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Evidence of unique idiotypic determinants and similar idiotypic determinants on human anti-thyroglobulin antibodies

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SUMMARY

This is a report on the production of heterologous anti-idiotypic antisera against human anti-thyroglobulin antibodies and on the detection of cross-reactivities among human anti-thyroglobulin antibodies by means of anti-idiotypic antisera. The results are evidence of the existence of unique idiotypic determinant on that anti-thyroglobulin antibody used for immunization and similar idiotypic determinants on all anti-thyroglobulin antibodies.

INTRODUCTION

Anti-idiotypic antiserum is a useful tool for testing the network theory in human autoimmunity. Moreover, as certain idiotypes recognized by anti-idiotypic antisera behave as genetic markers (Eichman, 1973), investigation of idiotypes may prove useful in studying the genetics of autoantibodies in man. In this paper, we shall describe the production of anti-idiotypic antisera against anti-thyroglobulin antibodies in rabbits and try to detect cross-idiotypic antigens on different IgG from patients with chronic thyroiditis.

MATERIALS AND METHODS

Serum samples. Serum samples from patients with chronic thyroiditis and healthy controls were used either to purify anti-thyroglobulin antibodies or as inhibitors of the reaction between anti-idiotypic antisera and idiotypes.

Purification of thyroglobulin. Semi-purified thyroglobulin was prepared from normal post mortem thyroid glands by means of differential ammonium sulphate precipitation according to the method of Derrien, Michel & Roche (1948). The substance was then chromatographed on a Sephadex G-200 column and a Sepharose 4B column.

Purification of anti-thyroglobulin antibody. The serum from a patient with chronic thyroiditis was precipitated with 33% $(NH_4)_2SO_4$, and the precipitate was dissolved in saline, dialysed against 0·01 M phosphate buffer (0·01 M pB), pH 7·5, fractionated over a large DEAE column, digested with pepsin in acetate buffer at pH 4·5 (protein to enzyme ratio 25:1, 37°C, 24 hr), and passed over a Sephadex G-150 column to obtain the IgG $F(ab')_2$ fragments.

Anti-thyroglobulin antibody was purified from IgG $F(ab')_2$ by means of a slight modification of the method of Davoli, Salabé & Andreoli (1978). Unbound proteins deprived of anti-thyroglobulin

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antibody activity by thyroglobulin antibody immunoadsorbent were referred to as $F(ab')_2(-)$. Elution of the anti-thyroglobulin antibodies specifically adsorbed by thyroglobulin immunoadsorbent was performed by means of 3 M NaSCN. The eluted antibody fraction was referred to as $F(ab')_2(+)$.

Preparation of immunoadsorbent. Proteins were coupled to cyanogen bromide activated Sepharose 4B according to the method of Axén, Porath & Ernback (1967).

Immunization of rabbits. Anti-idiotypic antisera were raised in Japanese white rabbits by means of four to six injections of 0·1 mg of purified IgG $F(ab')_2$ anti-thyroglobulin antibody in Freund's complete adjuvant at 2 week intervals. The rabbits were bled 1 week after last boosters.

Isolation and absorption of rabbit IgG. Rabbit IgG was repeatedly absorbed with Sepharose 4B cross-linked to Cohn FII, κ light chain myeloma protein, λ light chain myeloma protein, thyroglobulin and IgG $F(ab')_2(-)$ from the same donor.

Solid phase radioimmunoassay (Catt & Tregear, 1976). The antigen coated tubes (Falcon 2052 tube) were incubated together with the duplicate samples for 1 hr at 37°C, and then for one more hour at 4°C. Unbound proteins were then removed by washing the tubes with cold 0·01 M pB, 0·14 M

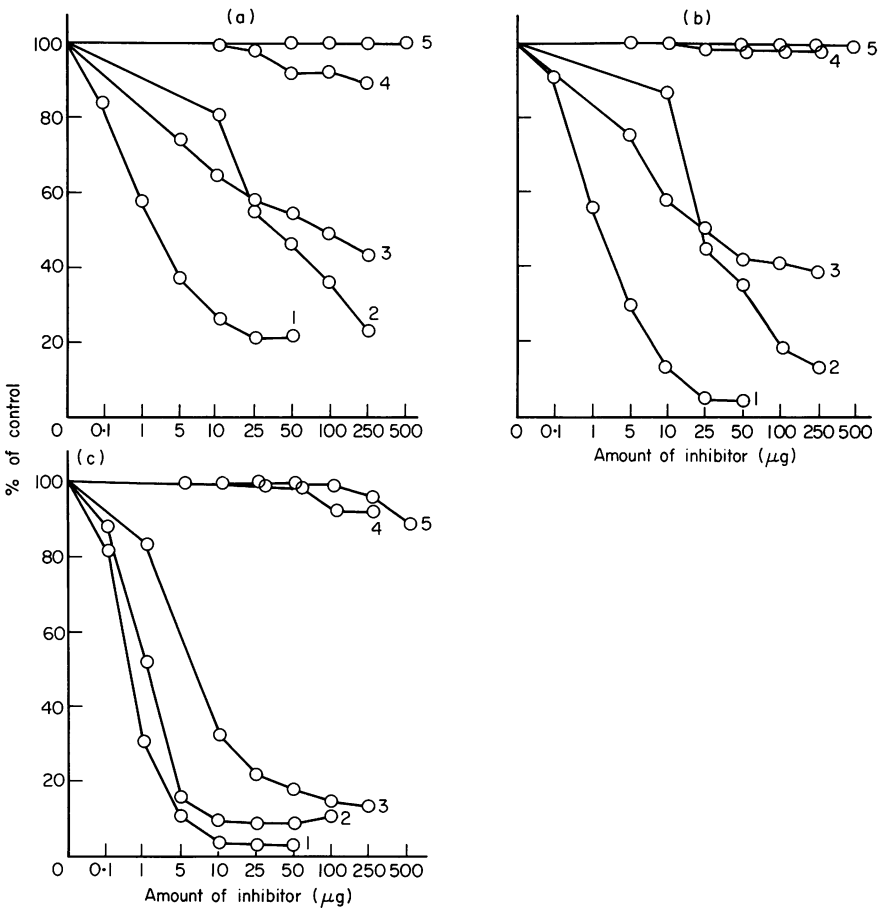

Fig. 1. Inhibition of binding of anti-idiotypic antiserum to $F(ab')_2(+)$ by various immunoglobulins and thyroglobulin. Panels (a), (b) and (c) show inhibition of binding of each anti-idiotypic antiserum to Na $F(ab')_2$, Ic $F(ab')_2(+)$ and Uc $F(ab')_2(+)$, respectively, by various immunoglobulins and thyroglobulin. 1, 2, 3, 4 and 5 represent the corresponding $F(ab')_2(+)$, the corresponding IgG, thyroglobulin, the corresponding $F(ab')_2(-)$ and pooled human IgG as inhibitors. Amounts of Na, Ic and Uc anti-idiotypic antisera were 0·1 mg, 0·05 mg and 0·015 mg, respectively.

Table 1. Inhibition of binding of anti-idiotypic antiserum* to F(ab′)2(+) by various IgG with anti-thyroglobulin antibodies

Chronic thyroiditis serum sample number	Thyroid test	Na anti-idiotypic antiserum to Na F(ab′)2(+) % inhibition IgG (μg)†				Ic anti-idiotypic antiserum to Ic F(ab′)2(+) % inhibition IgG (μg)†			
		10	50	250	500	10	50	250	500
101	> ×409,600	0	0	0	0	0	0	0	0
102	> ×409,600	0	0	0	0	0	0	0	6
103	> ×409,600	0	0	0	0	1	0	0	0
104	> ×409,600	0	0	3	5	0	0	11	0
105	> ×409,600	0	0	0	0	not tested			
106	> ×409,600	0	0	0	0	0	0	1	0

* Amounts of Na and Ic anti-idiotypic antisera were 0·1 mg and 0·05 mg, respectively.

† Amounts of IgG as inhibitors.

NaCl and pH 7·2. Rabbit immunoglobulin bound to the tubes were detected by incubating the tubes with ^{125}I-labelled goat anti-rabbit IgG.

Inhibition of binding of each anti-idiotypic antiserum to its corresponding idiotypes by various inhibitors. An inhibition test was conducted by mixing anti-idiotypic antisera with different dilutions of inhibitors.

Measurement of anti-thyroglobulin antibody and anti-microsome antibody. Anti-thyroglobulin antibody and anti-microsome antibody were assayed by means of both a thyroid test and a

Table 2. Inhibition of binding of Uc anti-idiotypic antiserum* to Uc F(ab′)2(+) by various IgG from patients with chronic thyroiditis

Chronic thyroiditis serum sample number	Thyroid test	% Inhibition IgG (μg)†					
		1	5	10	50	250	500
101	> ×409,600	16	39	41	58	62	60
102	> ×409,600	6	25	30	54	49	60
103	> ×409,600	5	24	46	49	64	61
104	> ×409,600	1	8	22	37	62	67
105	> ×409,600	0	20	16	35	52	53
106	> ×409,600	0	0	0	21	41	45
107	×6,400	0	0	8	30	57	61
108	×6,400	0	0	0	31	41	46
109	×400	0	0	7	12	34	43
110	×400	0	0	0	0	16	30
111	×20	0	0	5	15	27	41
112	< ×20	0	0	12	18	48	55
113	< ×20	0	0	4	17	31	26
114	< ×20	0	0	0	0	3	19
115	< ×20	0	0	0	0	4	0

* 0·015 mg of Uc anti-idiotypic antiserum.

† Amounts of IgG as inhibitors.

Table 3. Inhibition of binding of Uc anti-idiotypic antiserum* to Uc F(ab′)2(+) by F(ab′)2(+) or F(ab′)2(−) from chronic thyroiditis patients with high titres of thyroid test

Chronic thyroiditis serum sample number		% Inhibition F(ab′)2 (μg)†							
		0·1	1	5	10	25	50	100	250
101	F(ab′)2(+)	10	18	34	39	41	42		
	F(ab′)2(−)				0	0	9	26	41
102	F(ab′)2(+)	8	47	57	48	59	64		
	F(ab′)2(−)				16	21	27	28	49
103	F(ab′)2(+)	11	20	29	42	44	53		
	F(ab′)2(−)				0	6	11	13	21
104	F(ab′)2(+)	0	26	36	44	53	66		
	F(ab′)2(−)				0	0	0	0	4
105	F(ab′)2(+)	0	26	48	51	56	62		
	F(ab′)2(−)				0	3	4	10	22
106	F(ab′)2(+)	0	0	11	22	28	32		
	F(ab′)2(−)				0	6	9	10	19

* 0·015 mg of Uc anti-idiotypic antiserum.
† Amounts of IgG as inhibitors.

Table 4. Inhibition of binding of Uc anti-idiotypic antiserum absorbed with cross-reactive IgG to Uc F(ab′)2(+) by various IgG from patients with chronic thyroiditis

Chronic thyroiditis serum sample number	Cross-reactive 101* % inhibition IgG (μg)†						Cross-reactive 103* % inhibition IgG (μg)†					
	1	5	10	50	250	500	1	5	10	50	250	500
Uc	4	31	53	68	86	86	13	41	51	61	80	81
101	0	0	5	4	0	0	0	0	0	0	0	0
103	0	0	0	0	5	5	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	8	4	4	9	6	0	0	0	0	0	0
106	0	0	5	11	3	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	7	3	5	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0	0	0	0	0

* Anti-idiotypic antisera were absorbed with sample number 101 or sample number 103, and 0·015 mg of absorbed anti-idiotypic antisera were used in these reactions.
† Amounts of IgG as inhibitors.

microsome test kit (Fujizoki Pharmacentical Co. Ltd., Tokyo, Japan), using the tanned haemagglutination method.

RESULTS

Specificity of anti-idiotypic antisera

Three antisera from three different donors possessed the activities of anti-idiotypic antisera. Binding of each anti-idiotypic antiserum to the corresponding $F(ab')_2(+)$ was significantly inhibited by the corresponding IgG $F(ab')_2(+)$, thyroglobulin, but not by the corresponding $F(ab')_2(-)$ or pooled human IgG from 15 healthy donors unless very large quantities were added as inhibitors. (Fig. 1).

Detection of cross-reactivity among IgG isolated from the sera of patients with chronic thyroiditis

Binding of Na or Ic anti-idiotypic antiserum to the corresponding $F(ab')_2(+)$ was not inhibited by any IgG with high anti-thyroglobulin titres, as shown in Table 1. However, binding of Uc anti-idiotypic antiserum to the corresponding $F(ab')_2(+)$ was inhibited in a greater or lesser degree by various IgG from patients with chronic thyroiditis, as shown in Table 2. Table 3 shows that a large portion of inhibitions by various IgG with anti-thyroglobulin antibody activities are due to purified anti-thyroglobulin antibodies.

Evidence of unique idiotypes on Uc anti-thyroglobulin antibody

Binding of Uc anti-idiotypic antiserum absorbed with cross-reactive IgG to Uc anti-thyroglobulin antibody was inhibited by Uc IgG, but not by any other IgG. (Table 4).

DISCUSSION

Idiotypic cross-reactivity was detected in all anti-thyroglobulin antibodies. We excluded the possibility that idiotypic cross-reactivity may be caused by the contamination of soluble thyroglobulin–anti-thyroglobulin antibody immune complex in IgG from patients with chronic thyroiditis, firstly for the reason that IgG from patients with chronic thyroiditis were absorbed with the immunoadsorbents cross-linked to rabbit serum with anti-thyroglobulin antibody activity, and secondly because purified $F(ab')_2$ anti-thyroglobulin antibodies chromatographed on a Sephadex G-150 column showed idiotypic cross-reactivity. We also excluded the possibility of soluble thyroglobulin–anti-thyroglobulin antibody immune complexes in anti-idiotypic antisera, since rabbit sera with high anti-thyroglobulin titres did not inhibit the reaction of anti-idiotypic antiserum to Uc idiotype when used as an inhibitor (data not shown). Thus, Uc idiotypes were proved to consist of unique idiotypic determinants and similar idiotypic determinants on all anti-thyroglobulin antibodies.

In unrelated humans, idiotypic cross-reactivities of autoantibodies were described in cold agglutinins (Williams, 1968), monoclonal rheumatoid factors (Kunkel *et al.*, 1973) and polyclonal rheumatoid factors (Førre *et al.*, 1979). However, each group of cross-idiotypes in contrast to ours, is known to be divided into several groups. As idiotypes are inherited in animals, as mentioned above, evidence of similar idiotypes on all anti-thyroglobulin antibodies suggest that genetic controls play an important role in the occurrence of autoantibodies in humans.

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