

Rabbit Thyroid Autoantibody Produced by Intravenous Injections of Papain-Treated Rabbit Thyroid Extract* (32800)

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Circulating thyroid-specific autoantibody and characteristic inflammatory changes of thyroid gland have been produced by rabbits upon intradermal injection of rabbit thyroid extract incorporated in complete Freund adjuvant (1-3). Rose and Witebsky (4) found that intravenous injections of foreign, cross-reacting thyroglobulins would elicit autoantibodies in the rabbit without the use of Freund adjuvant. Prolonged immunization would sometimes result in the development of thyroid lesions. Weigle (5) was able to find precipitating autoantibodies and thyroid lesions in rabbits intravenously injected with rabbit thyroglobulin that had been coupled to arsanilic or sulfanilic acid.

Autoantibodies are thought to develop either by the interaction of an abnormal immune apparatus stimulated by some normal autologous antigen, or by a normal immune mechanism stimulated by an altered or related antigenic component. Freund adjuvant may induce changes in the immune response of an animal and may also alter the configuration of the antigen with which it is emulsified. Therefore, additional methods to induce the production of antibodies to autologous proteins without the use of adjuvants were sought.

Enzymatic cleavage of thyroglobulin may be considered to be a normal physiological process, and a thyroidal proteinase with specific action resembling pepsin has been isolated from thyroid epithelial cells (6). The question then arises whether partially degraded thyroglobulin can elicit autoantibody production in the absence of Freund adjuvant. We have digested normal rabbit thyroid extract with papain and have found that it

induces specific autoantibodies when injected intravenously into rabbits.

Materials and Methods. Saline extracts of normal rabbit thyroid glands (Pel-Freez Biologicals, Inc., Rogers, Arkansas) were prepared by a slight modification of a procedure previously reported (7). The protein concentration of the rabbit thyroid extract (RTE) was adjusted to 10 mg/ml with phosphate buffered saline, 1% by weight of papain (Nutritional Biochemicals Corp.) was added, and the mixture incubated at 37°C for 18 hours in the presence of 0.01 *M* cysteine and 2 mM EDTA. Enzymatic hydrolysis was stopped by dialysis against 0.01 *M* iodoacetamide. The digest was further dialyzed against phosphate buffered saline, centrifuged at 2000g for 5 min, the supernatant was filtered through a membrane filter, and stored in a sterile vaccine bottle at 4°C until used.

Eight male New Zealand white rabbits weighing approximately 2 kg were intravenously injected with 1 ml of papain-treated RTE (protein concentration about 5 mg/ml). The immunization schedule consisted of three injections a week for 2 weeks, followed by a rest period of 1 week; this cycle was repeated until maximum titers were obtained. This same immunization schedule was used with 4 additional rabbits in order to determine the effect of intravenous injections of either papain or RTE alone.

The ability of the rabbit to produce circulating thyroid-specific antibodies to intravenous injections of papain-treated RTE was measured by a micromodification of the tanned cell hemagglutination reaction (8). For these tests, tanned human erythrocytes (Group O) were coated with untreated RTE.

Results and Discussion. The tanned cell hemagglutination titers of the rabbits injected with papain-treated RTE are shown in Table I. Of the 6 rabbits that survived the testing period, 5 responded with positive tanned cell

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TABLE I. Tanned Cell Hemagglutination: Reactions of Rabbit Antisera Obtained by Intravenous Injections of Papain-Treated Rabbit Thyroid Extract.

Day:	0	11	21	32	43	50	67	70	85	92	103	110	117
Antiserum	Titer ^a												
Rabbit 2754	0		0	0				16	16	8	32	256	512
Rabbit 2813	0	0	0	4	64	64	256	1024					
Rabbit 2857	0	0	0	0	1024	1024	512						
Rabbit 2858	0	0	0	0	0	0	0						
Rabbit 2859	0	0	0	0	512	1024	1024						
Rabbit 2860	0	0	4	4	512	1024	512						

^a Values indicate the reciprocal of the greatest dilution of antiserum producing a definitely positive reaction; 0 = no reaction with 1:2 diluted antiserum.

hemagglutination reactions. Four of the rabbits showed maximum titers of 1024; the fifth, a titer of 512. The sera of the rabbits injected with RTE in one ear, papain in the other, failed to agglutinate cells coated with RTE.

Thyroid autoantibodies are known to react with thyroid extract and not with other tissue extracts of the rabbit (9). The tanned cell hemagglutination procedure was used to check the specificity of the rabbit antisera produced by intravenous injections of papain-treated RTE. The RTE-coated erythrocytes were added to serial dilutions of the positively reacting rabbit antisera (Table I) either unabsorbed or absorbed with rabbit organ extracts. The hemagglutination titers are shown in Table II. Absorption with thyroid extract removed all of the hemagglutination reaction of the five antisera. Extract from the thyroid of the antibody-producing rabbit itself was effective as absorbing agent.

Similar absorption with rabbit organs other than thyroid had no effect on the tanned cell hemagglutination titer, and indicates that the reaction is strictly specific for the thyroid antigen. The antisera produced by papain-treated RTE have been found identical in their organ-specific properties to the rabbit antiserum produced by intradermal injections of untreated rabbit thyroid extract incorporated in complete Freund adjuvant.

Thyroid-specific antibodies frequently cross-react with thyroid extract of other species (7). In order to determine the species-specificity of the rabbit antisera produced in response to papain-treated RTE, thyroid extracts of different species were used to coat tanned human erythrocytes. The results of this experiment are presented in Table III. Antiserum from rabbit 2754 cross-reacted with thyroid extracts of horse, and, in lower titers, with mouse, monkey, and goat. Antiserum from rabbit 2813, on the other hand, only

TABLE II. Tanned Cell Hemagglutination: Reactions of Rabbit Antisera Unabsorbed and Absorbed with Various Rabbit Organs.

Antiserum	Antigen	Unabsorbed	Titer after absorption with extract of rabbit					
			Liver (3.5) ^b	Lung (1.7)	Kidney (2.4)	Spleen (2.3)	Thyroid (2.0)	Thyroid (1.0)
R 2754 Trial Bleeding F	untreated	32 ^a	32	32	32	32	0	0
R 2813 Trial Bleeding E	papain-treated	64	64	64	32	64	0	0
R 2857 Trial Bleeding D	papain-treated	1024	1024	512	1024	1024	0	0
R 2859 Trial Bleeding D	papain-treated	512	512	512	256	512	0	0
R 2860 Trial Bleeding D	papain-treated	512	512	256	256	256	0	0

^a Values indicate the reciprocal of the greatest dilution of antiserum producing a definitely positive reaction.

^b Values in parentheses indicate the protein concentration (%) of the various rabbit organ extracts.

TABLE III. Tanned Cell Hemagglutination: Cross Reactions of Rabbit Antisera with Thyroid Extracts of Various Species.

Antiserum	Reaction with thyroid extract of								
	Guinea								
	Rabbit (2.0) ^b	Human (3.0)	Monkey (4.0)	Goat (1.5)	Horse (3.0)	pig (2.5)	Chicken (3.0)	Rat (2.6)	Mouse (3.0)
Rabbit 2754									
Trial Bleeding H	512 ^a	0	32	32	128	0	0	0	64
Rabbit 2813									
Trial Bleeding E	64	0	0	16	0	0	0	0	32

^a Values indicate the reciprocal of the greatest dilution of antiserum producing a definitely positive reaction; 0 = no reaction with 1:2 diluted antiserum.

^b Protein concentration (%) of the thyroid extracts.

cross-reacted with thyroid extracts of the mouse and goat but not with those of horse and monkey.

The results of Table III indicate that rabbit antisera produced by injection of papain-treated RTE cross-reacted with thyroid extracts of other species. Additional experiments showed that inhibition of tanned cell hemagglutination was accomplished only by rabbit thyroid extract. The lack of species-specificity of these antisera resembles that of antisera produced by intradermal injection of untreated rabbit thyroid extract incorporated with complete Freund adjuvant. These results, demonstrating the similar serological behavior of circulating antibody produced by intravenous injection with papain-treated RTE and by intradermal injection with Freund adjuvant, suggest that a thyroid antigen (probably thyroglobulin) acted on by proteolytic enzymes may stimulate autoimmunity in the absence of adjuvant. They may provide a clue to the event triggering auto-antibody formation in human disease.

Summary. Circulating autoantibodies were produced in rabbits by repeated intravenous injections of papain-digested rabbit thyroid

extract. Control experiments indicated that neither papain nor rabbit thyroid extract alone would induce these autoantibodies. The antibodies were shown to be organ-specific but not species-specific, and resembled closely those antibodies produced by injection of untreated rabbit thyroid extract emulsified in Freund adjuvant.

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