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THE DISTRIBUTION OF ANTI-THYROGLOBULIN ANTIBODIES IN THE IMMUNOGLOBULIN G SUBCLASSES

F. C. HAY AND G. TORRIGIANI*

Department of Immunology, Middlesex Hospital Medical School, Arthur Stanley House, 40–50 Tottenham Street, London, W1P 9PG

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SUMMARY

Using the coprecipitation technique with rabbit antisera specific for the human IgG subclasses, the antigen-binding capacity for thyroglobulin has been investigated in patients with Hashimoto's disease. Anti-thyroglobulin activity was found in all four subclasses. The proportion of the total activity in each subclass closely paralleled the relative amounts of each subclass in normal serum.

INTRODUCTION

In patients with Hashimoto's thyroiditis, autoantibodies to thyroglobulin have been demonstrated in all the three major immunoglobulin classes. The original studies were carried out by physically separating 7S and 19S fractions of serum by centrifugation, gel diffusion and by column chromatography (Pressman *et al.*, 1957; Korngold, Van Leeuwen & Brener, 1959; Fahey & Goodman, 1960). Both 19S and 7S antibodies were found in all these studies. However, in later work involving zone ultracentrifugation, Torrigiani & Roitt (1963) showed that the macroglobulin class always contributed $<1\%$ of the total antibodies to thyroglobulin. Fahey & Goodman (1964) and Goodman, Exum & Robbins (1964) used the technique of radioimmunoelectrophoresis to demonstrate qualitatively that antibodies were present in IgG, IgA and IgM classes. Soon after the discovery of the first three IgG subclasses, Terry & Fahey (1964) and Lichter (1964) extended these studies to show the presence of antibodies to thyroglobulin in IgG1, IgG2 and IgG3 in sera from patients with chronic thyroiditis. Torrigiani, Roitt & Doniach (1968) using a quantitative coprecipitation technique were able to measure the amount of antibodies to thyroglobulin in the IgG, IgA and IgM classes. Most of the antibody was found to be in the IgG class, with up to 20% being IgA but never $>1\%$ IgM.

Thyroglobulin antibodies have been found to be only weakly complement-fixing (Roitt,

* Present address: World Health Organisation, 1211 Geneva 27, Switzerland.

Correspondence: Dr F. C. Hay, Department of Immunology, Middlesex Hospital Medical School, Arthur Stanley House, 40–50 Tottenham Street, London W1.

Campbell & Doniach, 1958). This could either be a feature of the antigen or the antibody or both. To investigate the first possibility further, the presence of thyroglobulin antibodies in the different IgG subclasses were looked for, to determine whether there was any preponderance of antibody activity in the non-complement-fixing subclasses.

MATERIALS AND METHODS

Human sera

Sera from patients with Hashimoto's thyroiditis were stored at −20°C for up to 3 years.

Antisera

Rabbit antisera for the IgG subclasses were prepared as described previously (Hay, Hull & Torrigiani, 1971). Rabbits were immunized repeatedly with purified IgG myeloma proteins in Freund's complete adjuvant. All the antisera were made specific by absorption with kappa and lambda Bence-Jones proteins, and with myeloma proteins of all the other subclasses.

Human thyroglobulin

Thyroglobulin was prepared from the thyroid glands of humans by the ammonium sulphate precipitation method (Derrien, Michel & Roche, 1948), followed by gel filtration in Sephadex G-200 (Salvatore *et al.*, 1964).

Radiolabelling of thyroglobulin

Thyroglobulin was radiolabelled with ^{125}I (IMS4 Radiochemical Centre, Amersham) by the chloramine-T method of Hunter & Greenwood (1962) using the following conditions. 2·5 ml of human thyroglobulin solution (100 mg/ml in phosphate-buffered saline (PBS) was added to 500 μCi of ^{125}I in 0·1 ml PBS. 200 μg of chloramine-T in 0·05 ml PBS was added in 25°C and left for 1 min. Then 720 μg of sodium metabisulphite in 0·2 ml PBS was added. The labelled thyroglobulin was separated from the free iodine by gel filtration on a Sephadex G-200 column 20 cm × 1 cm made up in PBS. The radioactive protein was collected and its specific activity determined.

Treatment of thyroglobulin to prevent non-specific binding to immune complexes

To remove that fraction of labelled thyroglobulin that would bind to immune complexes non-specifically, an unrelated antigen–antibody reaction was performed in the labelled thyroglobulin solution. Two millilitres of rabbit antiovalbumin serum were added to the thyroglobulin solution. Then 0·5 ml of a 1 mg/ml solution of ovalbumin in PBS was added. The mixture was incubated at 37°C for 1 hr and then left in the cold overnight. The precipitate which formed was removed by centrifugation.

Antigen-binding capacity in each IgG subclass

The antigen-binding capacity was determined by the method of Torrigiani *et al.*, (1968). An excess of radiolabelled thyroglobulin was added to the patient's serum, followed by coprecipitation of the soluble complexes by specific rabbit anti-IgG subclass sera. A control serum lacking anti-thyroglobulin activity was included in all experiments and the values obtained for this serum subtracted from the test sera. The mixtures of serum plus antigen

were incubated at 37°C for 3 hr. 0·5 ml of PBS was added to each tube followed by an amount of the specific anti-subclass serum previously shown to precipitate all the related immunoglobulin. The solutions were then further incubated at 37°C for 1 hr and at 4°C overnight. The floccules which formed were spun down and washed twice with cold PBS. The amount of thyroglobulin bound was then determined by counting the precipitates in a Packard Auto-Gamma spectrometer.

RESULTS

The antigen-binding capacities of sera from patients with Hashimoto's thyroiditis were determined by the co-precipitation technique. The results are shown for all four IgG subclasses in Table 1. The antibody activity was found predominately in IgG1 with a mean antigen-binding capacity of 8·42 mg thyroglobulin bound per ml of serum. The other

TABLE 1. Antigen-binding capacities for thyroglobulin in Hashimoto's disease patients

Patient	IgG1	IgG2	IgG3	IgG4
SM	4·12*	2·64	0·72	0·60
DU	4·24	1·96	1·14	1·31
SC	1·00	1·69	0·42	0·63
SL	0·74	0·40	0·44	0·36
CA	40·24	8·84	0·86	1·06
LO	59·54	8·00	6·35	3·54
FE	3·91	1·15	1·14	0·40
MO	0·93	0·31	0·18	0·42
WA	2·98	2·00	0·98	0·59
BAT	2·00	1·86	0·24	0·62
WI	1·20	1·66	0·52	1·16
HAN	0·10	1·87	0·04	0·80
BI	0·50	0·53	0·00	0·07
EL	2·00	0·93	0·00	0·22
BAI	2·80	1·33	0·48	0·15
Mean	8·42	2·34	0·90	0·78
SD	17·27	2·55	1·55	0·79
Median	2·00	1·69	0·48	0·60

* Mg thyroglobulin bound/ml serum.

TABLE 2. Contribution of each subclass to total IgG and anti-thyroglobulin binding capacity

	IgG1	IgG2	IgG3	IgG4
Total IgG in normal serum (%)	*64–70	23–28	4–7	3–4
Antigen-binding capacity (mean %)	68	19	7	6

* Schur, 1972.

subclasses had lower antigen-binding capacities of 2·34 mg/ml for IgG2, 0·90 mg/ml for IgG3 and 0·78 mg/ml for IgG4.

The proportion of antibody found in each subclass corresponds closely with the proportion of these subclasses in normal serum IgG (Table 2).

DISCUSSION

The co-precipitation technique is based on the method of Farr (1958) in which the antibody content of a serum is measured in terms of the antigen-binding capacity by co-precipitating radioactive antigen with antibody by precipitating the antibody with 50% ammonium sulphate. This technique was modified by precipitating the antibody with antiglobulin so that antigens which were insoluble in 50% ammonium sulphate could be used (Skom & Talmage, 1958; Feinberg, 1958). This made it possible to measure the antigen-binding capacity in separate immunoglobulin classes (Torrigiani, Roitt & Doniach, 1968) by coprecipitating the immunoglobulins with class-specific antisera.

Extending this approach, the present investigation has shown that over 70% of the antigen-binding capacity is in subclasses that fix complement. Therefore it seems likely that the reason for the poor complement fixation observed in this system is dependent on the nature of the thyroglobulin molecule rather than on the antibodies. Roitt, Campbell & Doniach (1958) and Schulman & Witebsky (1960) showed that the human auto-antibodies could only react with a maximum of four determinants on the thyroglobulin molecule. This is in contrast to the findings in the heterologous thyroid immune system in which Heidelberger (1938) found a ratio of forty antibody molecules to one thyroglobulin molecule in immune precipitates made from sera of rabbits immunized with hog thyroglobulin. This limited number of antigenic sites in the human system may be the factor responsible for the lack of complement fixation as close proximity of bound IgG molecules is necessary for the binding of $C1_q$ (Humphrey & Dourmashkin, 1965; Borsos & Rapp, 1965).

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