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Effects of Rheumatoid Factor and Complement on Soluble Immunoglobulin G Complexes: $^{51}CrCl_3$ Labeling and Precipitation Studies[1] (39994)

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Introduction. Soluble immunoglobulin G complexes which react with macromolecular rheumatoid factor have been detected in the synovial fluid of patients with rheumatoid arthritis (1). This reaction has been thought to accentuate the complement diminution in the synovial fluid of these patients (1). Recent studies (2, 3) have suggested that although insoluble complexes are capable of producing functional and metabolic changes in normal neutrophils, soluble complexes are not. These studies (2, 3) utilized rheumatoid factor to convert soluble complexes into insoluble complexes capable of stimulating the biologic activity of normal neutrophils. The present studies explore the effects of rheumatoid factor and complement on the precipitation of $^{51}CrCl_3$-labeled and unlabeled soluble immunoglobulin G complexes.

Materials and methods. Human immunoglobulin G, obtained commercially (U.S. Biochemical Corp., Cleveland, Ohio) as human Cohn Fraction II, was solubilized in 0.9% NaCl (pH, 7.4). This solution contained immunoglobulin G, immunoglobulin M, and immunoglobulin A in a ratio of 104:2:1 as determined by radial immunodiffusion (Behring, Marburg, Germany). Upon passage through a Sephadex G-200 column (2.6 × 60.0-cm column at 25°), 85% of the total immunoglobulin G was eluted in a fraction corresponding to a molecular weight of approximately 150,000; the remainder was eluted as the macromolecular fraction (void volume). A 1 g/dl solution of this human immunoglobulin G was labeled with approximately 22.6 μCi of $^{51}CrCl_3$ (New England Nuclear, Boston, Mass., specfic activity approximately 375 Ci/g of Cr) per milliliter of immunoglobulin G at pH 7.4. After incubating for 1 hr at room temperature, the solution was passed over a Sephadex G-10 column (1.2 × 30.0 cm) to remove any unbound chromium. Tubes containing the maximum amounts of radioactivity and protein were pooled and subsequently heated at 63° for 7 min. After returning to room temperature, the solution was centrifuged at 10,000*g* for 15 min to remove any insoluble precipitate formed during the heating. The resulting slightly opalescent supernatant, upon passage over a Sephadex G-200 column, was found to contain a mean of 47.9% (range 37-60.5%) soluble immunoglobulin G complexes with the remainder of the protein in the form of 150,000 molecular weight immunoglobulin G. This solution was diluted with 0.9% NaCl to contain 2.4 mg/ml of soluble immunoglobulin G complexes and a mean of 1.13×10^5 cpm/mg of protein.

Rheumatoid serum was obtained with informed consent from patients with definite or classical rheumatoid arthritis and normal serum from healthy volunteers. The sera were either used immediately or frozen at −70° for use in future experiments. Rheumatoid factor titers were determined utilizing the commercial latex globulin reagent, Rheuma Quik (Biological Corp. of America, Port Reading, N.J.). All sera were spun at 10,000*g* for 15 min to remove any precipitate prior to use in the test system. In some studies, complement activity was removed from serum either by heat-inactivation for 30 min at 56° or by treatment with cobra venom factor (Cordis Corp., Miami, Fla.) for 30 min prior to use.

Complex reaction mixtures were prepared in a ratio of 1:2:5 by volume with

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soluble immunoglobulin G, normal serum, and rheumatoid serum, respectively. In studies using $^{51}CrCl_3$, these sera were previously treated with 0.05 ml of 1.3×10^{-3} *M* $CrCl_3$/5 ml of serum. Rheumatoid serum was utilized undiluted (Figs. 1 and 2) or was diluted with 0.9% NaCl to produce experimental sera with titers shown in Fig. 3. When either the rheumatoid or the normal serum was omitted from the mixture (Fig. 4), an equal volume of 0.9% NaCl was substituted to keep the reaction volume constant. After exposing the immunoglobulin G to 0.9% NaCl, rheumatoid serum, and/or normal serum, the respective tubes were centrifuged at 10,000*g* and washed twice in 0.9% NaCl. The supernates were discarded and the precipitates assayed in duplicate for radioactivity in a Nuclear Chicago gamma scintillation spectrometer. The precipitates were then resuspended in saline or phosphate-buffered saline and protein determinations were performed using the technique of Lowry *et al.* (4).

All data were statistically analyzed using Student's *t* distribution, since sample sizes were comparatively small in most of the experiments. Two-tailed Student's *t* tests were performed on all groups, and differences between these groups were considered to reach statistical significance when values of $P < 0.05$ were obtained.

Results. Figure 1 shows the effects of utilizing sera with increasing rheumatoid factor titers on the precipitation of ^{51}Cr-tagged soluble immunoglobulin G complexes. Although considerable variation occurred at each titer, differences in precipitation between negative sera and those containing rheumatoid factor became significant ($P < 0.05$) at titers of 1:80, were highly significant ($P < 0.001$) at titers of 1:160, and remained significant throughout the study to titers of 1:5120. Maximum precipitation apparently occurred in the region of rheumatoid factor titers of 1:1280, but there were not statistically significant

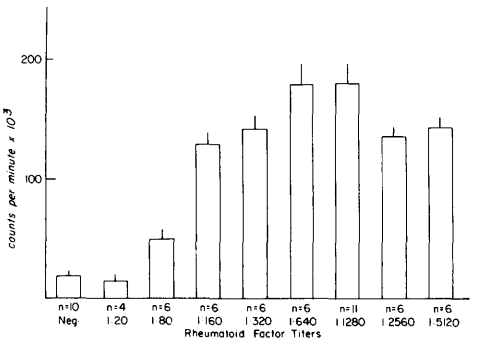

FIG. 1. Rheumatoid factor effects on soluble immunoglobulin G complex precipitation, $^{51}CrCl_3$ studies. Vertical lines on top of the bars = $\bar{X}$ + SEM. Differences between negative sera and those with rheumatoid factor become significant at titers of 1:80. Differences are not significant between any of the groups of sera with titers from 1:320 to 1:5120.

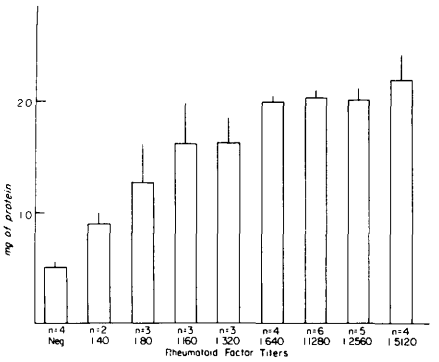

FIG. Rheumatoid factor effects on soluble immunoglobulin G complex precipitation, protein studies. Vertical lines on top of the bars = $\bar{X}$ + SEM. Differences between negative sera and those with rheumatoid factor become significant at titers of 1:160 and do not change significantly to titers of 1:5120.

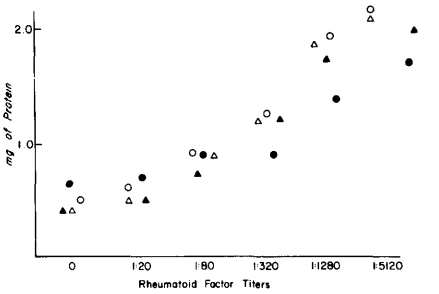

FIG. 3. Effect of rheumatoid factor dilution on soluble complex precipitation. ▲, ● = Two patients with initial rheumatoid factor titers of 1:5120; △,○ = 2 patients with initial rheumatoid factor titers of 1:2560; each symbol = $\bar{X}$ of four determinations. Differences between control and rheumatoid factor-containing sera become significant in the 1:80–320 range and there are no significant differences in precipitation from sera with titers from 1:1280 to 1:5120.

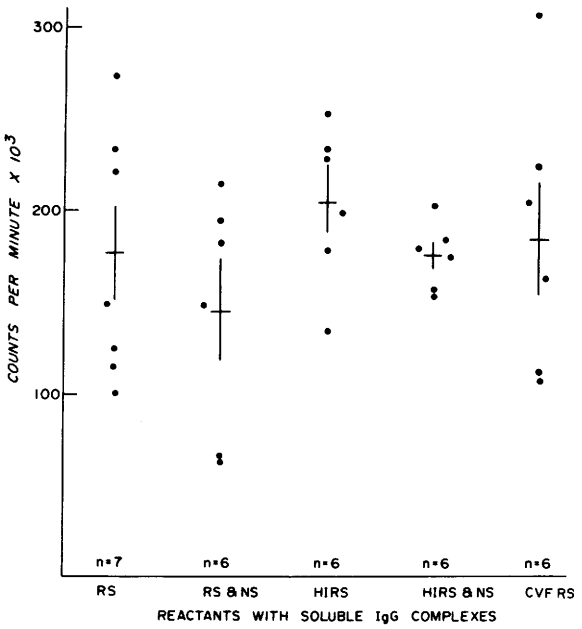

FIG. 4. Complement effects on soluble immunoglobulin G complex precipitation with rheumatoid factor. Crosses = $\bar{X}$ ± SEM, RS = rheumatoid serum, NS = normal serum, HI = heat-inactivated, CVF = cobra venum factor-treated. Treatments designed to decrease complement levels do not affect the ability of RS to precipitate ^{51}Cr-tagged soluble complexes.

differences between any of the sera groups exhibiting titers of 1:320 or above. Figure 2 shows similar results when nonradioactive soluble immunoglobulin G complexes are incubated with sera exhibiting rheumatoid factor titers up to 1:5120. Differences between negative and rheumatoid factor-containing sera became significant ($P < 0.05$) at titers of 1:160 and there were no statistically significant differences between any of the sera groups exhibiting titers of 1:160 or above. As shown in Fig. 3, serial dilutions of high-titered sera produced similar results. Differences between control and rheumatoid factor-containing sera became significant ($P < 0.05$) at titers of 1:80 (two patients) or 1:320 (two patients) and there were no statistically significant differences among any of the experimental sera exhibiting titers of 1:1280 or above. Figure 4 illustrates the precipitation obtained utilizing radioactively tagged soluble immunoglobulin G complexes and rheumatoid sera exhibiting a titer of 1:1280 but subjected to various treatments prior to incubation with the soluble complexes. As shown in this figure, there is no difference in the amount of precipitation from the soluble complex solutions whether rheumatoid serum (RS) is utilized alone, in the presence of normal sera (RS & NS), in the presence of rheumatoid factor sera which is heat-inactivated at 56° (HIRS), or in the presence of rheumatoid factor sera previously treated with cobra venum factor (CVFRS).

Discussion. $^{51}CrCl_3$ preferentially tags serum proteins (5) and has been utilized for *in vivo* studies of gastrointestinal protein loss (6). Previous studies (7) have shown that rheumatoid factor can cause specific precipitation when reacted with heat-aggregated γ-globulin but not with native immunoglobulin G. Previous precipitation studies (8, 9) have shown an inhibitory effect of native immunoglobulin G on soluble complex precipitation. Our studies utilize soluble complexes in equal volumes to native immunoglobulin G, and demonstrate increasing precipitation of the complexes from solutions incubated with rheumatoid sera to rheumatoid factor titers of 1:320 and 1:160 utilizing chromium-tagged or untagged complexes. A similar increase in precipitation with increasing amounts of rheumatoid fac-

tor to titers of 1:1280 is demonstrated in serial dilution studies of experimental sera and has been reported (10) in previous studies utilizing different methods. Our studies confirm these earlier findings and demonstrate that the chromium-tagged system yields a precipitin curve similar to that seen when nonradioactive soluble complexes are utilized. These studies suggest that increasing serum concentrations of rheumatoid factor, up to titers of 1:1280, yield increasing amounts of precipitate when exposed to a constant amount of soluble immunoglobulin G complexes in the presence of native γ-globulin.

Although rheumatoid factor alone (11) and rheumatoid factor–immunoglobulin G complexes have been shown to fix complemen (9), our studies utilizing ^{51}Cr-labeled complexes and high-titer (1:1280) rheumatoid sera confirm previous reports (12) suggesting that complement is not required for maximum precipitation of soluble complexes by high-titer rheumatoid factor-containing serum. This study has thus shown that a chromium tag applied to soluble immunoglobulin G complexes can be used to determine quantitatively the precipitation of these complexes. The measurable precipitate increases proportionately with increasing titers of rheumatoid factor up to 1:320 and is not affected by measures designed to eliminate complement from high-titer rheumatoid sera. These studies confirm previous suggestions that increasing titers of rheumatoid factor may increase the biologic activity of immunoglobulin G complexes, and demonstrate the usefulness of a ^{51}Cr tag in delineating the interaction of these complexes with rheumatoid factor.

Summary. $^{51}CrCl_3$-labeled soluble immunoglobulin G complexes have been shown to have similar precipitation characteristics to unlabeled complexes in the presence of increasing amounts of macromolecular rheumatoid factor. Complement depletion of high-titer rheumatoid sera did not appear to influence these interactions which may be important components of rheumatoid inflammation.

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