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EPIDERMAL LOCATION OF BETA-2-MICROGLOBULIN IN HUMAN SKIN

Urban Forsum and Ulla Malmnäs Tjernlund

From the Department of Dermatology, Uppsala University, University Hospital, Uppsala, Sweden

Abstract. Beta-2-microglobulin (beta$_2$-m) is associated with the epidermis in normal and pathological human skin and gives an immunofluorescence pattern similar to that of pemphigus antibodies and concanavalin A (Con-A). Inhibition experiments indicate, however, that the binding site of anti-beta$_2$-m is not identical with that of pemphigus antibodies or Con-A. It is suggested that beta$_2$-m might be an epidermal cell surface marker useful for the study of antigens associated with the epidermal cell surface.

Key words: Epidermal antigens; Beta-2-microglobulin, normal skin

Recently several groups of workers (2, 3, 4) have shown that concanavalin A (Con-A) binds to a surface saccharide in human epidermis. The relation of this surface marker to pemphigus antibodies has been investigated and found to be related to, though not identical with, the antigen that binds pemphigus antibodies (2).

A panel of such surface markers on epidermal cells detectable in cryostat sections using a suitable method such as immunofluorescence might be of great value when studying skin diseases involving the epidermis. If additional antigens can be defined, a better understanding of the cause of these diseases might evolve (6).

Recent studies on transplantation antigens on the cell surface of lymphocytes has implicated beta-2-microglobulin (beta$_2$-m) as the constant light chain of H-2 and HL-A alloantigens (6). The amino acid sequence of this protein is highly homologous to the various IgG heavy and light chain homology regions (6).

We have studied the binding of anti-beta$_2$-m to the epidermis in order hopefully to define another surface marker in epidermal skin sections. The results indicate that beta$_2$-m is a constant feature of the human epidermis.

MATERIALS AND METHODS

Skin and sera

Skin biopsies from normal and pathological skin were obtained by means of a 3 mm punch and quenching rapidly in isopentane with storage at −70°C until sectioned (usually within 24 hrs) at 6 μm and air dried before applying the staining reagents. The antisera used were stored in aliquots at −70°C until used.

Immunofluorescence technique

Fluorescein-isothiocyanate (FTC) conjugated anti-human IgG (Lot No. 075), IgM (Lot No. 044), IgA (Lot No. 015), C_3 (Lot No. 124) and beta$_2$-m (Lot No. 015) were purchased from Dakopatts A/S, Copenhagen, Denmark, and extensively tested for non-specific staining of cryostat sections of human skin, monkey esophagus, and rat liver. These reagents showed no appreciable non-specific staining. The conjugates are prepared from chromatographically purified rabbit immunoglobulin fractions, coupled with FITC isomer 1 and purified by gel and ion-exchange chromatography. Fractions with molar F/P ratio less than 4 are collected. The extinction ratio is given as E_{495}nm/E_{278}nm$=0.6\pm0.003$ by the manufacturer and the ratios found by us varied between 0.55 and 0.66. The specificity of the conjugates was tested before conjugation by crossed immunoelectrophoresis and afterwards on monoclonal bone marrow cells.

Direct and indirect immunofluorescence tests were performed following closely the directions given by Beutner et al. (1). The conjugates were used at a dilution of 1/10, 4% BSA (bovine serum albumin) in PBS, pH 7.0, served as diluent. The slides were incubated with conjugates for 30 min at room temperature, thoroughly washed in PBS, pH 7.0, and mounted in 10% glycerol PBS, pH 7.2. For indirect tests, the slides were first incubated with serum dilutions in PBS for 30 min at room temperature, followed by incubation with conjugates. The slides were read blind in a Zeiss fluorescence microscope with incident light and blue narrow band activation (chromatic mirror 510 and interference filters KP 490 and KP 500) illuminated with an Osram HBO 200 lamp.

To calibrate the human eye, serum diluted 1/40 from a patient with SLE and an ANA titre of 1/400 was applied to rat liver sections and followed by FTC-labelled anti-human IgG as control every workday. Slides with normal

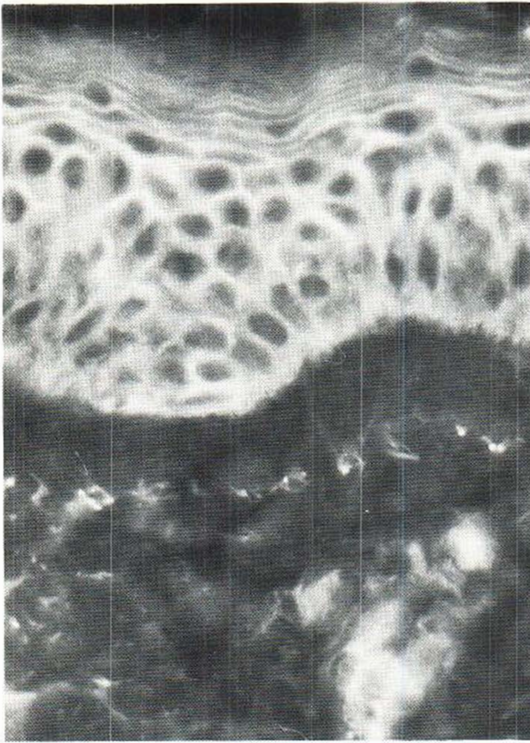

Fig. 1. Immunofluorescence pattern of normal human skin incubated with FTC-labelled anti-human beta$_2$-microglobulin, dilution 1/10.

skin and with pathological skin were read blind and decoded after determinations.

Conjugation of Con A

Concanavalin A (Calbiochem, USA) was conjugated with FITC (FTC-Con A) by adding 2.5 mg of FITC (BDH, Poole, England), dissolved in a small volume of phosphate buffer, pH 8.6, to 50 mg of Con A in 1 M NaCl under vigorous stirring, adjusted to pH 8.35 and the reaction mixture was incubated at room temperature for 2 hrs and then dialysed against 1 M NaCl overnight. Separation of the conjugated protein from free dye was achieved by gel filtration on Sephadex G-25 equilibrated with 1 M NaCl and eluted with a solution of 0.1 M glucose in 1 M NaCl. The conjugate was stored in aliquots at −70°C and dialysed to remove the glucose before use (8).

RESULTS

Thirty-four normal skin biopsies (healthy medical students and staff members) showed a strong inter-epithelial fluorescence with anti-beta$_2$-m from the basal layer upwards in a titre of 1/40 to 1/80 (Fig. 1). One biopsy showed weak fluorescence at the basal membrane for anti-IgM.

The fluorescence was as strong as the one usually found in the basal membrane of positive biopsies from patients with pemphigoid or SLE and by deposits in the epidermal papillae in biopsies from patients with dermatitis herpetiformis. Biopsies from lesions and normal-appearing skin of patients with pemphigoid, psoriasis vulgaris, and a panel of other dermatoses (including dermatitis herpetiformis, SLE, contact dermatitis, erythema multiformis, and cold urticaria) showed the same degree of reactivity with beta$_2$-m in the same titres. In addition, there were findings specific for dermatoses such as dermatitis herpetiformis, SLE, and pemphigoid (Table I).

The immunofluorescence pattern was strikingly similar to that found in biopsies from patients with pemphigus using anti-IgG and that produced by Con A. Inhibition experiments were performed to elucidate the possible relationship of these antigens. It is evident from Table II that no such relationship could be found; however, the partial identity of pemphigus antigen with Con A binding sites was confirmed.

Table I. *Immunofluorescence reactions of normal and pathological human skin to anti Ig, C_3 and beta$_2$-microglobulin at a conjugate dilution of 1/10*

Diagnosis	No.	IgG[a]	IgM[a]	IgA[b]	C_3[a]+[b]	Beta$_2$-m[c]
Normal skin	34		1			34
Pemphigoid	3	2	1		3	3
Psoriasis vulgaris	4					4
Miscell. dermatoses	20	1	1	2	3	20

[a] Basal membrane. [b] Deposits in dermal papillae. [c] Intercellular.

Table II. *Reciprocal blocking experiments of intercellular binding of beta$_2$-microglobulin, concanavalin A and pemphigus antibodies in normal human skin and monkey esophagus*

+ = Weak fluorescence. + + = Bright fluorescence.

	Normal human skin incubated with						Monkey esophagus
Titre	1st Step ... 2nd Step[b] ...	Pemph. Ser. FTC anti-IgG	Pemph. Ser. FTC anti-β_2m	Con A[a] FTC anti-β_2m	Anti-β_2m Pemph. Ser.[c]	Anti-β_2m FTC Con A[a]	FTC anti-β_2m
1/10		++	++	++	++	++	–
1/20		++	++	++	++	++	–
1/40		+	++	++	++	++	–
1/80		+	++	++	++	++	–
1/160		+	+	++	+	++	–
1/320		+	+	++	+	++	–

[a] Concanavalin A, 4 mg/ml. [b] Dilution 1/10. [c] Third step FTC anti-IgG.

DISCUSSION

Solheim et al. (7) showed that epithelial cells in trypsinized monolayers or tissue culture had HL-A and beta$_2$-m determinants associated with the cell membrane. Our results confirm the strong binding of anti-beta$_2$-m to epithelial cells. The function of beta$_2$-m on the cell membrane thus far remains unknown; however, the fact that it is a surface marker of epidermal cells that are easy to demonstrate in tissue sections deserves further interest. In most lymphoid cell lines studied, HL-A and beta$_2$-m determinants appear as what seems to be a tetrameric macromolecule resembling IgG with beta$_2$-m corresponding to the light chains (6). However, Daudi cells have only HL-A polypeptide and no beta$_2$-m, which suggests that beta$_2$-m and alloantigenic HL-A polypeptide are under separate genetic control and that beta$_2$-m does not constitute a membrane component that is absolutely necessary for the integrity of the cell membrane (5).

If, as Solheim speculates (7), beta$_2$-m and/or HL-A alloantigens are necessary for recognition of the tissue, beta$_2$-m might be an epidermal cell surface component that is lost in some epidermal disorders. Further screening of epidermal binding of beta$_2$-m might be fruitful, as failure to find this protein might indicate defective alloantigens in that disease.

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U. Forsum, M.D.
Department of Dermatology
University Hospital
S-750 14 Uppsala
Sweden