+$) in normal tissues might occur together on dendritic cells involved in cutaneous inflammatory reactions.

The results presented demonstrate that in psoriasis, allergic contact dermatitis and atopic dermatitis a proportion of the inflammatory dendritic cells express both antigens.

Keywords inflammatory dendritic cells Langerhan's cells interdigitating cells RED1 and NA1/34 monoclonal antibodies

INTRODUCTION

Interdigitating reticulum cells (IDC) and Langerhan's cells (LC) are both considered as macrophage-like dendritic cells which act as antigen presenting cells (APC) in immunological reactions (Poulter 1983a; Poulter & Janossy 1985). In many respects these two cell types have a similar morphology, including the possession of dendrites. They both bear HLA-DR antigen (Klareskog *et al.*, 1977; Forsum *et al.*, 1985), exhibit positive reactions for S-100 protein (Rowden, Bourdreau & Higley, 1985) and are both derived from the bone marrow (Katz, Tamaki & Sachs, 1979; Humphrey, 1981). Despite this, the precise relationship between these two cells remains obscure.

Immunohistological studies have revealed phenotypic distinctions between LCs which are stained with the thymocyte marker OKT6 (Fithian *et al.*, 1981) and IDCs which are identified with the monoclonal antibody (MoAb) RFD1 (Poulter *et al.*, 1984a; Poulter & Janossy 1985). In normal tissues LC and IDC occupy different tissue distributions, the LCs appearing in the epidermis and other epithelia, while IDCs are restricted to the T cell areas of lymphoid tissue and the cortico-medullary junction of the thymus (Janossy *et al.*, 1981). In diseased tissues, however, both cell types may appear in the same location (Poulter *et al.*, 1984a).

It has been suggested that surface antigen characteristics of functionally mature dendritic cells may be influenced by the local microenvironment (Balfour *et al.*, 1981; Poulter & Janossy, 1985) and that the differences between LCs and IDCs may be more morphological than functional. Recent studies of various dermatoses, in which both LCs and IDCs occurred, offered the opportunity to determine whether in diseased tissues LC and IDC specific membrane antigens could both occur on

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the same cell. Using double immunofluorescence labelling we have studied the pattern and distribution of antigens identified with MoAbs RFD1 and NA1/34 (a T6 equivalent) on dendritic cells in inflammatory skin lesions of atopic dermatitis, allergic contact dermatitis and psoriasis.

MATERIALS AND METHODS

Samples. The investigation used lesional tissue from four patients with atopic dermatitis, four patients with allergic contact dermatitis and four patients with psoriasis. The diagnosis of atopic dermatitis was established by standard clinical criteria (Hanifin & Rajka, 1980) and histopathological features. The presence of contact dermatitis was based on clinical history and confirmed by positive patch test examinations. The diagnosis of psoriasis was based on clinical features supported by typical histopathological findings. In all cases recent acute lesions were selected for biopsy.

Double immunofluorescence studies. Double immunofluorescence labelling was performed using as first layers a combination of MoAbs RFD1 of IgM class and NA1/34 (OKT6 equivalent; kindly supplied by Dr A. McMichael, Oxford, UK) of IgG class. These were followed by incubation with anti-IgG/FITC (Fluorescein isothiocyanate) and anti-IgM/TRITC (Tetraethyl rhodamine isothyocyanate) as second layers. Reactions were performed as fully described previously (Poulter *et al.*, 1983b). The tissue reactivity patterns of MoAbs RFD1 and NA1/34 in normal samples has been fully described, (Poulter *et al.*, 1984a; Poulter & Janossy, 1985). Preparations were mounted in buffered glycerol and viewed in a Zeiss microscope equipped with epifluorescent illumination and selective filters for FITC and TRITC. By counting the total number of cells positive for each antibody and those that were doubly labelled, the proportions of cells possessing both markers were quantified.

RESULTS

Each specimen studied exhibited a mononuclear cell infiltration of the dermis typical of the respective clinical condition. In all cases the dermal inflammatory infiltrate contained a significant number of IDCs labelled with MoAb RFD1 which were also labelled with MoAb NA1/34. The majority of cells positive for both antibodies were located near the dermo-epidermal junction with a few scattered in the epidermis (Fig. 1). The percentage of RFD1$^+$ cells that were also NA1/34$^+$ was between 30 and 40% in atopic dermatitis, between 40 and 50% in psoriasis and between 25 and 35% in allergic contact dermatitis. The percentage of NA1/34$^+$ cells that were also positive for RFD1 represented 80% of NA1/34$^+$ cells in the dermis, but only 10–20% of those cells located in the epidermis. There was no significant difference between the percentages of NA1/34$^+$/D1$^+$ cells found in atopic dermatitis, psoriasis nor allergic contact dermatitis. Although difficult to determine using immunofluorescence, it was estimated that the double labelled cells constituted about 5% of total infiltrating cells in atopic dermatitis and allergic contact dermatitis, and 5–10% of infiltrating cells in psoriasis.

DISCUSSION

In this study we have demonstrated that a number of dendritic cells bear antigenic characteristics of both LC and IDC in inflammatory skin lesions of atopic dermatitis, allergic contact dermatitis and psoriasis, conditions in which increased numbers of both IDCs and LCs have repeatedly been reported (Poulter *et al.*, 1984b; Uno & Hanifin, 1980; Zachary *et al.*, 1984; Silberberg-Sinakin *et al.*, 1976). In normal skin, LC are mainly located in the epidermis (Wolff & Stingl, 1983) but occasionally are observed in the dermis (Poulter *et al.*, 1982). In lesional skin of inflammatory dermatoses, dermal LCs are numerous (Poulter & Janossy, 1985). On the other hand normal skin contains no detectable IDCs, (i.e., no RFD1$^+$ cells; Poulter *et al.* in preparation). However, in the skin lesions of patients with dermatoses IDCs are abundant in the dermis (Poulter & Janossy, 1985),

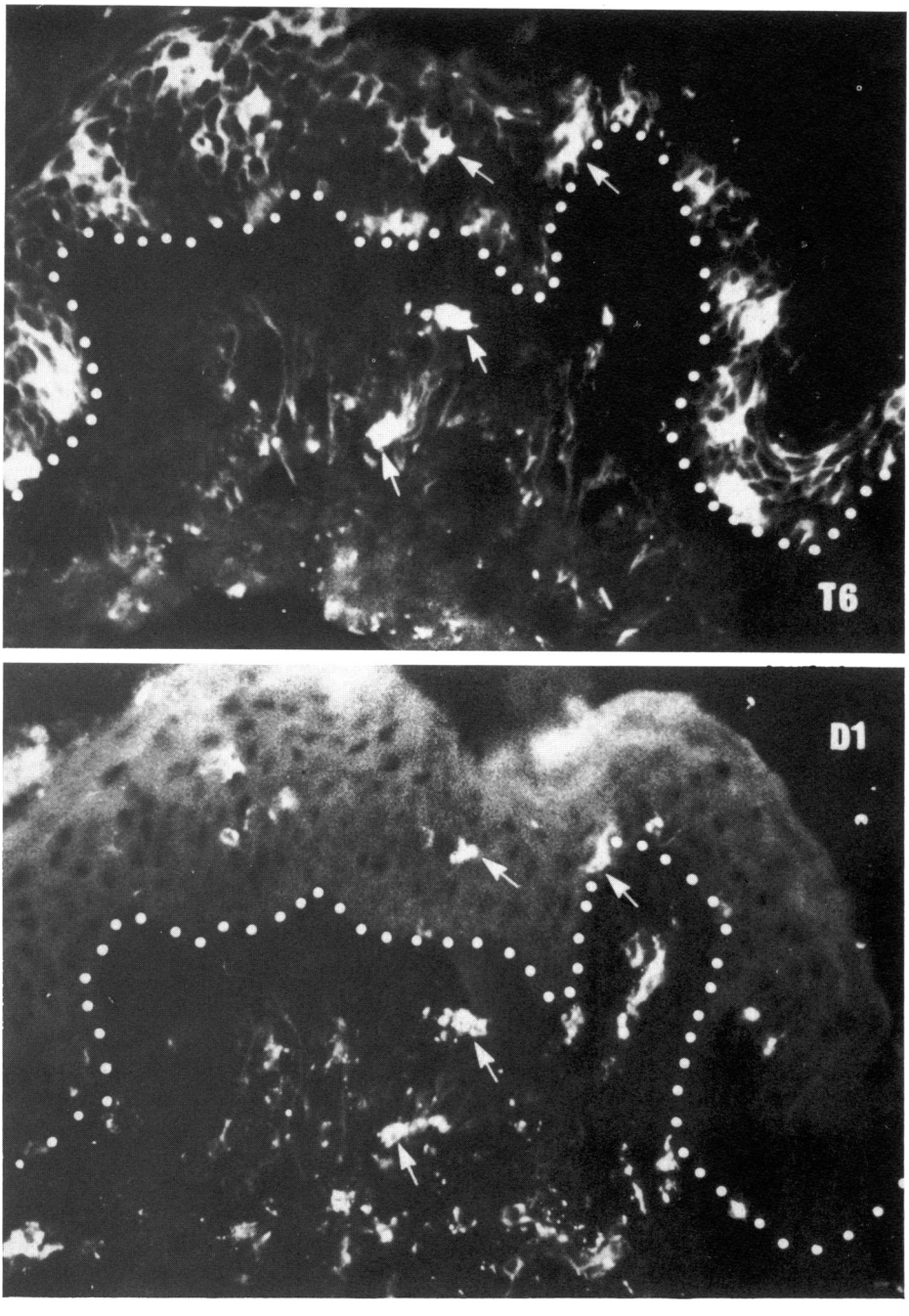

Fig. 1. Combined immunofluorescence study using MoAbs RFD1 and NA1/34 (T6 antigen) on lesional skin from a patient with atopic dermatitis. The same area is photographed using selected filters for FITC and TRITC. It is revealed that a proportion of dendritic cells both in the dermis and epidermis express both antigens (some examples are arrowed). × 250.

and have been observed occasionally in the epidermis (Bos *et al.*, 1985). Migration of LCs across the dermo-epidermal junction is assumed to occur and may be observed ultrastructurally. There may also be similar traffic of IDCs. The numerous common properties of LCs and IDCs including ultrastructural features have already been discussed. Until recently, however, a distinctive ultrastructural marker described by Birbeck, Breatnach and Everall (1961) was considered to occur only in LCs. However, this so-called Birbeck granule has been demonstrated in a proportion of IDC (Breathnach, 1980) which produce these granules to a variety of stimuli (Breathnach, 1977).

Our finding that antigenic determinants previously considered to be exclusive to one or other type of APC, may occur in the same dendritic cell strengthens the concept of close histogenetic and functional links between LCs and IDCs and provides further evidence to support the concept of inter-differentiation between these cells dependent upon biological stimulus or environment.

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REFERENCES

BALFOUR, B.M., DREXHAGE, H.A., KAMPERDIJK, E.W.A. & HOEFSMIT, E.C.M. (1981) Antigen-presenting cells, including Langerhans cells, veiled cells and interdigitating cells. In: *Microenvironments in Haemopoietic and Lymphoid Differentiation.* (ed. by R. Porter & J. Whelan). p. 281, Ciba Foundation Symposium, 84. Pitman, London., UK.

BIRBECK, M.S., BREATNACH, A.S. & EVERALL, J.D. (1961) An electron microscope study of basal melanocytes and high level clear cells (Langerhans cells) in vitiligo. *J. Invest. Dermatol.* **37,** 51.

BOS, J.D., HUISMAN, P.M., KRIEG, S.R., & FABER, W.R. (1985) Pityriasis rosea (Gibert): abnormal distribution pattern of antigen presenting cells *in situ. Acta Dermatoven,* **65,** 132.

BREATHNACH, A.S. (1977) Electron micrographs from a collection. *Clin. exp. Dermatol.* **2,** 1.

BREATHNACH, A.S. (1980) Branched cells in the epidermis: an overview. *J. Invest. Dermatol.* **75,** 6.

FITHIAN, E., KUNG, P.C.L., GOLDSTEIN, G. & RUBENFELD, M. (1981) Reactivity of Langerhans cells with hybridoma antibody. *Proc. natn. Acad. Sci. USA.* **78,** 2541.

FORSUM, V., CLAESON, K., HJEIM, E., KARLSSON-PARRA, A., KLARESKOG, L., SCHEYNIUS, A, & TJERNLUND, U. (1985) Class II transplantation antigens: distribution in tissues and involvement in disease. *Scand. J. Immunol.* **21,** 389.

HANIFIN, J.M. & RAJKA, G. (1980) Diagnostic features of atopic dermatitis. *Acta Dermatovener (Suppl.)* **92,** 44.

HUMPHREY, J.H., (1981) Differentiation of function among macrophages. In: *Microenvironments in Haemopoietic and Lymphoid Differentiation.* p. 302, Ciba Foundation Symposium 84, Pitman, London, UK.

JANOSSY, G., THOMAS, J.A., GOLDSTEIN, G. BOLLUM, F.J. (1981) The human thymic microenvironment. In: *Microenvironments in Haemopoietic and Lymphoid Differentiation.* p. 193, Ciba Foundation Symposium 84, Pitman, London, UK.

KATZ, S.I., TAMAKI, K. & SACHS, D.H. (1979) Epidermal Langerhans cells are derived from cells originating in bone marrow. *Nature,* **284,** 324.

KLARESKOG, L., TJERNLUND, U., FORSUM, V. & PETERSON, P.A. (1977) Epidermal Langerhans cells express Ia antigen. *Nature,* **268,** 248.

POULTER, L.W., SEYMOUR, G.J., DUKE, O., JANOSSY, G. & PANAYI, G. (1982) Immunohistological analysis of delayed-type hypersensitivity in man. *Cell. Immunol.* **74,** 358.

POULTER, L.W. (1983a) Antigen presenting cells *in situ*: their identification and involvement in immunopathology. *Clin. exp. Immunol.* **53,** 513.

POULTER, L.W., CHILOSI, M., SEYMOUR, G.J., HOBBS, S. & JANOSSY, G. (1983b) Immunofluorescence membrane staining and cytochemistry, applied in combination for analysing cell interactions in situ. In: *Immunocytochemistry: Practical Applications in Pathology and Biology.* (ed. by J. M. Polak & S. van Noorden). p. 233, J. Wright PSG, London, UK.

POULTER, L.W., COLLINGS, L.A., TUNG, K.S. & WATERS, M.J. (1984a) Parasitism of antigen presenting cells in hyperbacillary leprosy. *Clin. exp. Immunol.* **55,** 511.

POULTER, L.W., RUSSELL-JONES, R. & HOBBS, S. (1984b) The significance of antigen presenting cells in psoriasis. In: *Immunodermatology.* (ed. by D. C. MacDonald) Butterworth, Guildford, UK.

POULTER, L.W. & JANOSSY, G. (1985) The involvement of dendritic cells in chronic inflammation. *Scand. J. Immunol.* **21,** 401.

ROWDEN, G., BOURDREAU, S., HIGLEY, H. (1985) Langerhans cells and extra-epidermal dendritic cells. *Scand. J. Immunol.* **21,** 471.

SILBERBERG-SINAKIN, I., THORBECKE, G.J., BAER, R.L., ROSENTHAL, S.A. & BEREZOWSKY, V. (1976) Antigen bearing Langerhans cells in the skin dermal lymphokines and lymph nodes. *Cell. Immunol.* **25,** 137.

UNO, H. & HANIFIN, J. (1980) Langerhans cells in acute and chronic epidermal lesions of atopic dermatitis, observed by L-Dopa histofluorescence, glycol methacrylate thin section and electron microscopy. *J. Invest. Dermatol.* **75,** 52.

WOLF, K. & STINGL, G. (1983) The Langerhans cell. *J. Invest. Dermatol.* **80,** 0175.

ZACHARY, C.B., RAUCH, H.-J., ALLEN, M.H. & MACDONALD, D.M. (1984) Antigen presenting cells in atopic dermatitis—evidence for a cell mediated immune response in the pathogenesis. *Br. J. Dermatol.* **111,** 716.