

INTERCELLULAR AND CIRCULATING ANTIBODIES IN PATIENTS WITH DYSKERATOSIS FOLLICULARIS, DARIER'S DISEASE

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Abstract. Skin biopsies from 6 patients, and biopsies of the palatal mucosa of 4 of these patients with dyskeratosis follicularis (DF) (Darier's disease) were examined for in vivo bound antibodies by means of a direct immunofluorescence (IF) technique. Antibodies located in the intercellular substance of the epidermis were found in the skin lesions of all patients. Immunoglobulins of the classes IgG, IgA and IgM as well as C3 were found in all lesions. No antibodies reacting with the palatal mucosa were found. Sera from 6 patients with DF and 10 control persons were tested by an indirect IF technique for circulating antibodies. Guinea pig lip and normal oral mucosa and skin were used as antigens. All patients sera and one control serum reacted with the basal cells of the guinea pig lip. Three DF sera—but no control sera—reacted with the basal cells of human oral mucosa. None of the sera reacted with human skin.

Key words: Immunofluorescence technique; Intercellular antibodies; Basal cell antibodies; Dyskeratosis follicularis

Dyskeratosis follicularis (Darier's disease) (DF) is a rare hereditary disease of unknown etiology (12). The disease primarily affects the epidermis but may involve the oral mucous membranes. The histopathological changes in DF are characterized by a disturbance in the keratinization process resulting in the formation of *corps ronds* and grains (8). Furthermore, acantholysis of the supra-basal cells leading to formation of clefts and lacunae is a prominent feature. Similar acantholysis of the supra-basal cells is found in pemphigus vulgaris. In this mucocutaneous disease, circulating antibodies reacting with the cell membrane of epithelial cells are believed to be of significance in the pathogenesis (1, 3). Immunofluorescence techniques (IF) have demonstrated in vivo bound immunoglobulins in the skin as well as in the oral mucosa (1).

The supra-basal cell acantholysis found in DF as in pemphigus vulgaris indicates that immunopathological changes may be present in the stratified squamous epithelium of patients with DF. IF investigations of DF have, to the authors' knowledge, only been mentioned in a single report (3), where no evidence of immunopathological changes were found. The purpose of the present investigation was to establish whether circulating and/or in vivo bound immunoglobulins are to be found in patients with DF. Direct and indirect IF methods were used.

MATERIAL AND METHODS

The material included 6 patients with dyskeratosis follicularis. Biopsies from involved skin were obtained from all patients and from the palatal mucosa of 4. One of the patients refused to have a biopsy taken from the palatal mucosa and no visible lesions were found in the other. The skin biopsies were taken from eruptions of hyperkeratotic papules. The tissue was immediately frozen and stored at -20°C . Sera from the DF patients and from 10 healthy control persons were collected and kept at -20°C .

The search for in vivo bound immunoglobulins was made by a direct IF technique. Unfixed cryostat sections, 5–8 μm thick, were air dried for 15 min and incubated in a moist chamber with a drop of conjugate for 30 min at 4°C . After staining with conjugates, the sections were washed in phosphate-buffered saline (PBS) pH 7.2, three times for 5 min. To ensure that the sections evaluated by the IF technique showed typical histopathological changes they were later examined under a light microscope after staining with haematoxylin and eosin.

The sera were examined for circulating antibodies by an indirect IF method. The following serum dilutions were used: 1/4, 1/8, 1/16, 1/32, 1/64, and 1/128. Controls in which the patients' sera were replaced by PBS, pemphigus vulgaris sera, or bullous pemphigoid sera were included in each staining procedure.

Cryostat sections of guinea pig lip and normal human

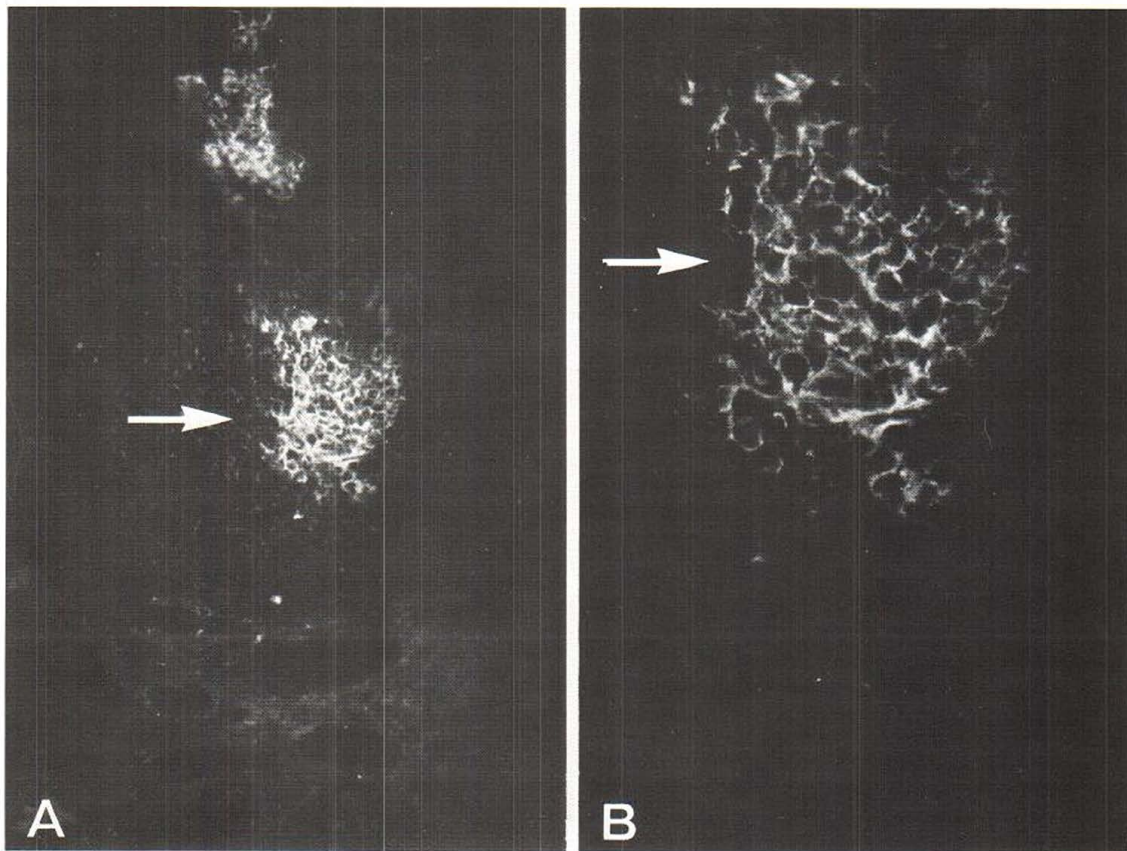


Fig. 1. (A) Direct immunofluorescence staining of a skin lesion from a patient with dyskeratosis follicularis $\times 95$. (B) The same section at higher magnification, illustrating

the staining of the intercellular areas of the squamous epithelium $\times 250$. The arrows indicate an area with acantholytic cells.

buccal mucosa as well as normal skin from an individual of blood group O were used as antigens. The tissue sections were incubated with serum at 4°C for 30 min, washed in PBS, pH 7.2, three times for 5 min and then incubated with conjugate for 30 min.

Heavy chain specific rabbit antihuman fluorescein isothiocyanate (FITC) conjugated IgG, IgA, and IgM (Dakopatts[®], Denmark) were used in both IF techniques. The specificity of the conjugates was tested on monoclonal bone marrow cells. For location of in vivo bound complement, rabbit antihuman FITC conjugated C3 was used. In the direct technique, a FITC-conjugated rabbit immunoglobulin to sheep serum IgG was used as a control conjugate. The conjugates have a protein concentration of 2.5 mg/ml and an antibody titre of 100 (1 ml conjugate absorbs 100 μg pure human immunoglobulin). The extinction ratio E_{495}/E_{278} was 0.63 ± 0.03 for all preparations. Unconjugated immunoglobulin molecules and molecules to which more than four molecules of FITC were bound were removed by gel-filtration and ion-exchange chromatography. A working titre of 1:20 was used for all conjugates.

All stainings were examined under a Leitz Orthoplan

fluorescence microscope with a Tiyoda darkfield oil immersion condensor. The primary filter was a FITC interference filter with red contrast band (Laboratory of Technical Optics, Denmark) and the secondary filter was matched to fit the primary filter (9).

RESULTS

In vivo bound immunoglobulins were found in the epidermal intercellular areas of the skin lesions in all patients (Fig. 1). Immunoglobulins of the classes IgG, IgA, and IgM as well as C3 were found in all lesions. There were no differences in the distribution of the antibodies. Positive staining reactions were confined to areas in which histopathological changes typical of the disease were located. In the majority of the sections the fluorescence was seen above the basal cell layer. The cells with fluorescent membranes appeared in haematoxylin-eosin



Fig. 2. Section of skin lesion from patient with Darier's disease stained with haematoxylin and eosin, $\times 120$. The section is identical with the one demonstrated in Fig. 1. The arrow indicates an area with acantholytic cells.

stained sections as acantholytic cells and grains (Fig. 2). In the sections stained with the control conjugate, no fluorescence of the intercellular areas of the epithelium was seen.

In the palatal mucosa no *in vivo* bound antibodies were found in any case.

The sera from all patients with DF gave staining with the cytoplasm of the basal cells in all cases when guinea pig lip was used as antigen. Even one of the control sera proved positive. When human oral mucosa was used as antigen, three of the DF sera—but none of the control sera—reacted with the basal cells (Fig. 3). No evidence of circulating antibodies against human skin components was found in any case. The circulating antibodies were of the IgG class in all sera. Titrations revealed end-point titres varying between 1/8 and 1/128. The results of the indirect IF investigations are summarized in Table I.

Table 1. Circulating antibodies to basal cells in a patient with *dyskeratosis follicularis* and in controls, related to type of antigen

	Antigen		
	Guinea pig lip	Human oral mucosa	Human skin
<i>Patients</i>			
Number with antibodies	6	3	0
Number without antibodies	0	3	6
<i>Controls</i>			
Number with antibodies	1	0	0
Number without antibodies	9	10	10

DISCUSSION

The experiments demonstrate that in the skin lesions, immunoglobulins are bound *in vivo* to the intercellular areas of the squamous epithelium. The antibodies were strictly confined to the cell membranes of the acantholytic cells and the grains in the lesions. No involvement of the neighbouring epidermis was found. This is an important immunopathological difference between DF and pemphigus vulgaris. It is noteworthy that immunoglobulins of the classes IgG, IgA, IgM, and C3 were found in all patients. The FITC-conjugated rabbit immunoglobulin to sheep serum IgG included as control in the direct IF test gave no staining reactions in sections from any of the patients. There



Fig. 3. Indirect immunofluorescence staining of human oral mucosa with sera from patient with Darier's disease, showing staining of the basal cell cytoplasm. $\times 162$.

is therefore no reason to believe that the in vivo bound antibodies are attributable to unspecific binding of rabbit immunoglobulin to human epidermis.

The demonstration of in vivo bound antibodies in this study, in contrast to a previous report, may be explained by differences in the type of lesion selected for biopsy (3). In the present study the biopsies were taken from eruptions of hyperkeratotic papules. As some of the DF lesions were rather small, care was taken that only representative sections were used. This was ensured by staining the tissue sections evaluated in the IF test with haematoxylin and eosin after the IF examination.

The lesions of the oral mucosa differed from those on the skin, as no in vivo bound antibodies were found. A possible explanation for this may be that very few cells of the grain type are found in these lesions (10, 14).

All normal human sera have been shown to contain antibodies against stratum corneum (5, 6). Studies of the permeability of squamous epithelium by horseradish peroxidase and lanthanum have demonstrated the existence of an intercellular barrier in the superficial layers of the epithelium (2, 11). The possibility therefore exists that under normal circumstances the circulating antibodies against stratum corneum are prevented from coming into contact with the antigen by the permeability barrier. In conditions like DF, with altered intercellular adhesion and in which keratinized cells are found closer to the basement membrane, the diffusion of antibodies reacting with keratinized cells might be enhanced. It is, in fact, possible that in DF the in vivo bound antibodies are identical with the antibodies against stratum corneum.

The antibodies present in the intercellular substance of the grains and parakeratotic cells are comparable to those found in the parakeratotic cell layer of psoriatic lesions (7).

Antibodies against basal cell cytoplasm have been reported to occur in approximately 25% of the examined sera when guinea pig lip is used as antigen (13). The sera in that study were from normal persons as well as patients with psoriasis, bullous pemphigoid leg ulcers, or cardiovascular diseases. The large number of positive sera in this investigation is in contrast to a study of circulating antibodies reacting with the basal cell cytoplasm in patients with drug reactions (4). Seventeen per cent of the sera were positive when guinea pig lip and human skin were used as antigens. In the con-

trol group, consisting of healthy individuals and patients with skin diseases, no positive sera were found. Even if the basal cell staining in patients with DF is very similar to that found in patients with drug reactions, its nature must be different, as no reaction with human skin was found.

Although the number of sera examined in this study is limited, circulating antibodies against basal cell cytoplasm seem to occur more often in patients with DF than in other individuals. The significance of the basal cell antibodies is unknown, especially since the target of these antibodies is different from the antibodies demonstrated in vivo.

The fact that no circulating antibodies react with the cells in which the in vivo bound antibodies are located and the absence of in vivo bound antibodies in the oral lesions, support the idea that the immunological changes demonstrated are secondary phenomena. Although not of primary significance, immunological mechanisms may be involved in the pathogenesis of DF.

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Received February 9, 1977

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