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Ia^+ dendritic cells lining the dermo-epidermal junction in bullous skin disorders, associated with the deposition of immune complexes

M. Drijkoningen [1], C. de Wolf-Peeters [1], J.J. van den Oord [1], A. Vanneste [1], H. Degreef [2] and V. Desmet [2]

[1] Department of Pathology and [2] Department of Dermatology, U.Z. St. Rafael, University of Leuven, Belgium

Summary. Skin biopsies from 34 patients, presenting with a variety of bullous skin disorders were investigated, using routine light microscopy and immunohistochemistry.

In bullous skin diseases characterized by deposition of complement factors (CF) and/or immunoglobulins (Ig), a monolayer of $OKIa_1^+$, OKT_6^-, OKM_1^- dendritic cells was found at the dermo-epidermal junction. Retrospectively, these cells were easily recognized on paraffin embedded, H & E stained material. In bullous skin disorders, showing no deposition of CF and/or Ig, this monolayer of dendritic cells was lacking.

It is suggested that these $OKIa_1^+$, OKT_6^-, OKM_1^- dendritic cells at the dermo-epidermal junction represent some type of antigen presenting cells, not corresponding to Langerhans cells, veiled cells or indeterminate cells.

Key words: Bullous skin disorders – Immunohistochemistry – Antigen presenting cells – Langerhans cells

Introduction

Several skin disorders of varying aetiology are characterized by the formation of bullae, resulting from different pathogenic mechanisms. In some of these bullous skin disorders, immunoglobulins (Ig) and/or complement factors (CF) can be demonstrated, the pattern of deposition being characteristic for the disease entity (Table 1). Other bullous dermatopathies do not show this deposition of Ig and/or CF within the skin biopsy.

In order to elucidate this diversity, we investigated the cellular composition of the inflammatory infiltrate and the appearance of epidermal Langer-

Offprint requests to: M. Drijkoningen at the above address

[1] Aspirant NFWO (Nationaal Fonds voor Wetenschappelijk Onderzoek)

Table 1

Diseases	Immunofluorescence results	References
Pemphigus vulgaris Pemphigus foliaceus	Interkeratinocytic positivity for IgG, C_3 and properdin in a honeycomb pattern	Chorzelski et al. (1979a)
Bullous pemphigoid	Linear positivity for IgG, C_3, properdin and fibrinogen at the dermo-epidermal junction	Chorzelski et al. (1979b)
Dermatitis herpetiformis	Granular deposits of IgA, and to a lesser degree, of IgG, C_3 and properdin, in the tip of the dermal papilla	Fry et al. (1979)
Linear IgA bullous dermatosis	Linear deposition, predominantly of IgA, in the subepithelial region	Chorzelski et al. (1979c)
Herpes gestationis	Linear positivity for C_3 at the dermo-epidermal junction and sometimes for C_{1Q}, properdin and IgG	Provost et al. (1979)

hans cells in various bullous skin diseases, applying monoclonal antibodies directed against lymphocytes and monocytes/macrophages.

Materials and methods

Thirty four skin biopsies, taken for diagnostic purposes from patients presenting with a variety of bullous skin disorders were investigated. These included: bullous pemphigoid (BP; 11 cases), pemphigus vulgaris (PV; 6 cases), erythema exsudativum multiforme (EEM; 3 cases), pemphigus foliaceus (PF; 2 cases), toxic epidermal necrolysis (TEN; 1 case), epidermolysis bullosa (EB; 1 case), insect bite (1 case), dermatitis herpetiformis (DH; 6 cases), herpes gestationis (HG; 1 case), linear IgA dermatitis (1 case) and herpes virus infection (1 case).

The biopsies were taken from different skin areas. Part of the material was fixed in Bouin's solution and processed for routine light microscopy. A representative part of the biopsy was quickly frozen in liquid nitrogen-cooled isopentane and stored at −80° C until used for immunohistochemistry.

The presence of Ig and CF was demonstrated by an indirect immunofluorescence technique, using rabbit-antisera directed to IgA, IgM, IgG, C_3, C_{1Q}, C_4, properdin and fibrinogen as the first, and FITC-labelled goat-antirabbit Ig as the second layer. The antisera against C_3, C_4 and fibrinogen were purchased from Behringwerke, Marburg, Western Germany; those against C_{1Q}, IgA and properdin from Nordic, Tilburg, The Netherlands; and those against IgG and IgM from Dakopatts s/a, Copenhagen, Denmark. The rabbit-antihuman IgM and the FITC-labelled goat-antirabbit Ig were purchased from Organon Teknika, Oss, The Netherlands.

For the demonstration of cell-surface antigens, an indirect immunoperoxidase procedure as described by Mason et al. (1982) was performed. The following monoclonal antibodies were applied: $OKIa_1$, directed against the common framework of HLA-Dr or Ia-like antigens (Reinherz et al. 1979a); OKT_6, reactive with Langerhans cells (Fithian et al. 1981; Murphy et al. 1981; Chu et al. 1982) and 70% of the cortical thymocytes (Reinherz et al. 1980a); BA_1, identifying mature B-lymphocytes (Abramson et al. 1981); OKT_4, detecting the helper/inducer T-lymphocyte subset (Reinherz et al. 1979b; Ledbetter et al. 1981); OKT_8, directed to the suppressor/cytotoxic T-lymphocyte subset (Reinherz et al. 1980b); OKM_1, reacting with monocytes and polymorphonuclear granulocytes (Breard et al. 1980) and DRC_1, identifying dendritic reticulum cells (Naiem et al. 1983). The OK-series of monoclonal antibodies was purchased from Ortho Pharmaceuticals Co., Raritan, NJ; BA_1 from Hybritech, La Jolla, CA; and DRC_1 from Dakopatts s/a, Copenhagen, Denmark. A monoclonal antibody TO_5,

directed against complement (C_{3b}) receptors (Gerdes et al. 1982), was kindly donated by D.Y. Mason, Nuffield Department of Pathology, Oxford, U.K.

Sections were incubated with monoclonal antibodies for 30 min, washed in three changes of phosphate buffered saline, pH 7,2 and incubated for another 30 min with peroxidase-conjugated rabbit-antimouse Ig (Dakopatts s/a, Copenhagen, Denmark). After a second wash in three changes of phosphate buffered saline, pH 7,2, the reaction product was developed, using 3-amino-9-ethyl-carbazole and H_2O_2, according to Graham et al. (1965).

Results

From the immunofluorescence results, the biopsies were divided into two groups. Group I contained bullous skin disorders lacking demonstrable deposition of Ig and/or CF and consisted of cases of erythema exsudativum multiforme (3), toxic epidermal necrolysis (1), epidermolysis bullosa (1), insect bite (1) and herpes virus infection (1). Group II was composed of bullous dermatopathies characterized by the deposition of Ig and/or CF at the dermo-epidermal junction or within the epidermis and consisted of cases of bullous pemphigoid (11), pemphigus vulgaris (6), pemphigus foliaceus (2), herpes gestationis (1), dermatitis herpetiformis (6) and linear IgA dermatitis (1).

In most cases of both groups, OKT_6^+ epidermal Langerhans cells showed well developed dendritic processes and were evenly distributed both in the bullous region and in the adjacent intact epidermis. In all cases, $OKIa_1$ visualized a fewer number of epidermal Langerhans cells than did OKT_6. In both groups, few inflammatory cells, mainly corresponding to OKT_4^+ and OKT_8^+ T-lymphocytes and OKM_1^+ polymorphonuclear granulocytes were present in between the keratinocytes.

In the majority of cases, a perivascular inflammatory infiltrate of variable density was found in the upper part of the dermis, that consisted mainly of $OKIa_1^+$ lymphocytes with a predominance of T-cells. OKT_4^+ helper/inducer T-cells outnumbered OKT_8^+ cytotoxic/suppressor T-cells. A few OKM_1^+ monocytes were also present. Variable numbers of OKT_6^+ cells, intermingled with the dermal infiltrate, were observed. These dermal OKT_6^+ cells showed dendritic processes, mainly in biopsies of group II.

At the dermo-epidermal junction in the area surrounding the bulla $OKIa_1^+$, OKT_6^-, OKM_1^- cells showing easily recognizable dendritic processes, were observed in 23 out of 27 cases of group II (in 2 cases of bullous pemphigoid, 1 case of dermatitis herpetiformis and 1 case of pemphigus vulgaris these cells were absent). They formed a one-cell thick, more or less continuous layer, lining the dermo-epidermal junction (Fig. 1). They reacted neither with antibodies detecting dendritic reticulum cells (DRC_1), nor with antibodies detecting complement receptors (TO_5). On paraffin embedded, H & E stained sections, these mononuclear elements could be recognized retrospectively as a monolayer of elongated cells with clear cytoplasm and a fusiform nucleus. On top of this monolayer, vacuolar alteration at the dermo-epidermal interface was found (Fig. 2). These cells could not be visualized on biopsies of group I, neither with immunostaining, nor on paraffin embedded material.

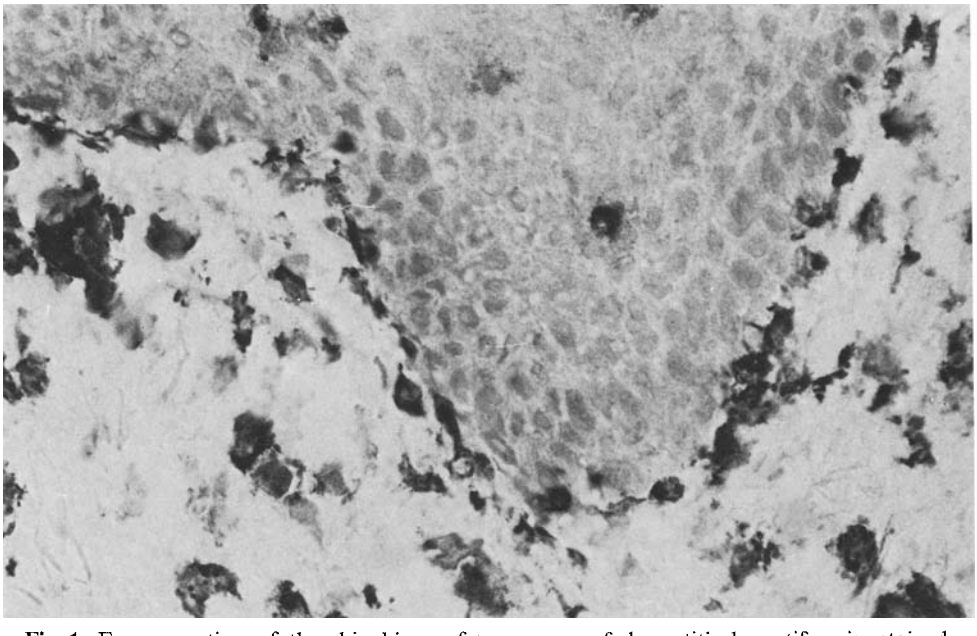

Fig. 1. Frozen section of the skin biopsy from a case of dermatitis herpetiformis, stained with monoclonal antibody $OKIa_1$. A monolayer of dendritic $OKIa_1^+$ cells, localized at the dermo-epidermal junction is easily recognized

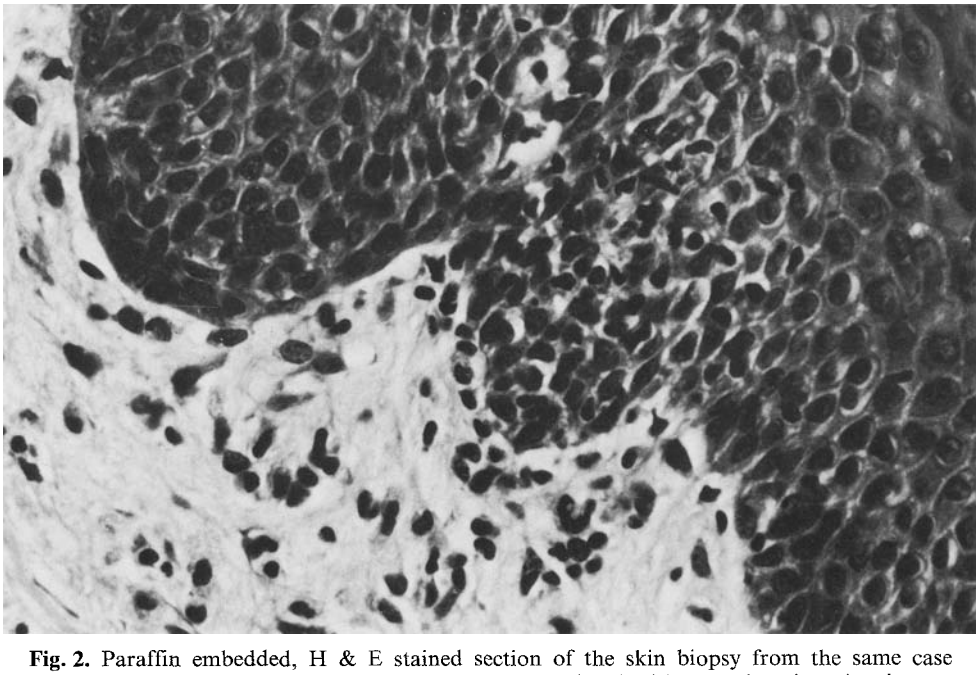

Fig. 2. Paraffin embedded, H & E stained section of the skin biopsy from the same case as in Fig. 1. A monolayer of mononuclear cells associated with vacuolar alteration is seen at the dermo-epidermal junction

Discussion

The number of epidermal Langerhans cells and the composition of the dermal inflammatory infiltrate was very similar in both groups of bullous skin disorders, except for the more pronounced dendritic appearance of the dermal OKT_6^+ cells in group II. These dermal OKT_6^+ dendritic cells may represent dermal Langerhans cells, migrating from the epidermis towards the draining lymph nodes or vice versa (Silberberg-Sinakin et al. 1976).

In almost all cases of group II, we observed hitherto undescribed $OKIa_1^+$ dendritic cells at the dermo-epidermal junction in the perilesional area. These cells did not react with OKM_1 nor with OKT_6, thus lacking the characteristic phenotype of monocytes (Breard et al. 1980) and epidermal Langerhans cells/indeterminate cells (Fithian et al. 1981; Murphy et al. 1981 a; Chu et al. 1982; Murphy et al. 1981 b) respectively. They could not be visualized by monoclonal antibody DRC_1, identifying dendritic reticulum cells (Naiem et al. 1983), and did not present C_{3b} receptors (TO_5^-), as do Langerhans cells (Stingl et al. 1977) and veiled cells (Hoefsmit et al. 1982). On paraffin embedded, H & E stained sections, these dendritic cells were easily recognizable as elongated cells with clear cytoplasm and a fusiform nucleus. In only four cases of group II these Ia^+ dendritic cells were lacking, the reason for their absence being unclear.

We do not know the exact nature of these cells. Relying on their cytologic aspect, their immunophenotype ($OKTa_1^+$, OKT_6^-, OKM_1^-, TO_5^-, DRC_1^-) and their localization at the dermo-epidermal junction in association with the deposition of Ig and/or CF, we can speculate that these cells belong to the group of dendritic, antigen-presenting cells.

In man, 4 types of dendritic antigen presenting cells have been described: dendritic reticulum cells (=follicular dendritic cells) present in lymphoid follicles; interdigitating reticulum cells present in the T-dependent area of spleen and lymph nodes, and in the thymus; epidermal Langerhans cells present in epidermis, squamous epithelia and draining lymph nodes; and lymphoid dendritic cells (Tew et al. 1982). The latter cells correspond to Steinman's dendritic cells in the mouse (Tew et al. 1982) and are supposed to be present in connective tissues of most human organs (Daar et al. 1983). These dendritic cells represent a distinct population of bone-marrow derived, thymus independent cells, not belonging to the mononuclear phagocytic system (Tew et al. 1982; Steinman and Nussenzweig 1980; Hart and Fabre 1981). They are functionally capable of presenting antigens and inducing a lymphocyte response (Poulter 1983). Lymphoid dendritic cells carry the $OKIa^+$, TO_5^- immunophenotype (Tew et al. 1982) as do the dendritic cells at the epidermal-dermal junction.

Since Ia-like glycoproteins do not define stable subsets of antigen presenting cells, but rather demarcate cells, activated in a specific manner (Unanue et al. 1984), we can speculate as well that the dendritic cells, observed at the dermo-epidermal junction represent freshly arrived, bone-marrow derived monocytes in transition to Langerhans cells (Katz et al. 1979), hav-

ing already lost the monocyte-related antigen recognized by OKM_1, but having not yet acquired the Langerhans cell related antigen recognized by OKT_6.

The localization of these $OKIa_1^+$ dendritic cells at the dermo-epidermal junction adjacent to the bulla on the one hand, and their association with the deposition of Ig and/or CF on the other, suggests their involvement in either or both of these two phenomena.

Regardless of their origin and function, these $OKIa_1^+$ dendritic cells lining the dermo-epidermal junction can be recognized on paraffin embedded, H & E stained sections. As such, the recognition of these cells in the absence of immunohistochemical data can be of some additional help in the differential diagnosis of bullous skin disorders, since their presence is related to the deposition of Ig and/or CF in the skin biopsy.

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