

## SERUM IMMUNOGLOBULINS IN PEMPHIGUS\*

PETER J. GROB, M.D. AND THEODOR M. INDERBITZIN, M.D.

Sera from patients with the bullous skin disease pemphigus contain immunoglobulins with a specific affinity to a component in stratified epithelia (1, 2).

In order to obtain information about a possible immunological mechanism underlying pemphigus, the concentrations of the immunoglobulins (Ig) A, M, G and D were determined in the sera of such patients (3, 4). Lin and Fusaro (3) analyzed the sera from 12 pemphigus patients with the semiquantitative immunoelectrophoretic method. They found depressed IgM levels, whereas the IgA levels were above normal. Waldorf and Rogentine (4) studied sera from 21 pemphigus patients with the agar-gel diffusion technique. They found that all mean values of the IgA, IgM, IgG and the IgD were below normal.

In the present study these contradictory findings were analyzed. In addition we wish to report on comparisons made between the immunoglobulin concentrations and either the antiepithelial activity of the sera or the activity of the disease.

### MATERIALS AND METHODS

Twenty four serum samples from 15 patients with pemphigus were studied.

The concentrations of the immunoglobulins A, G and M were determined in immunoplates (Hyland Laboratories, Inc. Los Angeles, Calif.). The method used was described by Fahey and McKelvey (5).

The concentrations of the immunoglobulins in the sera of our patients were compared with the values given for normal sera [Schwartz (6) and Fahey and McKelvey (5)].

Immunoelectrophoresis of all sera was carried out (micromethod of Scheidegger (7)). The immunoelectrophoretic pattern of the sera was evaluated qualitatively.

The indirect immunofluorescent test was performed with the technique described by Coons (8): Rabbit esophagus epithelium served as test substrate. Isothiocyanate labelled rabbit antihuman gammaglobulin immune serum was pre-

pared with the method of Marshall, Eveland and Smith (9). Isothiocyanate labelled goat antihuman IgA, IgG and IgM immune sera were obtained from the Hyland Laboratories Inc. Los Angeles Calif. and were used to determine type and titer of the immunoglobulin which reacted with the rabbit oesophagus epithelium in the indirect immunofluorescent test.

The total protein concentrations were estimated by the Biuret method (10).

### RESULTS

The immunoelectrophoretic pattern of all sera were normal. The mean immunoglobulin concentrations in sera from healthy persons were  $2.3 \pm 1.09$  mg/ml for IgA,  $1.19 \pm 0.55$  mg/ml for IgM and  $9.03 \pm 2.15$  mg/ml for IgG (Schwartz (6)). In comparison to this, the mean IgA concentration was elevated in the sera from our patients (p-value  $<0.05$ ). The mean IgM concentration was decreased (p-value  $<0.01$ ). The mean IgG concentration was normal (Table 1).

The labelled anti-IgA and anti-IgM immune sera failed to react with the tissue fixed pemphigus immunoglobulin. The antiepithelial titers in the pemphigus sera obtained either with the isothiocyanate labelled antihuman gammaglobulin or the isothiocyanate labelled antihuman IgG sera were identical.

The concentrations of the various immunoglobulins varied greatly in the sera analyzed. A similar variability became evident when comparing the ratios between the three classes of immunoglobulins (Table 2).

Neither the activity of the disease nor the antiepithelial serum activity was reflected by obvious changes in the concentrations of the A, M and G immunoglobulins.

The sera of all patients were analyzed twice within 2 to 9 months (Table 2). During this time the individual immunoglobulin concentrations remained practically constant. The activity of the disease (patients No. 2, 3, 5, 15) as well as the antiepithelial serum activities (patients No. 1, 2, 3, 7, 8, 15), however, changed considerably.

### COMMENT AND CONCLUSION

The mean immunoglobulin concentrations in the sera of our patients with pemphigus

This investigation was supported by Public Health Service Grants Ti-Am-5220-07 and AM-04486-07

Received for publication February 20, 1967.

\* From the Department of Dermatology, Tufts University School of Medicine, 136 Harrison Av. Boston, Mass. 02111

were compared with the mean concentrations found for normal sera by Schwartz (6). Schwartz determined the immunoglobulin concentrations in the sera from 100 healthy blood donors. We used the same technique and the same lot of immunoplates for the present study. In the sera of our pemphigus patients the mean IgM concentrations were below normal, the IgA concentrations were elevated and the IgG concentrations were normal. This confirms the findings by Lin and Fusaro (3). The mean immunoglobulin concentrations in the sera of our patients with pemphigus were close to those found in pemphigus patients by Waldorf (4). Waldorf, however, compared the pemphigus Ig concentrations with the normal values given by Fahey and McKelvey (5) (Table 2). On the basis of this comparison all mean immunoglobulin concentrations in his pemphigus sera were below normal.

The serum immunoglobulin concentrations showed wide ranges. Equally variable were the ratios between the concentrations of the three immunoglobulins studied in each serum. The anti-epithelial serum titer and disease activity was not reflected by changes in the concentrations of the immunoglobulins. This was also demonstrated by the fact that the immunoglobulin levels remained fairly constant, while the anti-epithelial serum or disease activities changed considerably during the time of observation.

The IgG seems to be the immunoglobulin which in the indirect immunofluorescent test reacts with the epithelium. The anti-epithelial serum activity, however, was not reflected by significant changes in the concentrations of the IgG.

TABLE 1  
Mean values of immunoglobulins (mg/ml)

Immunoglobulin class	In sera of pemphigus pat.		In sera of normal individuals	
	Our results	Waldorf (4)	Schwartz (6)	Fahey (5)
IgA	2.8 ± 1.28*	2.35 ± 0.87	2.3 ± 1.09	2.8 ± 0.7
IgM	0.76 ± 0.39	0.94 ± 0.87	1.19 ± 0.55	1.2 ± 0.35
IgG	9.1 ± 2.61	8.33 ± 1.66	9.02 ± 2.15	12.4 ± 2.2

\* S-value.

TABLE 2  
Serum immunoglobulin concentrations (mg/ml) in patient with pemphigus

Patient No.	Serum investigated months 1-12, 1966	IgA	IgM	IgG	Total protein g/100 ml	Activity of disease*	Titer of indirect IF†
1	8	4.7	1.0	9.5	7.0	0	0
	10	4.0	1.0	9.5		0	40
2	7‡	3.5	0.6	10.5	7.3	++	320
	12	3.5	1.1	8.0		+	80
3	3	3.0	1.6	13.5	7.7	0	40
	12	3.5	1.6	10.5		++	640
4	7	2.5	0.4	12.0	6.5	0	640
	11	2.5	0.6	9.5		+	640
5	7‡	2.2	0.6	13.5	8.0	++	160
	12	1.9	0.3	12.0		+	160
6	12	5.5	1.6	12.0	7.8	+	160
7	4	1.5	0.4	8.0	6.9	0	10
	12	1.2	0.6	6.5		0	0
8	4	1.0	0.9	6.0	5.7	++	320
	10	0.7	0.3	3.8		++	80
9	12‡	4.0	0.6	8.0	7.0	++	80
10	12‡	3.7	0.4	12.0	7.4	++	1280
11	12	2.8	1.0	8.0	7.3	+	80
12	1	3.7	0.6	10.2	6.9	++	80
	9	3.5	0.7	13.5		++	160
13	12	2.0	0.9	10.5	6.9	+	320
14	12	4.3	0.7	8.0	5.5	+	80
15	6	1.4	0.4	6.0	7.3	0	0
	12	1.2	0.4	7.8		+	10

\* Activity of disease: 0 no lesions; + few lesions, patient not seriously sick; ++ wide spread lesions, patient severely sick.

† Titer expressed as the reciprocal value of the highest dilution of serum still leading to a positive indirect IF-test.

‡ Serum sample taken before initiation of corticosteroid therapy.

The IgM levels in the pemphigus sera were very often below normal. Lowered IgM levels are frequently observed in consuming diseases. Elevated IgM as well as elevated IgG and IgA concentrations have been observed in autoimmune diseases, such as lupus erythematosus, ulcerative colitis and Hashimoto's thyroiditis (11).

The IgA concentrations were elevated in many of our patients. This type of immunoglobulin is "excreted" by the body fluids into skin and mucous membranes (12). But again, concentrations of the IgA immunoglobulins did not reflect the activity of the disease.

Waldorf (4) has indicated that prolonged corticosteroid treatment could change the serum immunoglobulin concentrations. The Ig levels which we found in 4 of our patients before such therapy was begun (patients No. 2, 5, 9, 10) were not significantly different from those after prolonged treatment with corticosteroids.

#### REFERENCES

1. Beutner, E. H. and Jordan, 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., Jordan, R. and Cherlock, B.: Autoantibodies in pemphigus vulgaris. *JAMA*, 192: 682, 1965.
3. Lim, S. D. and Fusaro, R. M.: Pemphigus vulgaris I. Analysis of B<sub>2</sub>A and B<sub>2</sub>M serum proteins by immunoelectrophoresis. *J. Invest. Derm.*, 39: 303, 1962.
4. Waldorf, D. S. and Rogentine, G. N.: Serum immunoglobulin in pemphigus vulgaris. *J. Invest. Derm.*, 47: 227, 1967.
5. Fahey, J. L. and McKelvey, E. M.: Quantitative determination of serum immunoglobulin in antibody-agar plates. *J. Immun.*, 94: 84, 1965.
6. Schwartz, R. S.: Immunologic Service, New England Medical Center, Tufts University, School of Medicine: Personal communication.
7. Scheidegger, J. J.: Une micromethode de l'immunoelectrophorese. *Int. Arch. Allergy*, 7: 103, 1955.
8. Coons, A. H. and Kaplan, M. H.: Localisation of antigen in Tissue cells. *J. Exp. Med.*, 91: 1, 1950.
9. Marshall, J. D., Eyeland, W. C. and Smith, C. W.: Superiority of Fluorescein isothiocyanate (Riggs) for Fluorescent-antibody technic with a modification of its application. *Proc. Soc. Exp. Biol. Med.*, 98: 898, 1958.
10. Kabat, E. A. and Mayer, U. M.: *Experimental Immunochemistry*. Published by Charles C. Thomas, Springfield, Illinois.
11. Alper, Ch. A., Rosen, F. S. and Janeway, C. A.: Gammaglobulins II. *New Engl. J. Med.*, 275: 591, 1966.
12. Hong, R., Pollara, B. and Good, R. A.: A model of colostral IgA. *Biochemistry*, 56: 602, 1966.