Inhibition of *Trichinella spiralis* Complement Fixation by Immune Macroglobulin (33435)

ROY W. STEVENS, JULIA T. UTTER, AND MARGIT S. RUBIN
(Introduced by W. Kaufmann)

Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201

Latex particle–agglutination (LPA) and complement-fixation (C'F) techniques, each with a heat-stable extract of *Trichinella* larvae as antigen, serve as serologic tests for trichinosis (1). In a study of LPA and C'F test reactivity in early disease, it was noted that LPA activity was markedly reduced by exposure of the serum to a dilute sulfhydryl reducing agent although fixation of complement (C') was not significantly altered by this treatment (2). This selective reduction in serologic activity suggested that at least two qualitatively different antibodies are involved in the response to ingestion of *Trichinella spiralis*.

This report describes the serologic activity of two immunoglobulins: one agglutinates the extract of larvae but does not incorporate C' in the complex; and one causes both agglutination and fixation of C'. The evidence indicates that these immunoglobulins are M (IgM) and G (IgG) respectively. In the presence of IgM, the quantity of C' fixed in an IgG-antigen complex may be reduced by the loss of antigen to an IgM-antigen union. This competitive inhibition is demonstrable in a suitable C'F system by addition of specific IgM to antigen prior to addition of C' and immune serum.

Materials and Methods. Portions of human serum were obtained from samples submitted to a diagnostic serology service. Rabbit serum was obtained from an animal that had been fed viable *Trichinella* larvae. Sephadex G-200 chromatography with Tris (hydroxymethyl) aminomethane - HCl buffer (0.1 *M*, pH 8.0) was employed for isolation of *Trichinella* antibody from whole serum samples. Fractions obtained were dialyzed against 0.15 *M*, pH 7.3 triethanolamine buffered saline (TBS)[1] and were reacted with larval carbohydrate (3)[2] antigen in LPA, qualitative C'F, and microcomplement-fixation (MC'F) (4) tests.

Partially purified IgM preparations for inhibition studies were obtained by repeated Sephadex filtration of whole human immune serum. The protein of the ascending portion of the peak first eluted from the G-200 gel was recycled and the similarly eluted protein was collected as IgM. This fraction pool was pervaporated to original sample volume and dialyzed against TBS prior to protein determination (5) and MC'F test. Immunoglobulin content of partially purified preparations was determined by a quantitative gel-diffusion technique with antiserum incorporated in agar,[3] and by immunoelectrophoresis using specific antisera.[4]

The MC'F test was conducted in a total volume of 1.2 ml with TBS as diluent. Reagents were combined in 0.2-ml amounts; TBS was used to balance all test tubes to full volume, and was substituted whenever one or more test components were omitted for reagent control. Tests were incubated at 3–6° for 18 hr; 0.2 ml sensitized erythrocytes were added, and the system was heated at 37° for 30 min. The MC'F system controls were supplemented by mixtures for (1) detection of direct C'F by IgM, and (2) for detection of nonspecific inactivation of C' by IgM in the presence of the immune whole

[1] The TBS contains Ca^{++} 1.5×10^{-4} *M* and Mg^{2+} 5.0×10^{-4} *M* optimal for complement fixation and immune hemolysis.

[2] Antigen concentration was determined by the phenol-sulfuric acid method of J. E. Hodge and B. T. Hofreiter.

[3] Immuno-plate, Hyland Laboratories, Los Angeles, California.

[4] Antiserum to whole human serum (IEP antiserum) and antisera to gamma G, M, and A immunoglobulins, Hyland Laboratories, Los Angeles, California.

serum. The second of these was designated as an inhibitor-serum control. The inclusion of inhibitor-serum control readings in the calculation of C′ fixed nullified the nonspecific loss of C′ due to isolated macroglobulin. Complement fixation in the inhibition system was calculated as the optical density (OD) of this control minus the OD of the macroglobulin-antigen-serum (reaction) mixture. The direct MC′F system calculation was serum control OD minus reaction mixture OD.

For inhibition test, macroglobulin and antigen mixtures were left at room temperatures with occasional mixing for 30–60 min prior to the addition of optimum concentrations of whole immune serum, C′, and TBS diluent. Mixtures without added macroglobulin were manipulated in the same fashion to establish the level of direct C′F by the immune serum. The amount of C′ fixed (ΔOD) in the inhibition system was compared with that obtained in the absence of added IgM, and the percentage of inhibition was calculated.

$$1 - \left(\frac{\Delta\text{OD inhibition test}}{\Delta\text{OD direct test}} \right) \times 100$$

For a test of the influence of sulfhydryl reducing reagent on the inhibitory capacity of IgM preparations, equal volumes of pH 8.0 Tris buffer solutions of macroglobulin K.B. (20 mg/ml) and 0.01 *M* dithiothreitol (DTT) (6) were mixed and left at room temperature for 1 hr. Globulin K.B. in buffer served as a control on the DTT treatment. The treated and control globulins were dialyzed first against 0.015 *M* iodoacetamide and second against TBS prior to inhibition test.

Results and Discussion. Two distinct serum fractions obtained from Sephadex filtration of whole immune serum exhibit activity in LPA and C′F tests (Fig. 1). The protein of the first peak eluted from the gel contains IgM; the second contains IgG (7). As illustrated, antigen unites with each of these globulins although C′ is incorporated only in the IgG-antigen complex.

Quantitative gel immunodiffusion of the partially purified inhibitor preparations indicated an IgM content of from 8 to 17% of

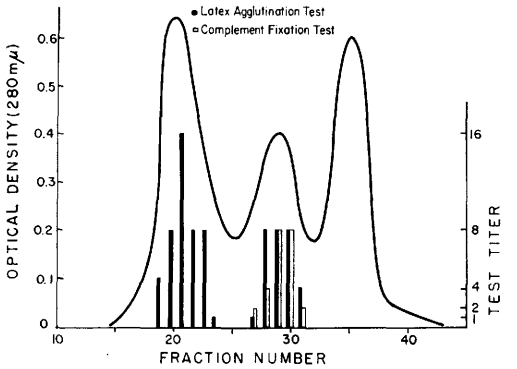

FIG. 1. Sephadex G-200 fractionation of antiserum. A 5-ml sample of whole human serum (agglutination titer 64, fixation titer 32) was applied to the 100 × 1.5-cm column and a 5-ml fraction was collected with Tris buffer (0.1 *M*, pH 8.0) each hour. After dialysis against TBS (0.15 *M*, pH 7.3) fractions 18 through 34 were examined in agglutination and fixation tests.

the total protein. No precipitation was observed in the IgA or IgG test. A single line of precipitation, an IgM anti-IgM line, occurred at the cathodal end of immunoelectrophoretic patterns developed with the inhibitor preparations.

In the presence of immune macroglobulin, C′F is reduced in regions of antibody excess, equivalence, and antigen excess (Fig. 2). Similar titrations with macroglobulin derived from normal human serum or serum pools failed to reduce the amount of C′ fixed with *Trichinella* carbohydrate and a variety of whole immune human or rabbit sera. As illustrated, inhibition is relatively constant from a region of equivalence into the zone of antigen excess. Additional experiments were conducted with single antiserum and antigen concentrations selected to provide maximum C′F in slight antigen excess.

Differences in the capacity of human serum macroglobulins to inhibit C′F were indicated by tests with rabbit antiserum (Fig. 3a). The differences in degrees of inhibition of this antiserum undoubtedly reflect the amount of specific anti-larva carbohydrate IgM contained in the globulin preparations.

Results of inhibition tests with macroglobulin exposed to a sulfhydryl reducing agent

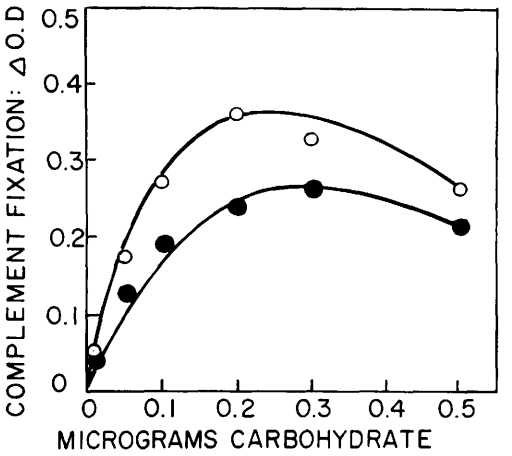

FIG. 2. Microcomplement fixation. Reactivity of whole human serum with *Trichinella* carbohydrate antigen. Complement fixation with a 1:150 dilution of serum M.B.○ and with the same M.B. serum dilution in the presence of 100 μg of M.B. macroglubulin ●. The zones of antibody excess, antibody-antigen optimum, and antigen excess are apparent in each of the curves.

compared with globulin similarly treated without the agent, confirmed that the integrity of the macroglobulin structure is essential for complex with the maximum quantity of antigen (Fig. 3B). Globulin exposed to DTT lost approximately two thirds of the original inhibitory capacity.

IgM has frequently been recognized as antibody synthesized early on antigenic stimulus. This report of the failure of an IgM-antigen union to fix C′ therefore defines a mechanism for competitive inhibition of C′F tests conducted on early phase antisera. The reduced availability of antigen for an IgG-antigen-C′ complex partially explains the C′F test serum prozone and the weakly reactive or nonreactive C′F test result frequently obtained in the presence of significant amounts of LPA test reactive antibody.

IgM has been shown to be more efficient than IgG in fixation of C′ with erythrocytes (8). Recent studies have indicated, however, that this C′F efficiency may be related to the physical state of the antigen, and that little or no C′ is fixed by IgM with some soluble antigens of relatively simple structure (9). Maximal C′F with erythrocytes or other particulate antigens, and minimal or no fixation with soluble antigens suggest that C′F with IgM may require a number and distribution of determinants on an antigen of relatively large molecular volume (9). Failure of the IgM-*Trichinella* carbohydrate complex to fix C′ is consistent with this hypothesis, and this failure is effectively illustrated by the MC′F inhibition test.

Summary. *Trichinella spiralis* immune human serum macroglobulin inhibits complement fixation with *Trichinella* carbohydrate and whole serum. The macroglobulin preparations possess specific agglutination activity

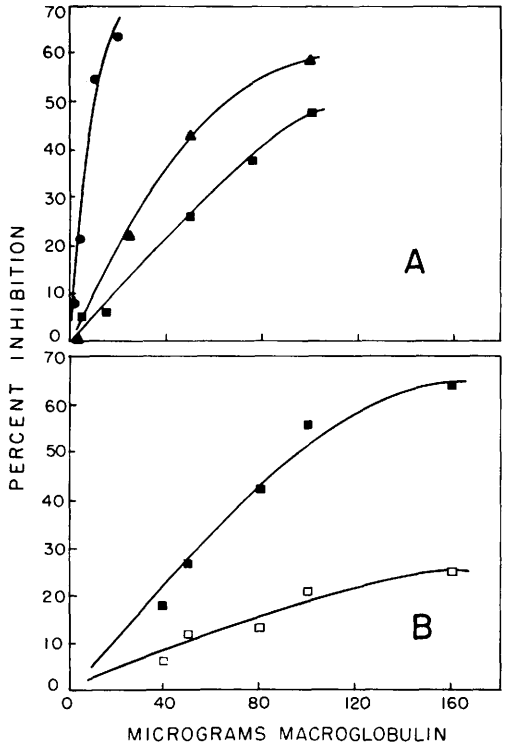

FIG. 3. Inhibition of microcomplement fixation. (A) Percentage of reduction in complement fixed with 0.4 μg *Trichinella* carbohydrate and a 1:2000 dilution of immune rabbit serum: inhibition by human macroglobulin from serum pool R.S., ●; serum M.B., ▲; and serum S.J., ■. (B) Influence of sulfhydryl compound on inhibitory activity of immune macroglobulin: inhibition of fixation with 0.4 μg carbohydrate and a 1:150 dilution of human serum K.B. by macroglobulin K.B., ■; and by macroglobulin K.B. treated with 0.01 *M* dithiothreitol □.

and the inhibitor is characterized as immunoglobulin M (IgM) which does not incorporate complement in its union with antigen.

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Influence of Carbon Tetrachloride, Vitamin E, and Protein Upon Liver Slice Respiratory Activity (33436)

E. M. BLENDERMANN AND LEONARD FRIEDMAN (Introduced by P. L. Harris)

Division of Nutrition, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C. 20204

The relation of nutritional state to certain toxic manifestations caused by carbon tetrachloride (CCl_4) has been the subject of several investigations. For instance, lowering dietary vitamin E or protein has been shown to increase the susceptibility of rats to the lethal or necrogenic effects of CCl_4 (1–3), but a protein-free diet was demonstrated by McLean and McLean (4, 5) to have the reverse effect.

Although several studies have been concerned with the effects of CCl_4 intoxication on *in vitro* liver carbohydrate metabolism (6–8), little attention has been paid to the influence of diet therein. Therefore, studies were initiated to determine how different dietary treatments, particularly those known to afford some protection against the gross effects of CCl_4, influence the animal's response to this toxic chemical at the cellular level. The influence of vitamin E and of protein level on some aspects of *in vitro* liver carbohydrate metabolism as affected by CCl_4 administered *in vivo* was investigated.

Materials and Methods. Animal treatments. Weanling male albino rats of the Osborne-Mendel strain[1] were maintained on a purified diet of the following composition (%): vitamin-free casein,[2] 18; sucrose, 63.8; stripped lard, 9; cod liver oil, 1; Jones-Foster salt mixture, 4; vitamin E-free vitamin mixture (9), 4; and choline chloride, 0.2. Usually, half of the rats were fed this vitamin E-deficient diet and the other half received the same diet supplemented with 100 mg of DL-alpha-tocopherol acetate/kg of diet. In an-

[1] Division of Nutrition colony, Food and Drug Administration, Washington, D.C.

[2] General Biochemicals, Chagrin Falls, Ohio.

[3] New England Nuclear Corporation, Boston, Massachusetts. The specific activity of D-glucose-^{14}C (uniformly labeled) was 8.8 mCi/mmole.

[4] Reagents obtained in Biochemica Test Combinations (C. F. Boehringer & Soehne GmbH., Mannheim, Germany).