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Humoral Antibodies in Sera of Patients with Bullous Skin Diseases* (32689)

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It has been shown that sera of patients with pemphigus vulgaris contain antibodies to an intercellular antigen of stratified squamous epithelium demonstrable by indirect immunofluorescent (IF) staining (1). More recently, evidence was presented that these antibodies combine with the patient's own skin and may be justifiably called autoantibodies (2).

This study shows that sera of some patients suffering from bullous skin diseases react with established human cell lines such as HEp-2 cells, and that some of the sera also contain antihuman gamma globulin antibodies resembling the rheumatoid factor.

Materials and Methods. The study included 11 sera of patients with bullous skin diseases, all of which were previously examined by IF staining (1,2). Monolayer cell cultures of heteroploid human cell lines: HeLa, HEp-2, and AV-3 were prepared in test tubes, as described previously (3). Cell cultures of diploid-cell lines originating from human embryonic lung, WI-26, WI-38, and MA, as well as from human embryonic skin and muscle, MAF, were purchased from Microbiological Associates, Inc., Bethesda, Md. Primary cell cultures of kidney and lung of rhesus monkey and guinea pig origin were prepared following the procedure described by Karzon *et al.* (4).

In order to detect antibodies combining with

cell-surface antigens, the mixed-agglutination test was employed. Similar procedure has been used in this laboratory for demonstration of human transplantation antibodies (5-7). Briefly, monolayer cell cultures were incubated with the tested serum at various dilutions. Thereafter, indicator erythrocytes were added to the cell cultures. For most experiments these were sheep erythrocytes sensitized by human antiserum to sheep erythrocytes and agglutinated by monospecific rabbit antiserum to human IgG. For some experiments, instead of anti-IgG serum, a monospecific anti-IgM serum or a multispecific antiserum against human globulins were used to prepare the indicator erythrocytes (7). Adherence of the indicator erythrocytes to the cell cultures was interpreted as a positive reaction.

To demonstrate anti-human gamma globulin factors, the hemagglutination test with human O Rh-positive erythrocytes sensitized by a human anti-CD serum "Ripley" was used (8). The serum "Ripley" was kindly supplied by Dr. M. Waller of the Medical College of Virginia, Richmond, Va. The procedure of the test was described previously (9).

Results. Table I lists results of a representative mixed-agglutination study with sera of four patients suffering from bullous skin diseases and HEp-2 cell cultures. It may be noted that in this particular experiment three sera gave positive results. Interestingly, some positive sera showed prozones which in Table I are exemplified by reactions of sera M.C. and S.M. Serum of patient A.T. who received multiple transfusions served as a positive con-

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trol. As expected on the basis of previous experience with this serum, it gave a strong positive mixed-agglutination test.

Table II summarizes the results obtained in mixed-agglutination test with four human cell lines as well as results of agglutination test with sensitized erythrocytes. The serum of a patient with multiple transfusions gave strong positive results in the mixed agglutination test with all four cell lines under study. Of 11 pathologic sera, six sera from patients with various forms of pemphigus and

one serum from a patient with dermatitis herpetiformis gave positive mixed-agglutination tests with cultures of epithelial cells HeLa, HEp-2, and AV-3, but not of fibroblastic cells WI-38. Since antibodies combining with human cell cultures may follow multiple transfusions or pregnancies (5), it was important to find that none of the seven patients whose sera gave positive results in mixed agglutination ever received a blood transfusion. Three patients were males and of four female patients, three had no chil-

TABLE I. Mixed-Agglutination Tests with HEp-2 Cell Cultures and Sera of Patients with Bullous Skin Diseases.

Dilution of serum 1 to:	Sera of patients				Multitransfusion serum	Normal human sera	
	M.C.	S.M.	S.C.	Y.E.	A.T.	D.T.	M.B.
5	—	—	—	3+	ND	—	—
10	—	±	—	3+	3+	—	—
30	±	2+	—	3+	3+	—	—
90	3+	3+	—	3+	3+	—	—
270	3+	±	—	3+	3+	—	—
810	3+	—	—	3+	3+	—	—
2430	1+	—	—	2+	3+	—	—
7290	—	—	—	±	2+	—	—
Diluent				—			

TABLE II. Comparison of Results Obtained in Three Serologic Tests with Sera of Patients with Bullous Skin Diseases.

Initials	Diagnosis	Titer of mixed agglutination with cell cultures of				Titer of agglutination of erythrocytes sensitized by Rh antibodies	Titer of indirect IF staining of intercellular areas
		HeLa	HEp-2	AV-3	WI-38		
M.C.	Pemphigus vulgaris	2430	2430	810	<30	160	320
L.A.	Pemphigus vegetans	810	810	270	<30	10	<30
F.C.	Pemphigus erythematousus	810	270	90	<30	<10	<30
K.W.	Pemphigus vulgaris	270	270	810	<30	<10	30
W.D.	Pemphigus vulgaris	90	90	90	<30	10	<30
S.M.	Pemphigus vulgaris	90	90	90	<30	40	<30
Y.E.	Dermatitis herpetiformis	2430	2430	810	<30	20	<30
S.C.	Pemphigus vulgaris	<30	<30	<30	<30	10	30
B.D.	Pemphigus vulgaris	<30	<30	<30	<30	<10	90
P.M.	Pemphigus vulgaris	<30	<30	<30	<30	80	90
F.R.	Pemphigus vulgaris	<30	<30	<30	<30	<10	30
A.T.	Multiple transfusions	7290	7290	2430	2430	<10	ND
Twelve normal subjects		<30	<30	<30	<30	<10	<30

dren. It may also be noted in the table that five of seven sera positive in the mixed agglutination test gave negative results in the IF test, whereas of four sera negative in mixed agglutination, all were positive in IF. Furthermore, absorption of the serum M.C. with HEP-2 cell suspensions resulted in the disappearance of the activity against cell cultures while the antibodies to the intercellular antigen detectable in the IF test remained unaffected. It was also found in this study that seven of 11 pathologic sera contained antibodies to human gamma globulin as shown by the hemagglutination test with erythrocytes sensitized by Rh antibodies. It was quite interesting that as many as five sera possessed antibodies combining with both human cell cultures and human gamma globulin.

It appeared interesting to obtain more information concerning the previously noted negative results of the mixed-agglutination test with fibroblastic cell cultures. Because of limited volume of positive sera available, only two pemphigus sera were included in this study. Significantly, both these sera gave negative results with all four fibroblastic cell cultures under investigation (Table III). Subsequently, three positive pemphigus sera were tested against primary cell cultures of kidney and lung of rhesus monkey and guinea pig. As shown in Table IV, all sera gave positive reactions with kidney cell cultures of both species, whereas negative or weakly positive results were obtained with lung cell cultures. This was consistent with the fact that the former cultures were rich in epi-

TABLE III. Mixed-Agglutination Tests with Two Pemphigus Sera and Human Cell Cultures.

Type of sera tested	Titer with cultures of				
	Epithelial cells		Fibroblastic cells		
	HEp-2	WI-38	WI-26	MA	MAF
Pemphigus vulgaris M.C.	810	<30	<30	<30	<30
Pemphigus vegetans L.A.	810	<30	<30	<30	<30
Multiple transfusions A.T.	7290	7290	2430	2430	7290
Normal human serum	<30	<30	<30	<30	<30

TABLE IV. Mixed-Agglutination Tests with Three Pemphigus Sera and Primary Cell Cultures of Kidney and Lung.

Type of sera tested	Titer with cell cultures of				
	HEp-2	Rhesus monkey		Guinea pig	
		Kidney	Lung	Kidney	Lung
Pemphigus vulgaris M.C.	810	810	<30	810	30
Pemphigus vegetans L.A.	810	90	<30	270	<30
Pemphigus vulgaris K.W.	270	270	30	270	90
Multiple transfusion A.T.	7290	<30	<30	<30	<30
Normal human serum	<30	<30	<30	<30	<30

thelial cells and the latter were mostly composed of fibroblasts.

As shown in Table V, treatment of positive

TABLE V. Mixed-Agglutination Test with HEp-2 Cell cultures (A) and Agglutination Test with Erythrocytes Sensitized by Rh Antibodies (B). Effect of Mercaptoethanol Treatment and Ultracentrifugal Fractionation of Pemphigus Vulgaris Serum M.C.

	A	B
Native serum M.C.	810	160
ME-treated serum	810	<10
UC fractions ^a		
Top	9	<1
Middle	27	<1
Bottom	<1	9

^a Protein concentration of fractions was adjusted to 0.1 mg/ml.

serum M.C. with 2-mercaptoethanol (10) had no effect on titer of the reaction with cell cultures while it resulted in complete abolishment of the anti-gamma globulin activity. Upon separation of the serum by sucrose density-gradient ultracentrifugation (11), the antibodies combining with cell cultures were recovered in the top and middle fractions, whereas antibodies to gamma globulin were demonstrated only in the bottom fraction. Double-diffusion gel precipitation tests using multispecific and monospecific antiglobulin sera confirmed the presence of IgG in the top and middle fractions and the presence of IgM in the bottom but not in other fractions. The nature of the antibodies combining with cell cultures was further studied by comparing results of mixed-agglutination tests performed with three different indicator erythrocytes (7). Strong positive results were obtained in tests employing indicator erythrocytes prepared with either the monospecific anti-IgG serum or the multispecific antiserum to human gamma globulins, whereas negative results were obtained when indicator erythrocytes prepared with monospecific anti-IgM serum were used.

These results would indicate that the antibodies combining with cell cultures are associated with the IgG class of immunoglobulins and the antibodies to gamma globulin are of IgM nature.

Discussion. The present study demonstrated that many patients with bullous skin diseases form antibodies combining with human epithelial but not fibroblastic cell cultures. Twofold evidence was presented that these antibodies are of IgG nature: (1) Upon ultracentrifugal separation of the serum, the antibodies could be recovered in the top and middle fractions but not in the bottom fraction; and (2) the binding of the antibodies to the cell cultures has been demonstrated by using indicator erythrocytes specifically designed to detect IgG antibodies but not by an indicator detecting IgM antibodies.

The antibodies combining with epithelial cell cultures were different from antibodies to the intercellular antigen of epidermis demonstrable by immunofluorescent staining technique (1). This was obvious from a lack of correlation in appearance of these two types of antibodies in pathologic sera as well as from absorption experiments.

The antibodies to cell cultures found in patients with bullous skin diseases resembled antibodies demonstrable by mixed-agglutination procedure in sera of homograft recipients, multitransfused patients, and multiparous women (5,6). However, the distinct characteristic of antibodies encountered in bullous skin diseases was their exclusive reactivity with epithelial cells and their cross reactions with cell cultures of nonhuman origin.

Beside antibodies to cell cultures, sera of many patients with bullous skin diseases were shown to contain antibodies to human gamma globulin, which resembled the rheumatoid factor. Simultaneous occurrence of these two types of antibodies was rather striking. Antibodies to gamma globulin were of IgM nature as evidenced by their mercaptoethanol sensitivity and ultracentrifugal separation in the bottom fraction.

Simultaneous occurrence of IgG antibodies to cell surface antigens and IgM antibodies to human gamma globulin with anti-IgG specificity suggest that these two types of antibodies may interact with each other. This possibility appears likely in view of the fact that some sera containing both types of antibodies gave prozone inhibition upon titration in the mixed-agglutination test. It seems

probable that such inhibition may be caused by the reaction between IgG antibodies bound to cell cultures and the antibodies to gamma globulin; at high serum concentrations this reaction may result in complete covering of antigenic sites of IgG, which prevents the binding of indicator erythrocytes. Future experiments will have to show whether antibodies demonstrable by mixed agglutination may play some pathogenic role. This would seem conceivable since these antibodies are directed against "accessible" antigens of the cell surface. However, before any speculation along this line can be made, evidence has to be presented that these antibodies are auto-antibodies. To this end, studies performed on cultures of the patient's own cells will be necessary. Thus far, we did not succeed in obtaining such material.

Summary. Eleven sera of patients with bullous skin diseases were studied for antibodies to cell surface antigens by means of mixed-agglutination tests with cell cultures. It was shown that seven sera contained IgG antibodies reacting with epithelial cell cultures but none of them had antibodies combining with fibroblastic cell cultures. The antibodies reacting with cell cultures were different from antibodies to intercellular antigens of epidermis detectable by immunofluo-

rescent staining technique. Besides these antibodies, some sera of patients with bullous skin diseases were shown to contain IgM antibodies with anti-gamma globulin activity resembling the rheumatoid factor.

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Cold Stimulation of Organs in Relation to Potassium Concentration and Drug Action (32690)

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The different reactivity of various organs to thermal stimulus (1) led us to investigate more closely the correlation between cold contraction and K^+ concentration, for a better understanding of the mechanism involved. With the same purpose, we studied the effect of various pharmacological agents on cold contraction and K^+ liberation.

It has been established already (2) that cold stimulation of smooth muscles induces intracellular variations of K^+ concentration

which appear also in the perfusion fluid.

Materials and Methods. Segments (3–4 cm) of colon and vas deferens of white rat, and guinea pig colon were used. The organs were suspended in tissue bath containing 15 ml of Tyrode's solution, maintained at a temperature of 37°C and oxygenated with a mixture of 95% O_2 and 5% CO_2 . The composition of the Tyrode's solution (in gm/liter of distilled water) was NaCl 8.0; KCl 0.2; $CaCl_2 \cdot 2H_2O$ 0.26; $MgCl_2 \cdot 6H_2O$ 0.21; NaH_2