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IgG, IgA, IgM, and IgD antiglobulins in juvenile rheumatoid arthritis

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Usual techniques for the detection of antigammaglobulin factors (DAT, SCAT, HEAT, Latex FII) are based upon agglutination reactions and are therefore useful when IgM antibodies are involved. It is well known that antiglobulins also belong to IgG and IgA classes.

In our experience, 50 per cent. of patients with JRA are persistently seronegative when sera are tested only with conventional agglutination techniques (García Morteo, Hübscher, and Arana, 1970). Recent papers reported elevated IgG antiglobulins in seronegative RA and JRA demonstrated by means of immunoadsorption techniques employing human, horse, and rabbit gammaglobulin rendered insoluble either with glutaraldehyde or with bis-diazobenzidine. These adsorbents had non-specific elution of immunoglobulins (Torrigiani and Roitt, 1967; Torrigiani, Ansell, Chown, and Roitt, 1969; Torrigiani, Roitt, Lloyd, and Corbett, 1970; Bianco, Panush, Stillman, and Schur, 1970).

The purpose of this work was to evaluate antigammaglobulin factors in seronegative JRA using a quantitative immunoadsorption technique which would have no non-specific elution of immunoglobulins.

Material and methods

Sera were collected from fifty patients with JRA selected according to the criteria of Ansell and Bywaters (1959) and from fifty healthy individuals matched for age and sex. Of the patients 21 were under 10, twenty were between 10 and 20, and nine were over 20 years of age. There were 33 females and 17 males. All the sera in both groups proved to be negative in Latex FII and SCAT tests (Singer and Plotz, 1956; Roitt and Doniach, 1969).

The immunoadsorbents consisted of human (heat-aggregated Cohn's FII) or horse (ammonium sulphate precipitate) gammaglobulin linked to Bentonite through the action of a carbodiimide, prepared according to Carpenter, Barsales, and Reisberg's original methods (Carpenter and Barsales, 1967; Carpenter and Reisberg, 1968). 1 ml. serum was incubated with 3 mg. of each adsorbent antigen for 1 hr at 37°C. and left overnight at 4°C. After the complex had been thoroughly washed, elution was carried on for 1 hr in 0·05 M glycine-HCl buffer pH 2·5. The eluates were then neutralized with 0·5 NaOH and their immunoglobulin contents were estimated by Mancini's single radial immunodiffusion method, using specific antisera to IgG, IgA, IgM, and IgD.* Results were expressed in IU per ml. serum, according to WHO 67/97 Ig reference serum.

Results

The control sera had no elution of any class of immunoglobulin, while 48/50 JRA sera were positive for at least one of the immunoglobulin classes (Table). Using human FII as a substrate, 34 sera (68 per cent.) had IgG antiglobulins, seventeen (34 per cent.) had IgA, sixteen (32 per cent.) had IgM, and fifteen (30 per cent.) had IgD. When horse

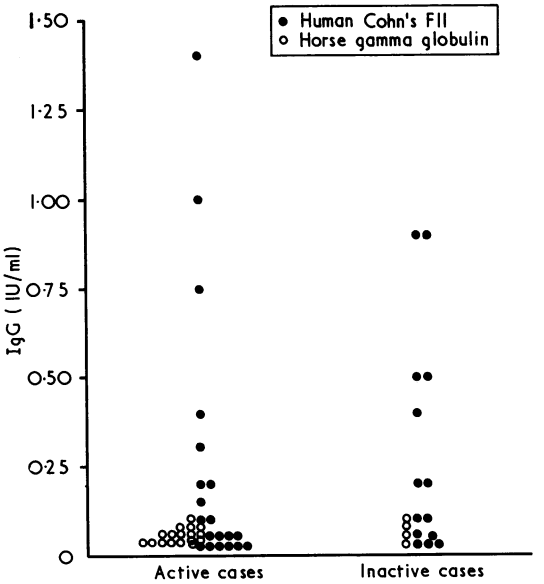

FIGURE *IgG antiglobulins (IU/ml.) demonstrated by human Cohn's FII and horse gammaglobulin in active and inactive cases*

* Immunoplates were obtained from Hoechst and Hyland Laboratories.

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Table *Particulars of fifty patients*

Patient no.	*Age (yrs)*	*Sex*	*Disease activity*	*Antiglobulins (IU/ml.) Human FII*				*Horse gamma globulin*			*Serum gamma globulin*
				IgG	*IgA*	*IgM*	*IgD*	*IgG*	*IgA*	*IgM*	
1	12	M	Yes	0·20	—	0·80	2·80	0·06	—	—	↑
2	11	M	Yes	—	—	—	—	0·05	—	—	↑
3	17	F	No	0·50	—	—	—	—	—	—	N
4	12	F	No	—	—	—	2·80	—	—	—	↑
5	10	M	No	0·90	—	—	2·80	—	—	—	N
6	9	F	Yes	0·15	0·30	—	1·50	0·05	—	—	↑
7	13	F	Yes	0·10	—	—	—	0·05	—	—	N
8	5	F	Yes	0·02	—	—	—	—	—	—	N
9	9	F	No	0·10	—	—	5·00	—	—	—	N
10	7	F	Yes	0·05	—	—	2·80	0·06	—	—	↑
11	17	F	No	0·05	—	—	—	—	—	—	↑
12	8	M	Yes	0·75	—	—	—	—	—	—	↑
13	2	F	Yes	0·02	—	0·12	—	—	—	—	N
14	14	M	No	0·02	0·60	0·17	—	—	—	—	N
15	17	F	Yes	0·20	0·35	1·30	—	—	—	—	↑
16	4	M	Yes	0·10	—	—	—	0·07	—	—	N
17	17	M	Yes	1·40	0·50	—	—	0·06	—	—	N
18	8	F	Yes	—	—	—	—	0·06	—	—	N
19	30	F	Yes	—	0·50	—	—	—	—	—	N
20	26	F	Yes	—	1·20	—	—	—	—	—	↑
21	14	M	Yes	0·02	0·50	—	—	0·05	—	1·80	↑
22	27	F	Yes	—	—	—	—	0·05	—	—	N
23	34	F	No	—	—	—	—	—	—	—	↑
24	3	M	Yes	—	—	1·50	1·50	—	—	—	N
25	9	M	Yes	0·30	—	—	—	0·07	—	—	N
26	12	F	No	0·40	—	3·50	1·50	—	—	—	N
27	12	M	Yes	0·02	0·05	—	0·75	0·07	—	—	↑
28	11	F	Yes	0·05	0·27	1·30	2·80	—	—	—	N
29	13	F	No	—	1·00	—	—	0·05	—	—	↑
30	30	M	No	—	—	—	3·75	—	—	—	N
31	18	F	No	0·20	—	—	2·80	—	—	—	↑
32	20	M	No	0·50	—	—	—	—	—	—	↑
33	8	F	No	—	0·27	1·30	—	0·02	—	—	↑
34	5	M	Yes	0·05	—	—	—	0·04	—	—	N
35	6	F	Yes	—	1·20	3·50	—	0·05	—	—	↑
36	19	F	No	—	—	—	—	—	—	—	N
37	10	F	Yes	1·00	—	0·90	—	—	—	—	N
38	8	M	Yes	0·40	—	—	—	—	—	—	N
39	16	F	No	0·90	0·35	—	—	—	—	—	N
40	42	F	No	0·10	—	0·80	—	—	—	—	N/↓
41	13	F	No	0·02	—	1·30	—	—	—	—	N
42	27	F	No	0·02	—	—	—	—	—	—	N
43	18	F	No	—	1·00	1·30	—	0·10	—	2·00	N
44	43	F	No	0·20	—	—	—	—	—	2·00	N
45	10	F	No	0·03	—	—	2·80	0·07	—	—	N
46	7	M	Yes	—	—	—	4·40	0·10	—	—	↑
47	7	M	Yes	0·02	1·35	0·90	—	—	—	—	↑
48	37	F	Yes	0·02	0·50	0·70	—	0·06	—	2·00	N
49	8	F	Yes	0·05	1·00	—	2·80	0·05	—	—	↑
50	5	F	Yes	—	—	0·12	—	—	—	—	↑

gamma globulin was used, 21 sera (42 per cent.) had IgG and four (8 per cent.) had IgM antiglobulins; the presence of IgD antiglobulins in these eluates was not investigated. The immunoplates were able to recognize a minimum immunoglobulin concentration of 0·02 IU/ml. for IgG, 0·05 IU/ml. for IgA, 0·12 IU/ml. for IgM, and 0·75 IU/ml. for IgD. The highest concentrations of antiglobulins were observed in the human-FII eluates (Figure, p. 32).

The presence of IgG antiglobulins in the eluates from sera adsorbed with horse gammaglobulin was strictly related to disease activity ($P < 0.001$), but, with this exception, the presence and level of antiglobulins showed no relation to the disease activity, the severity of its evolution, or systemic involvement. Nevertheless, no relation was found with serum levels of gammaglobulin; 50 per cent. of the patients had hypergammaglobulinaemia, and the rest had normal or low levels, as evidenced by paper electrophoresis.

Discussion

The use of the present immunoadsorbent allows the use of homologous and heterologous gamma globulins as substrates without non-specific elution and achieves a high degree of positivity (96 per cent.) with conventionally negative RA sera for antiglobulins, but gives negative results in normal subjects. It has also yielded evidence of the existence of antiglobulins belonging to the IgD class.

A disparity in immunoglobulin class and concentration of antiglobulins was often obtained from a single serum with both antigens. This could possibly be related to different specificities or affinities of antibodies.

The results of our work suggest, according to the findings of Torrigiani and Roitt (1967), that the immunological distinction between seronegative and seropositive rheumatoid disease depends on the class and specificity of antiglobulins and the methods used for their detection, rather than on some fundamentally different pathogenic mechanism.

Summary

An immunoadsorption technique has been used to demonstrate the presence of antigammaglobulin factors in 48 out of fifty sera from patients with juvenile rheumatoid arthritis that were negative when tested with conventional agglutination techniques. The existence of IgD antigamma globulin factors is reported.

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References

ANSELL, B. M., AND BYWATERS, E. G. L. (1959) *Bull. rheum. Dis.*, **9**, 189 (Prognosis in Still's disease)

BIANCO, N., PANUSH, R., STILLMAN, J., AND SCHUR, P. (1970) *Arthr. and Rheum.*, **13**, 306 (Serum antigammaglobulins in juvenile rheumatoid arthritis)

CARPENTER, R. R., AND BARSALES, P. B. (1967) *J. Immunol.*, **98**, 844 (Uptake by mononuclear phagocytes of protein-coated bentonite particles stabilized with a carbodiimide)

—— AND REISBERG, M. (1968) *Ibid.*, **100**, 873 (Carbodiimide-induced Bentonite antigen complexes: readily prepared immunoadsorbents)

GARCÍA MORTEO, O., HÜBSCHER, O., AND ARANA, R. (1970) *Medicina (B Aires)*, **30**, 384 (Autoanticuerpos en artritis reumatoidea juvenil)

ROITT, I. M., AND DONIACH, D. (1969) 'WHO Manual of Autoimmune Serology'. (Sheep cell agglutination test (SCAT) for the detection of rheumatoid factors)

SINGER, J. M., AND PLOTZ, C. M. (1956) *Amer. J. Med.*, **21**, 888 (The latex-fixation test. I. Its application to the serologic diagnosis of rheumatoid arthritis)

TORRIGIANI, G., AND ROITT, I. (1967) *Ann. rheum. Dis.*, **26**, 334 (Antiglobulin factors in sera from patients with rheumatoid arthritis and normal subjects)

——, ANSELL, B. M., CHOWN, E. E. A., AND ROITT, I. M. (1969) *Ibid.*, **28**, 424 (Raised IgG antiglobulin factors in Still's disease)

——, ROITT, I. M., LLOYD, K. N., AND CORBETT, M. (1970) *Lancet*, **1**, 14 (Elevated IgG antiglobulins in patients with Still's disease)