

EPIDERMAL SURFACE SACCHARIDES REACTIVE WITH PHYTOHEMAGGLUTININS AND PEMPHIGUS ANTIGEN

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Abstract. In order to study the relationship between epidermal surface saccharides and pemphigus antigen(s), fluorescein-labelled Concanavalin A (ConA) and Phytohemagglutinin-P (PHA-P) were used. These phytohemagglutinins were found to bind with the intercellular areas of human epidermis. Alpha-methyl-D-mannoside and α -methyl-D-glucoside inhibited the epidermal intercellular staining pattern produced by ConA-FITC, while N-acetyl-D-galactosamine blocked the same staining pattern produced by PHA-P-FITC. Normal human skin reacted with pemphigus antibody and pemphigus skin with the deposition of IgG both gave a positive intercellular staining pattern with fluorescein labelled phytohemagglutinins. Our data indicated the non-identity of the binding site of ConA and PHA-P, and pemphigus antigen(s).

Key words: Epidermal surface saccharides; Glycocalyx; Concanavalin A; Phytohemagglutinin-P; Pemphigus antigen

Immunofluorescent studies have revealed the presence of antibodies in the sera of patients with pemphigus vulgaris which reacts with the intercellular areas of the stratified epithelium and the in vivo bound immunoglobulins in lesions of the same condition (1, 2, 3). The demonstration of this pemphigus antibody has now become a practical and useful aid in the diagnosis of bullous skin diseases. Further, elution studies of the lesion of this condition supplied direct evidence that the antibody eluted had the same reactivity as that in free circulation (9). To what antigen(s) these antibodies are directed is, however, a question for which a definitive answer is not yet available, though it has been suggested that an extracellular carbohydrate coat or glycocalyx may play a role in immunity (10). Recently, an epidermal intercellular staining pattern was demonstrated by the use of the fluorescein-labelled phytohemagglutinins, Concanavalin A and Phytohemagglutinin-P, suggesting the presence of epidermal surface saccharides (8).

In the present study, we have attempted to determine whether or not any relation exists between pemphigus antigen(s) and the binding site of these hemagglutinins in the intercellular areas of the epidermis.

MATERIALS AND METHODS

Skin and sera. A 66-year-old woman (M. H. 36634/73) had a 3 month history of generalized bullae which easily dried up to scales. The condition was clinically diagnosed as pemphigus foliaceus. Biopsy from her fresh bulla revealed subcorneal blister formation with acantholysis. The sera gave a high circulating antibody titer to the intercellular substances of human epidermis up to 1:640 dilution by standard indirect immunofluorescent (IF) staining (4). A fresh bulla was taken from this patient by the standard biopsy technique and was quickly frozen to -20°C and cut in a cryostat at $6\text{ }\mu\text{m}$ thickness. The sections were used unfixed.

Concanavalin A-FITC (ConA-FITC) and Phytohemagglutinin-P-FITC (PHA-P-FITC). The conjugated products of ConA-FITC and PHA-P-FITC were obtained from Cappel Laboratories, Downingtown, Pa., USA. The details of the procedure for conjugation of these phytohemagglutinins with fluorescein isothiocyanate were described elsewhere (8, 12). Both ConA-FITC and PHA-P-FITC were diluted 1:40 with phosphate-buffered saline (PBS) at pH 7.4 prior to use. Six-micron frozen sections from normal human skin were used as substrate. The sections were stained on glass slides in a humidified chamber at room temperature for 45 min, washed 3 times with PBS for 15 min and mounted with coverslips in 50% glycerol-PBS. The specimens were examined with a Nikon fluorescence microscope.

For the study of the neutralization of binding with ConA-FITC and PHA-P-FITC, various monosaccharides (Sigma) at a concentration of 0.5 M were added to an equal amount of solution of ConA-FITC and PHA-P-FITC respectively and incubated in a water bath at 37°C for 30 min. These phytohemagglutinins containing various monosaccharides were then applied to normal human skin. Blocking experiments were performed in which sections were incubated with ConA (Sigma) and PHA-P (Sigma) respectively at a concentration of 1 mg/ml in PBS for 15 min at 37°C prior to incubation with ConA-FITC and PHA-P-FITC. A saline control was run with every experiment.

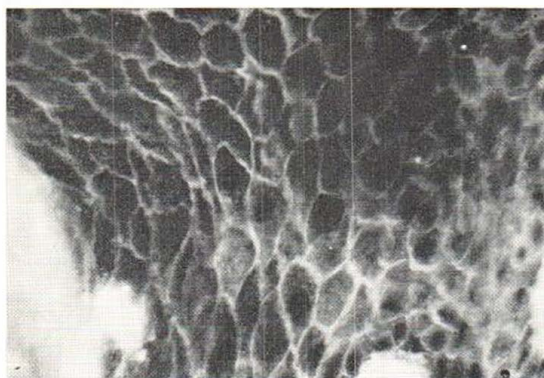


Fig. 1a. Normal human skin stained with ConA-FITC. $\times 320$.

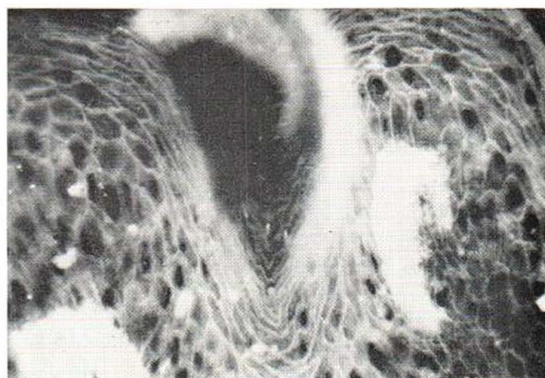


Fig. 1c. Normal human skin reacted with pemphigus sera and stained with ConA-FITC. $\times 160$.

In order to see whether ConA and PHA-P would compete with pemphigus antihody for its binding site in the squamous epithelium, two staining procedures were carried out. Firstly, 6 μ m frozen sections from normal human skin were reacted with the sera from the patient described above at 1:8 dilution for 45 min at room temperature in a humidified chamber and then stained with ConA-FITC and PHA-P-FITC. As a control, indirect IF staining was carried out in the same manner as reported by Beutner *et al.* (4). FITC-labelled goat antihuman IgG (Hyland Lab., Calif.; Lot No. 2201-T003a1) was used at 1:10 dilution. Secondly, pemphigus skin of the patient whose sera showed 1:640 antibody titer at the time of biopsy was stained with these two fluorescein-labelled phytohemagglutinins in the same manner as described above. As a control, direct staining was done with FITC-labelled goat anti-human IgG.

RESULTS

Both ConA-FITC and PHA-P-FITC gave an intercellular staining pattern on the normal human epidermis (Fig. 1a & 1b). No significant differences were found in the patterns produced by the two

phytohemagglutinin conjugates except that some staining of the nuclear membranes of squamous cells was occasionally produced and the basement membrane staining was stronger in the case of ConA-FITC. This intercellular staining pattern was indistinguishable from that of direct IF staining of IgG on the pemphigus skin (Fig. 2). Prior incubation of the sections with the solutions of unlabelled ConA blocked subsequent staining with ConA-FITC, as well as in the case of PHA-P-FITC.

Results of the neutralization studies of various monosaccharides are summarized in Table I. With ConA-FITC, staining was completely inhibited by α -methyl-D-mannoside, α -methyl-D-glucoside. On the other hand, staining was blocked by *N*-acetyl-D-galactosamine in the case of PHA-P but not by any of the other sugars tested. This intercellular staining of both ConA-FITC and PHA-P-FITC was

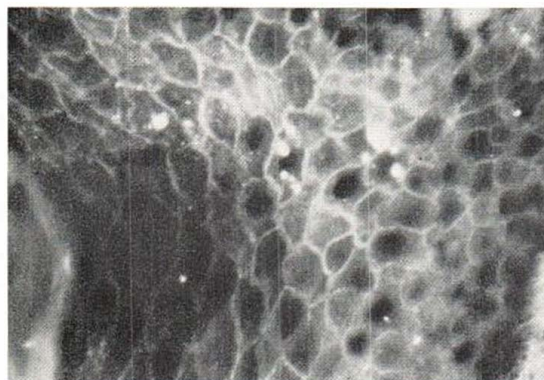


Fig. 1b. Normal human skin stained with PHA-P-FITC. $\times 320$.



Fig. 1d. Normal human skin reacted with pemphigus sera and stained with PHA-P-FITC. $\times 160$. All photographs (1a-1d) show indistinguishable intercellular staining pattern.

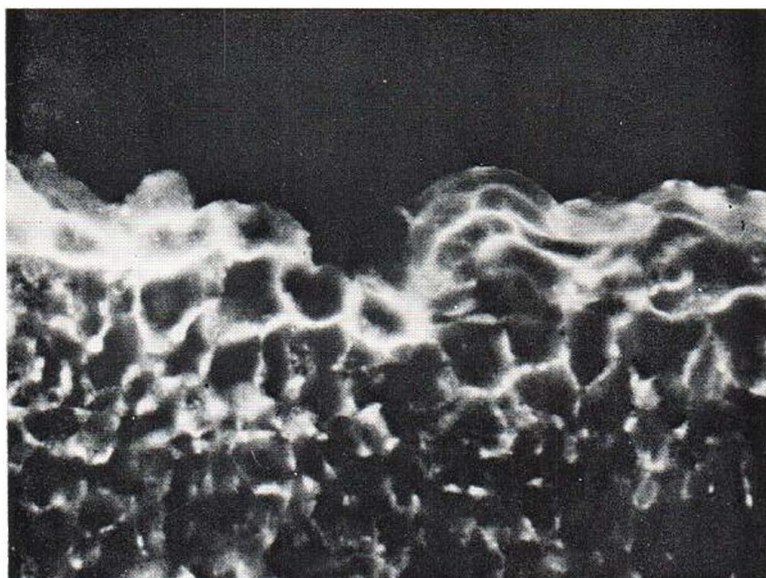


Fig. 2. Direct IF staining of pemphigus skin with FITC- labelled anti-human IgG to show intercellular deposition of IgG. $\times 320$.

Table I. Neutralization of epidermal intercellular staining of Concanavalin A and Phytohemagglutinin-P with various monosaccharides

Each monosaccharide was incubated with ConA-FITC, PHA-P-FITC for 15 min at 37°C prior to application on normal human epidermis. + = negative fluorescence (neutralization occurred), - = positive fluorescence (no neutralization)

Monosaccharides (0.25 M)	ConA-FITC	PHA-P-FITC
α -methyl-D-glucoside	+	-
α -methyl-D-mannoside	+	-
β -methyl-D-glucoside	-	-
N-acetyl-D-galactosamine	-	+
N-acetyl-D-glucosamine	-	-

not influenced by the prior application of pemphigus sera to the sections of normal human skin (Fig. 1c & 1d). Further, direct staining of the pemphigus skin with ConA-FITC and PHA-P-FITC gave identical intercellular staining patterns. Control sections produced a marked deposition of IgG in the intercellular areas of the denuded epidermis (Fig. 2).

DISCUSSION

The binding affinities of ConA and PHA-P for saccharides and their chemical nature have been well defined. Our present results are in agreement with the previous report (8). Alpha-D-manno-

syl and α -D-glucosyl residues are the binding sites for ConA, as was shown by the inhibition of the staining of the section of normal human skin by prior application of ConA. On the other hand, PHA-P was shown to bind with N-acetyl-D-galactosamine. This confirms that carbohydrate-containing substances were present in the intercellular areas of the human epidermis (6, 7, 8).

Blood group antigens consisting of saccharides are known to be present as surface components in the intercellular areas of human epidermis (11). Grob & Inderbitzin reported the non-identity of the blood group substances with pemphigus antigen (5). Nieland (8) did not draw any definite conclusion as to whether or not ConA reacted independently with epidermal surface saccharides. The experiments were technically unsatisfactory due to the precipitation of serum protein by ConA, since pemphigus sera was reacted in the presence of ConA. This was avoided in the present study by reacting the pemphigus sera prior to the application of ConA-FITC and, in addition, by the use of pemphigus skin where bound IgG were already present, as shown in Fig. 2.

Our data clearly indicate that the binding sites of the phytohemagglutinins used, namely, α -D-mannosyl, α -D-glucosyl residues and N-acetyl-D-galactosamine are not the components of the specific site of the antigenic substances which react with the pemphigus antibody, since these hemagglutinins were found to react independently to the binding site of the

epidermis in which antibodies were already fixed in vivo and also of the normal epidermis in which pemphigus sera reacted in vitro. Thus our present study gave us the non-identity of the binding site of Con A and PHA-P and pemphigus antigen(s).

REFERENCES

1. Beutner, E. H. & Jordon, R. E.: Demonstration of skin antibodies in sera of pemphigus vulgaris patients by indirect immunofluorescent staining. *Proc Soc Exp Biol Med* 117: 505, 1964.
2. Beutner, E. H., Lever, W. F., Witebsky, E., Jordon, R. E. & Chertock, B.: Autoantibodies in pemphigus vulgaris: response to intercellular substance of epidermis. *JAMA* 192: 682, 1965.
3. Beutner, E. H., Jordon, R. E. & Chorzelski, T. P.: The immunopathology of pemphigus vulgaris and bullous pemphigoid. *J Invest Derm* 51: 63, 1968.
4. Beutner, E. H., Chorzelski, T. P. & Jordon, R. E.: Autosensitization in Pemphigus and Bullous Pemphigoid. Thomas, Springfield, Ill., 1970.
5. Grob, P. J. & Inderbitzin, T. M.: Pemphigus antigen and blood substances A and B. *J Invest Derm* 49: 285, 1967.
6. Komura, J. & Ofuji, S.: Phosphotungstic acid stain and epidermal cell surface. *J Invest Derm* 49: 391, 1967.
7. Mercer, E. H., Jahn, R. A. & Maibach, H. I.: Surface coats containing polysaccharides on human epidermal cells. *J Invest Derm* 51: 204, 1968.
8. Nieland, M. L.: Epidermal intercellular staining with fluorescein-conjugated phytohemagglutinins. *J Invest Derm* 60: 61, 1973.
9. Nishikawa, T., Harada, T., Hatano, H., Ogawa, H. & Miyazaki, H.: Elution of antibody from the skin lesions of patients with pemphigus vulgaris. *Acta Dermatovenereol* (Stockholm) 54: 275, 1974.
10. Rambourg, A., Neutra, M. & Leblond, C. P.: Presence of a "cell coat" rich in carbohydrate at the surface of cells in the rat. *Anat Rec* 154: 41, 1966.
11. Szulman, A. E.: The histological distribution of the blood group substances A and B in man. *J Exp Med* 111: 785, 1960.
12. Tkacz, J. S., Cybulska, E. B. & Lampen, J. O.: Specific staining of wall mannan in yeast cells with fluorescein-conjugated Concanavalin A. *J Bacteriol* 105: 1, 1971.

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