

EPIDERMAL INTERCELLULAR BINDING OF CONCAVALIN A AND PEMPHIGUS ANTIBODY

Takeji Nishikawa, Takashi Harada, Hitoshi Hatano, Hideoki Ogawa, Akio Taneda and Hiroaki Miyazaki

From the Department of Dermatology, Keio University School of Medicine, Tokyo, and the Department of Dermatology, Juntendo University School of Medicine, Tokyo, Japan

Abstract. The binding sites of Concanavalin A (ConA) and pemphigus antibody in the intercellular spaces of the human epidermis have been investigated. Extraction of human skin with phosphate-buffered saline (PBS) and 70% ethanol resulted in complete loss of the binding sites of the pemphigus antibody, whereas the extracted skin still retained its reactivity to ConA. Preincubation with more than 0.25 mg/ml of ConA with normal skin caused a reduction in the binding of pemphigus sera. However, preincubation of pemphigus sera with normal human skin caused no inhibition of the subsequent binding of fluorescein-labelled ConA. These results indicate that ConA binding sites might be related to, but not identical with those of pemphigus antibody.

Key words: Intercellular substance; Epidermal saccharides; Concanavalin A; Pemphigus antibody

Recently, Nieland (5) demonstrated the presence of surface saccharides in the intercellular spaces of human epidermis by the use of fluorescein-labelled Concanavalin A (ConA), a lectin isolated from the jackbean. ConA has been shown to bind to simple sugars, glycoproteins, and complex polysaccharides containing, α -D-glucopyranosyl or α -D-mannopyranosyl groups in a non-reducing terminus (8). Since its staining pattern is identical with that of pemphigus antibody, it was suggested that the binding sites of ConA and pemphigus antibody might be closely related. Hashimoto et al. (4) concluded from reciprocal blocking experiments, competition experiments, and electron microscopic observations, that the binding sites of ConA in the glycocalyx of keratinocytes are identical with those of pemphigus antibody.

We, independently, have found that the pemphigus skin has the capacity to bind with ConA, where antibodies have already been fixed in the intercellular spaces of the epidermis. Based mainly upon this fact, we suggested that the binding sites of ConA

and those of pemphigus antibody are not identical (6).

In the present short study, we demonstrate supporting evidence that would seem to confirm our view that the binding sites of ConA might be inter-related with, but not identical with, those of pemphigus antibody.

MATERIALS AND METHODS

Skin and sera. Involved skin of a patient with pemphigus foliaceus (M. H. 36634/74) was taken by the standard biopsy technique, quickly frozen at -20°C and sectioned in a cryostat at 4-6 μm thickness. The sections were used unfixed. As a control, normal skin was obtained and was used in the same manner as above. The sera taken from the same pemphigus patient was used throughout this study. Standard indirect immunofluorescent staining (2) revealed the circulating antibody titre as 1:1024.

Fluorescein-labelled reagents. Fluorescein-labelled ConA (ConA-FITC) was obtained from Cappel Laboratories, Inc., Pa., USA. The details of the conjugated product have been described elsewhere (5). ConA-FITC was diluted 1:40 with phosphate-buffered saline (PBS) at pH 7.4 prior to use. Fluorescein-labelled goat anti-human IgG (Lot. 2201T003A1) was obtained from Hyland Laboratories, Calif., USA and was used at 1:10 dilution with the same buffer.

Removal of pemphigus antigens. Normal skin sections were rinsed in PBS and 70% ethanol respectively overnight. Subsequently, these sections were used as substrate for fluorescein staining, together with untreated normal skin sections. The sections were stained with ConA-FITC and pemphigus antibody and were examined by fluorescence microscopy as described previously (6).

Reciprocal blocking test of ConA and pemphigus antibody. Specific inhibition by ConA of the reaction of pemphigus antibody to the intercellular spaces of the epidermis was tested by preincubation of normal skin sections with serially diluted ConA (Sigma) for 30 min at room temperature, subsequent incubation with pemphigus sera at 1:8 dilution, and again with FITC anti-human IgG.

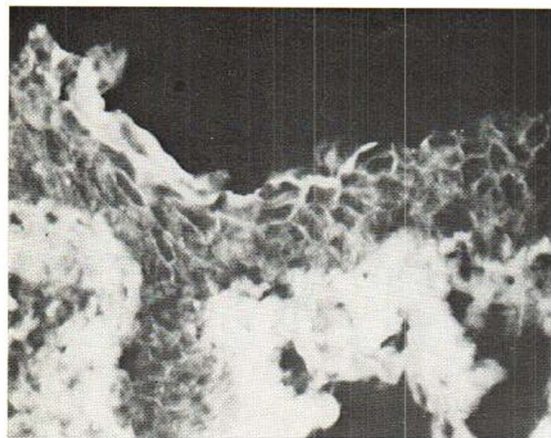


Fig. 1. The involved skin of the patient with pemphigus foliaceus stained with ConA-FITC to show positive fluorescence in the intercellular spaces of the epidermis. $\times 150$.

Inhibition of ConA binding was tested by preincubation of normal human skin sections with serially diluted pemphigus sera and subsequent incubation with ConA-FITC and FITC anti-human IgG.

RESULTS

As has previously been reported (6), the involved skin of the pemphigus patient has the capacity to react with ConA-FITC (Fig. 1), irrespective of the concentration of the labelled ConA. The results of the reciprocal blocking test of ConA and pemphigus antibody with the intercellular spaces of the epidermis are shown in Table I. Preincubation of

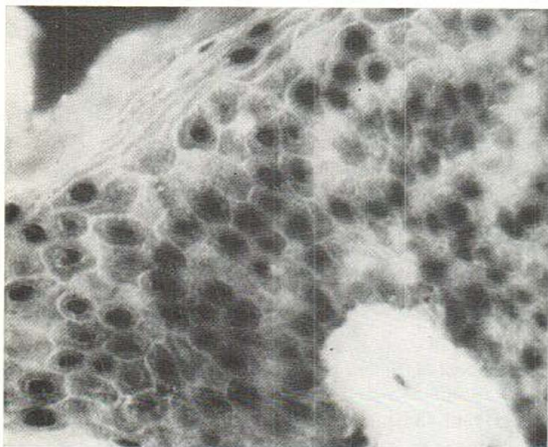


Fig. 2. Application of ConA-FITC to the normal human skin extracted with phosphate-buffered saline to show positive fluorescence. $\times 190$.

Table I. Reciprocal blocking of epidermal intercellular binding of Concanavalin A and pemphigus antibody

Normal skin preincubated with pemphigus sera	Subsequent incubation with	
	ConA-FITC	FITC-Antihuman IgG
Control	++	—
1:8	++	++
1:32	++	++
1:128	++	+
1:512	++	+
Normal skin preincubated with ConA	Subsequent incubation with pemphigus sera (1:8) and FITC-Antihuman IgG	
Control	—	++
0.031 mg/ml	—	++
0.062 mg/ml	—	++
0.125 mg/ml	—	++
0.250 mg/ml	—	+
0.50 mg/ml	—	+
1.00 mg/ml	—	+
2.00 mg/ml	—	+

normal skin sections with 0.031 to 0.125 mg/ml of ConA did not influence the subsequent reaction of pemphigus sera at 1:8 dilution. Concentration of 0.25 mg/ml or more up to 2 mg/ml of ConA weakened the subsequent reaction of pemphigus sera but never achieved complete blocking of the pemphigus antibody. On the other hand, previous treatment of normal skin sections with serially diluted pemphigus sera had no effect on the subsequent binding of ConA.

Normal skin extracted with PBS and 70% ethanol respectively overnight caused complete loss of fluorescence when pemphigus sera at 1:16 and 1:32 dilution were applied. Application of ConA-FITC to the extracted skin sections, however, gave positive fluorescence, as shown in Fig. 2.

DISCUSSION

Only limited information is available with regard to the physicochemical properties of pemphigus antigens, although these antigens were revealed by absorption studies (1, 3) and passive cutaneous anaphylaxis (7).

In our present study, human skin rinsed in PBS and 70% ethanol caused complete loss of reactivity to pemphigus sera, while ConA-FITC reacted well with these extracted sections. This result indicates that pemphigus antigens are soluble in both PBS and 70% ethanol and the binding sites of ConA in the intercellular spaces still retain their reactivity even after the extraction. Reciprocal blocking experiments suggest that the reduction of the binding of pemphigus antibody occurred only when the doses of ConA

were increased. Therefore, it would appear reasonable to conclude that the binding sites of pemphigus antibody are not identical with those of ConA, though there is a possibility that the sites are partially inhibited by the attachment of excess ConA. Thus, our short study offers additional data that ConA binding sites are not identical with pemphigus antibody binding sites (6) and suggests that the main components of the substances reactive with pemphigus antibody are less likely to be surface saccharides reactive with ConA. These data might support the recent physicochemical studies on pemphigus antigens (9) indicating that the pemphigus antigens are protein in nature.

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H. Hatano, M.D.
Department of Dermatology
Keio University School of Medicine
Shinjuku
Tokyo 160
Japan