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Thyroglobulin Antibody Activity in the γ-Globulins γ-A, γ-B and γ-C of Human Serum. (29304)

EDWARD A. LICHTER (Introduced by Maurice Landy)
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

In earlier work with primate antisera to human serum proteins, antigenic heterogeneity of normal human γ-globulin was described (1,2). Three proteins, γ-A, γ-B and γ-C, of differing electrophoretic mobility were identified with monkey antisera. Although these proteins are not yet separable by physical methods, immunoelectrophoresis followed by radioautography(3,4) provides a method for determining the presence of antibody activity in each of these 3 proteins. With these techniques, antibodies to thyroglobulin in the serum of patients with chronic thyroiditis have been found among the β_2A, β_2M and γ-globulin proteins(5,6). In the present study the precipitin lines of γ-A, γ-B and γ-C developed by immunoelectrophoresis of serum from the same patients with thyroiditis(5) were compared with those of normal sera in their ability to combine with radioactive labeled thyroglobulin.

Materials and methods. The γ-A, γ-B and γ-C proteins of 2 normal human sera (H.G. and R.C.) and sera from 3 patients with chronic thyroiditis (M.H., L.H. and H.M.) were studied for antibody activity to thyroglobulin. The titer of hemagglutinating antibody to thyroglobulin in the sera of the 3 patients was $>5 \times 10^5$; no antibody was demonstrable in the sera of the normal controls(5).

The thyroglobulin antigen in normal human thyroid tissue was included in a 0.15 M NaCl crude extraction which was then lyophilized. Ten mg of the lyophilized thyroglobulin extract was iodinated with I^{125} by the method of Talmage *et al*(7). The I^{125} in the thyroglobulin extract was 97% precipitable in 5% TCA. Contaminating iodinated serum proteins were removed by fractional addition of a monkey antiserum (T795) against human serum proteins to the iodinated thyroglobulin extract until no precipitate was formed when the supernatant was tested with monkey antisera to human serum or to human γ-globulin.

The monkey antiserum X354 was used to identify the γ-A, γ-B and γ-C γ-globulins in human serum. The horse antiserum Blue Boy was also used to identify γ-C although

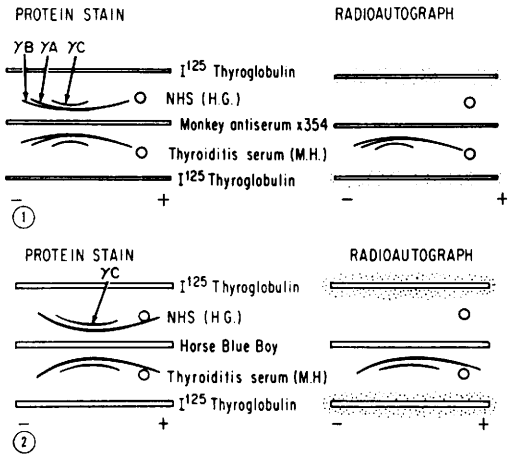

FIG. 1. Diagram of immunoelectrophoresis; protein stained (left) and radioautograph of the same precipitin arcs (right). Wells contain normal human serum (H.G.) or thyroiditis patient serum (M.H.). Antiserum trough (center) filled with *monkey antiserum X354* to human γ-globulin. Outer 2 troughs filled with I^{125} labeled thyroglobulin.

FIG. 2. Diagram of immunoelectrophoresis; protein stained (left) and radioautograph of same precipitin arcs (right). Wells contain normal human serum (H.G.) or thyroiditis patient serum (M.H.). Antiserum trough (center) filled with *horse antiserum Blue Boy* to human antibody globulins. Outer 2 troughs filled with I^{125} labeled thyroglobulin.

it did not distinguish between γ-A and γ-B (2).

Electrophoresis of the human sera was carried out in 1.5% Noble agar, 0.05 M Veronal buffer pH 8.4, for 3 hours at 4 volts/cm on a 3¼″ × 4″ glass plate. The antiserum was placed in the center trough and the precipitin arcs allowed to develop for 24 hours. The plate was washed in 0.15 M NaCl for 48 hours. The iodinated thyroglobulin was placed in the 2 outer parallel troughs (Fig. 1) and allowed to diffuse toward the precipitin arcs for 24 hours. After washing in 0.15 M NaCl for 24 hours, the agar plate was dried under wet filter paper and the dry plate placed on X-ray film for 7 days. The precipitin arcs were stained with Buffalo black(8) for comparison with the arcs seen on the X-ray film.

Results. Fig. 1 is a diagram of the immunoelectrophoretic plate stained for protein after a normal serum (H.G.) and a thyroiditis serum (M.H.) were subjected to electrophoresis and the precipitin arcs developed with monkey antiserum; the radioautograph of the same precipitin arcs which fix the I^{125} labeled thyroglobulin appears on the right. The diagram of the protein stain shows the 3 γ-globulins, γ-A, γ-B and γ-C, present in both the normal serum (H.G.) and in the thyroiditis serum (M.H.). In the diagram of the radioautograph, the radioactivity localized only in the 3 precipitin arcs formed with the thyroiditis serum. This indicates that the thyroglobulin is bound by antibodies present in all 3 γ-globulins and that the thyroglobulin antibody binding sites remain active after the γ-globulin molecules are precipitated in agar by a monkey antiserum to human γ-globulin. In other experiments (not shown) the thyroiditis sera L.H. and H.M. also localized radioactivity in the 3 arcs of γ-globulin while another normal serum R.C. did not show radioactivity in its 3 precipitin arcs of γ-globulin.

Fig. 2 shows results of experiments similar to those in Fig. 1 except that the precipitin arcs were developed with the horse antiserum Blue Boy. The protein stain diagram shows the precipitin arc characteristic of 7S γ-globulin in the normal and the thyroiditis serum. In addition a shorter arc is seen in both sera which identifies a protein with mobility similar to that of γ-C as identified by the monkey antiserum X354 in Fig. 1. This horse antiserum does not distinguish between γ-A and γ-B but seems to identify γ-C. When the radioautograph was developed, the I^{125} radioactivity was again found only in the precipitin arcs of the sera from the patients with thyroiditis and not in the arcs of the normal sera.

Discussion. The proteins γ-A, γ-B and γ-C are normal variants of human γ-globulin not usually detected by antibodies to γ-globulin prepared in sheep, goats, rabbits and horses. In this study, the specific binding of I^{125} labeled thyroglobulin to the γ-A, γ-B and γ-C precipitin arcs of the 3 thyroiditis sera indicates that the γ-globulins γ-A, γ-B and γ-C each have antibody activity to thyroglobulin. These 3 proteins with different antigenic determinants and slightly different electrophoretic mobilities have now been shown to have the common physiological function as antibody γ-globulins.

The similarity in the immunoelectrophoretic precipitin arcs and the demonstration of the presence of antibody activity for thyroglobulin is presumptive evidence that the γ-C proteins in human serum, identified by monkey antisera, is the same class of proteins identified as γ-C by the horse antiserum Blue Boy.

Goodman *et al* noted that in the thyroglobulin system, the sensitivity of the method combining immunoelectrophoresis and radioautography was less than that originally reported with insulin(5). In the present study the level of sensitivity was not a limiting factor for the qualitative findings reported because of the use of high titered thyroiditis sera.

In an attempt to improve the specificity of the methods, the iodinated thyroglobulin extract was precipitated in an excess of a monkey antiserum to human serum proteins (T795). This antiserum forms precipitin arcs with proteins of α and β mobility as well as with the immunoglobulins β_2A, β_2M and γ-globulin of normal human serum(2). Thus any contribution of radioactivity to the precipitin arc of the radioautograph by nonspecific binding of isotope labeled serum proteins in the thyroid extract would be minimized. This procedure diminished the faint radioactivity in the precipitin arcs of the normal sera to almost imperceptible arcs without notably diminishing the *very strong* radioactivity in the precipitin arcs obtained with the thyroiditis sera.

Conclusions. The human γ-globulins, γ-A and γ-B, identified by monkey antisera, and γ-C, identified by both monkey and horse antisera, have antibody activity. In 3 sera from patients with chronic thyroiditis, the γ-A, γ-B and γ-C were shown to have antithyroglobulin activity by radioautography with I^{125} labeled thyroglobulin after immunoelectrophoresis.

ADDENDUM: After submission of this paper, an abstract has appeared which confirms the observations made here (Fed. Proc. 23:454, 1964—W. D. Terry and J. L. Fahey).

Dr. Sheldon Dray provided monkey and horse antisera and Dr. Howard C. Goodman supplied thyroglobulin extract and thyroiditis sera.

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Immunological Sympathectomy and CCl_4 Hepatotoxicity.* (29305)

ROBERT E. LARSON, GABRIEL L. PLAA AND MICHAEL J. BRODY
Department of Pharmacology, College of Medicine, State University of Iowa, Iowa City

A causative role has been ascribed to the sympathetic nervous system in production of the hepatic lesion seen after CCl_4 intoxication(1-3). One of the principal observations that led to this suggestion was that in rats, spinal cord transection at the level of C-6 or C-7 prevented the appearance of the centrilobular necrosis after an oral dose of CCl_4 (1-5). We have since shown that an alternative explanation could be advanced for the protection afforded by cord-transection, *viz.* reduced hepatic metabolism resulting from the hypothermia induced by the cordotomy (5). Our observations cast considerable doubt on the idea that sympathetic discharge alone

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