

ELUTION OF ANTIBODY FROM THE SKIN LESIONS OF PATIENTS WITH PEMPHIGUS VULGARIS

Takeji Nishikawa, Takashi Harada, Hitoshi Hatano, Hideoki Ogawa 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. Immunoglobulins could be eluted from the fresh bullous lesions of two patients with known pemphigus vulgaris by treatment with acid citrate buffer (pH 3.0). Tzanck smears showed a marked reduction in immunofluorescence following the incubation of smears in an acid citrate buffer. Intercellular antibody to normal human epidermis was demonstrated in the eluates obtained from skin homogenates of bullous lesions. Direct evidence was thus obtained to suggest that *in vivo* bound immunoglobulin has an auto-antibody nature, that is, it binds with the intercellular space of human epidermis.

Since Beutner & Jordon (1) demonstrated the presence of auto-antibody to stratified squamous epithelium in the sera of patients with pemphigus, many workers have reported similar findings (2, 10, 11, 12). In addition, the demonstration of bound immunoglobulins (1, 2, 3) and complement (5) in lesions of the same condition, favours the concept that an antigen-antibody reaction may play an important role in the pathogenesis of the disease.

In the present study, we have investigated the possibility that pemphigus antibody might be a component of bound immunoglobulins, by elution studies with acid citrate buffer (pH 3.0) which dissociates antigen-antibody complexes.

MATERIALS AND METHODS

Tzanck smears. Fresh bullous lesions from a 49-year-old woman with pemphigus vulgaris were examined. The base of a fresh bulla was gently scraped with sterile metal forceps onto a glass slide and air dried. Slides were then stored at -20°C until used. Duplicate slides were soaked 4 h and overnight respectively, at room temperature in citric acid buffer (pH 3.0) and then placed in phosphate-buffered saline (PBS) at pH 7.5 for 60 min to adjust the pH before direct immunofluorescent (IF) staining for IgG. Control slides were soaked in PBS only. Direct IF staining was carried out in the same

manner as reported by Beutner et al. (3). FITC-labelled goat anti-human IgG (Hyland Lab., Calif., U.S.A., Lot No. 2232700 3A1) was used at 1:10 dilution.

Elution from bullae. Fresh bullous lesions from 2 patients with pemphigus vulgaris whose diagnosis had been confirmed by biopsy and standard indirect and direct immunofluorescence tests were used. Elution of immunoglobulins was carried out according to the method described by Koffler et al. (6). Fresh bullae were carefully excised with scissors and stored in the deep-freeze at -20°C until used. A total amount of 0.95 g of the skin (wet weight) was cut into small pieces in PBS and homogenized in an ice-jacketed Waring blender at 10 000 rpm for 3 min. The same procedure was repeated three times at 20 sec intervals. The homogenates were centrifuged at 6 000 g for 15 min at 4°C . The supernatant was removed and the residue was resuspended in PBS for further washing. The procedure of washing the residue was repeated 7 times until the supernatant became clear. At each washing the supernatant was stored to check the protein concentration and the presence of pemphigus antibody. After the final washing, citric acid buffer was added to the residue and stirred overnight at room temperature. Then the supernatant was removed after centrifugation and was dialysed against multiple changes of PBS for 48 h at room temperature. The final eluates and the washings at each stage were concentrated to 2 ml by negative pressure dialysis through a collodion bag. The indirect IF staining was carried out after the exposure of the eluates for 45 min to the frozen section of the normal human skin. The presence of intercellular antibody in the washings was also tested by the same method. The protein concentration of eluates and the washings was determined by the method of Lowry et al. (9).

RESULTS

Tzanck smears soaked in PBS gave brilliant immunofluorescence from the individual acantholytic cells, as well as clumps and sheets of adherent epidermal cells (Fig. 1). Most of this bright immunofluorescence was seen on the cell membrane of the acantholytic cells. The sheets of cells showed the same type of

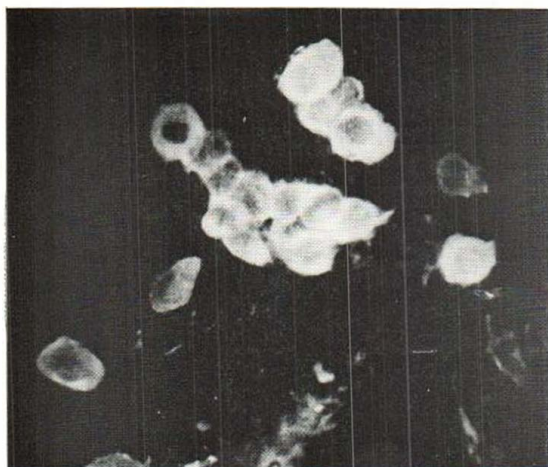


Fig. 1. Acantholytic cells from Tzanck smear of pemphigus vulgaris stained with FITC-labelled anti-human IgG to show positive immunofluorescence. $\times 160$.

intercellular fluorescence as observed in the frozen section of biopsied skin from pemphigus vulgaris. Compared with control preparations, smears treated with acid citrate buffer showed a marked loss of immunofluorescence after being incubated in acid citrate buffer overnight (Fig. 2), although 4 hours' incubation caused little reduction in fluorescence. The reduction in fluorescence was greatest at the periphery of the cell membrane. Most of the cells kept their shape fairly well at an acid pH.



Fig. 2. Reduction of immunofluorescence after treatment with acid citrate buffer (pH 3.0) overnight. $\times 160$.

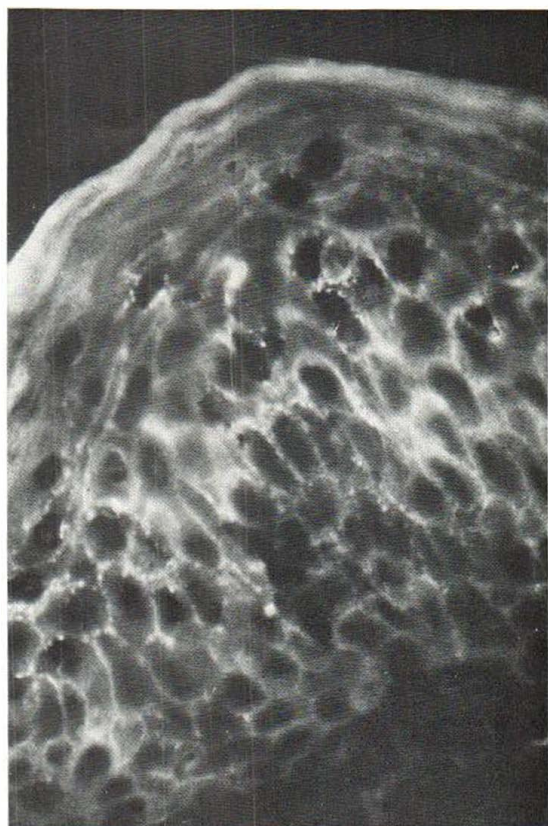


Fig. 3. Eluates from skin homogenates on normal human skin stained with FITC-labelled anti-human IgG to show positive intercellular immunofluorescence. $\times 320$.

The eluates from skin homogenates showed a strong intercellular immunofluorescence when the eluates were applied to the normal human skin for 45 min. This intercellular immunofluorescence of IgG is the same as that seen in the standard indirect IF staining of pemphigus sera (Fig. 3). The protein concentration of the eluates and washings are set out in Table I. The eluates contained 0.458 mg/ml of protein. Washing 1 showed a high protein concentration of 4.91 mg/ml with positive intercellular immunofluorescence but the concentration of protein declined at each washing and the final washing (Washing 7) showed only 0.03 mg/ml of protein with negative immunofluorescence to the human skin.

DISCUSSION

In vivo bound immunoglobulins deposited in the skin lesions of pemphigus have been regarded as

Table I. The protein concentration and intercellular immunofluorescence in the eluates and washings with several changes of phosphate-buffered saline

	Protein conc. (mg/ml)	Intercellular immuno- fluorescence
Eluates	0.458	+
Washings 1	4.91	+
Washings 3	1.19	-
Washings 7	0.03	-

auto-antibodies despite lack of definite evidence, because of the following findings; (a) antibodies present in patient's sera are reactive with the site of fixation, (b) these antibodies can bind in vivo when injected into experimental animals, and (c) complement is also fixed to the intercellular areas (4).

In the present study, the dissociation of antigen-antibody complexes was primarily detected by the application of acid citrate buffer to the Tzanck smears. A marked loss of immunofluorescence in the acantholytic cells was demonstrated, suggesting the dissociation of immune complexes at an acid pH. This was further corroborated by the elution studies on skin homogenates. The possibility of a contamination of eluates by free circulating antibody was excluded by careful changes of PBS during the process of homogenization of the bullae, as shown in Table I.

Thus, our present study supplied direct evidence that in vivo bound immunoglobulins had been dissociated and that the antibody eluted showed an ability to bind with the intercellular space of human epidermis. The intercellular staining pattern of the antibody eluted was the same as that of the antibody in free circulation. Landry & Sams (7) recently demonstrated two different types of antibody eluted from the skin of systemic lupus erythematosus and Landry et al. (8) very recently reported that the specificity of antibodies eluted from the skin of bullous pemphigoid was somewhat different from that in circulation.

Investigations are proceeding in our laboratories to establish the immunoglobulin class and specificity of the antibody eluted and also to determine whether or not this antibody is responsible for the actual production of acantholysis.

ACKNOWLEDGEMENTS

We are grateful to Dr R. Marks, Dr F. Schellander and Professor Y. Fukazawa for their review of the manuscript.

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 to pemphigus vulgaris. *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.: Auto-sensitization in Pemphigus and Bullous Pemphigoid. C. C. Thomas, Springfield, Ill. 1970.
5. Cormane, R. H. & Chorzelski, T. P.: Bound complement in the epidermis of patients with pemphigus vulgaris. *Dermatologica* 134: 463, 1967.
6. Koffler, D., Schur, P. H. & Kunkel H. G.: Immunological studies concerning the nephritis of systemic lupus erythematosus. *J Exp Med* 126: 607, 1967.
7. Landry, M. & Sams, W. M., Jr: Basement membrane, antibodies in two patients with systemic lupus erythematosus. *Lancet* April 15; 821, 1972.
8. Landry, M., Sams, W. M., Jr & Jordon, R. E.: Bullous pemphigoid: Elution of in vivo fixed antibody. Presented at the 34th Meeting of the Society for Investigative Dermatology, Inc., New York, June 22-24, 1973.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J.: Protein measurement with folin phenol reagent. *J Biol Chem* 193: 265, 1951.
10. Peck, S. M., Osserman, K. E., Weiner, L. B., Lefkowitz, A. & Osserman, R. S.: Studies in bullous diseases: Immunofluorescent serologic tests. *New Engl J Med* 279: 951, 1968.
11. Waldorf, D. S., Smith, C. W. & Strauss, A. J. L.: Immunofluorescent studies in pemphigus vulgaris: confirmatory observations and evaluations of technical considerations. *Arch Derm* 93: 28, 1966.
12. Wright, E. T., Epps, R. L. & Newcomer, V. D.: Fluorescent antibody studies in pemphigus vulgaris. *Arch Derm* 93: 562, 1966.

Received November 8, 1973

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