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Human sera with precipitating antibodies to human soluble immune complexes. A brief communication

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Introduction:

Previous numerous papers by the senior author dealt with the human serum factor referred to as anti-antibody which is specifically directed against IgG antibodies that underwent molecular transformation in the course of the reactions with their corresponding antigens. The reactions of this serum factor could be conveniently detected by means of agglutination of Rh-positive erythrocytes sensitized by anti Rh antibodies. No precipitation tests could be developed.

Materials and Methods:

Most studies were conducted by means of double diffusion in gel precipitation.

Results:

A rheumatoid arthritis serum, G, was noted that produced a strong reaction of double diffusion in gel precipitation with serum samples of a renal graft recipient, T. Further screening detected one more rheumatoid arthritis serum reacting with T; of 28 sera from renal graft recipients, 6 reacted in a similar way to T, but the reactions were considerably weaker and poorly reproducible. Evidence was presented that the precipitin in the two rheumatoid arthritis sera under study had properties of previously described anti-antibody.

Conclusions:

Sera with precipitating anti-antibodies may serve as exquisite reagents for detection of soluble immune complexes in human sera.

Key words:

rheumatoid arthritis • renal graft • circulating immune complexes

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INTRODUCTION

The studies on antigenicity of antibodies by the senior author of this paper were initiated in collaboration with T. Luszczynski in the 1950's and published in this journal⁴. They led to the description of a serum factor referred to as “anti-antibody” that reacted with antibodies altered in the course of serological reactions². Anti-antibodies could be conveniently detected in agglutination tests with Rh-positive erythrocytes sensitized by incomplete Rh antibodies. Inhibition of this agglutination could be employed for detection of circulating immune complexes in human sera¹. In the present study a serum factor was detected in two rheumatoid arthritis sera, which produced precipitation reactions with a few sera of renal graft recipients. Evidence was presented that the factor under study has properties of anti-antibody in that it can serve as a reagent for detection of circulating soluble immune complexes in human sera.

MATERIALS AND METHODS

The human sera originated from patients treated in hospitals in the Buffalo area. Many of the samples had been used in previous studies of this laboratory and preserved for several years at -20°C .

Double diffusion gel precipitation tests were performed in 0.5% agarose gel in Petri dishes. Charged plates were tightly closed and placed in a refrigerator at 4°C . Readings were recorded after 24 to 72 h.

RESULTS AND DISCUSSION

During our studies using double diffusion gel precipitation, we inadvertently noticed a strong precipitation arc that was produced in the reaction of a rheumatoid arthritis serum, G, with the serum of a renal graft recipient, T. The convexity of this arc was directed towards G (Fig. 1). Because of the remarkable strength and reproducibility of this reaction, we performed further studies to obtain more information about the observed phenomenon.

When serum T was tested in double diffusion gel precipitation against 36 rheumatoid arthritis serum samples, including G, only one more, serum R, produced a reaction which was very similar to the one produced by G. The precipitation lines produced by G and R with T merged into a reaction complete identity (Fig. 2). Serum T gave strong reaction up to a dilution of 1:8 (Fig. 3), whereas sera G and R reacted only up to a 1:2 dilution. Both G and R were positive for rheumatoid factor in the latex fixation test as well

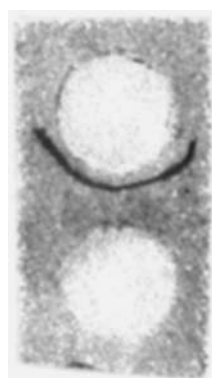


Figure 1. Upper well: transplantation serum T. Lower well: rheumatoid arthritis serum G.

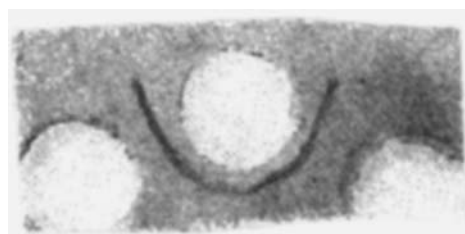


Figure 2. Upper well: transplantation serum T. Lower wells: rheumatoid arthritis sera G (left) and R (right).

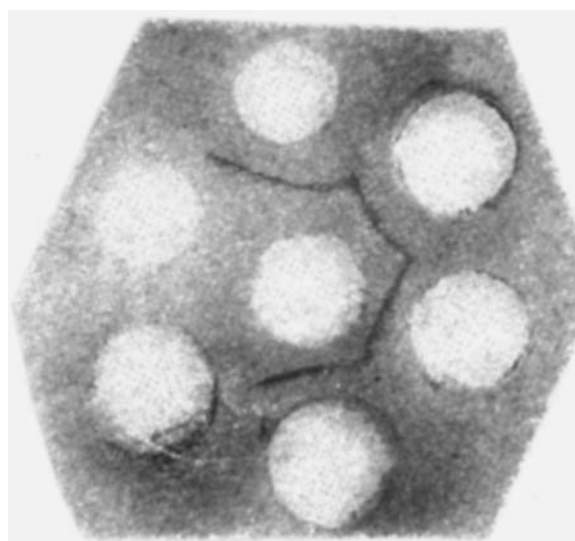


Figure 3. Central well: rheumatoid arthritis serum G. Peripheral wells: transplantation serum T at increasing dilutions, clockwise from the uppermost well, 1 to 1, 2, 4, 8, 16 and 32.

as in the agglutination of Rh-positive erythrocytes sensitized by Rh-antiserum “Ripley”⁷.

We also screened 28 transplantation sera for precipitation reaction with sera G and R. With six sera we obtained positive reactions similar to those produced by serum T; they were, however, considerably weaker and poorly reproducible. Heating of sera G and R as well as T at 62°C for 15 min resulted in complete disappearance of the reactions. Subsequently we found that adding one volume of 2% human FII to two vol-

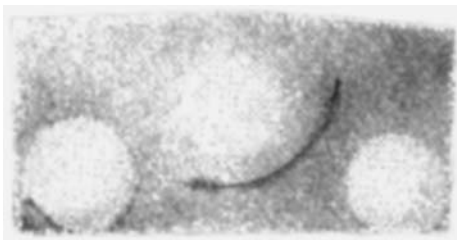


Figure 4. Upper well: transplantation serum T. Lower wells: rheumatoid arthritis serum R mixed with 2% FII of human serum (left) or with 2% human serum albumin (right).

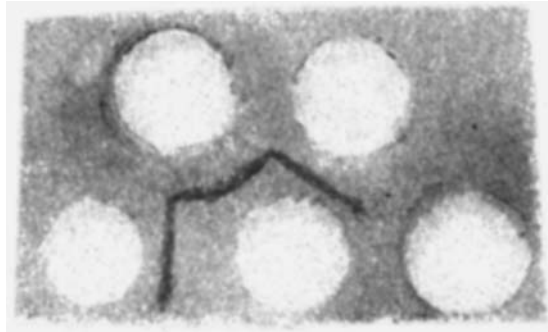


Figure 5. Central well: rheumatoid arthritis serum R. Peripheral wells: antidextran serum WR mixed with dextran at concentrations of (clockwise from the lower left well) 20, 13, 9 and 6 µg/ml.

umes of serum R abolished its reaction with T, whereas addition of a similar amount of human albumin failed to influence this reaction (Fig. 4).

The simplest explanation for the observed reactions was the assumption that the rheumatoid factors of the G and R sera react as allotypic antibodies and serum T has a corresponding allotypic antigen. This hypothesis would be confirmed by the demonstration that the antigen under study constantly appears in the donor of the T serum. Fortunately, we had at our disposal 5 samples of serum T, collected between January 1999 and January 2000. We found that the samples from January 8 and March 3, 1999, were negative, those from September 17 and December 12, 1999, were positive, and the sample from January 10, 2000, was again negative.

We concluded that we were not dealing with an allotypic reaction and advanced the hypothesis that the rheumatoid factor in sera G and R has properties akin to a previously described anti-antibody. The outstanding property of the anti-antibody is its reaction with human IgG antibody that has undergone molecular transformation in the reaction with its corresponding antigen². If this supposition were correct, then the “precipitogen” observed in the T serum would be a soluble immune complex formed transiently by patient T.

The crucial experiment to be conducted had to prove that G and R sera react with immune complexes produced *in vitro*. To this end we used the human anti-dextran serum WR available from our previous study⁵. Samples of this serum were mixed with dextran to attain dextran at concentrations of 6–20 µg/ml. When tested against R, precipitation lines were formed with samples containing dextran at concentrations of 20, 13 and 9 µg/ml, but not with the sample containing dextran at a concentration of 6 µg/ml (Fig. 5).

In further studies along these lines we conducted a “mixed serum” test (Table 1). As predicted, serum R mixed with anti-Rh serum “Ripley” behaved as a complete (i.e. agglutinating) Rh antiserum. Anti-antibody was not neutralized in the mixed serum and it could agglutinate Rh-positive erythrocytes after the Rh antibodies had reacted with them and undergone molecular transformation. In contrast, antibodies in a commercial antiglobulin reagent of rabbit origin which was tested for control were neutralized by the unaltered IgG of the Ripley serum and could not enter in reaction with Rh antibodies on erythrocytes.

The neutralization of sera G and R with commercial FII was not very surprising because such preparations may contain IgG that has been altered during the isolation procedure to the point where they resemble IgG antibodies transformed in a serologic reaction or otherwise such preparation may contain immune complexes.

Thereafter, we screened several dozen pathologic sera for serum factors resembling those present in G and R. The only positive result was obtained with 1 out of 30 sera from patients with GI carcinomas. This

Table 1. Mixed serum reactions. Agglutination of Rh-positive erythrocytes by serum R and by rabbit anti-human-globulin serum mixed with anti Rh serum “Ripley”

	Dilution of Ripley serum 1 to:			Saline
	20	60	80	
Dilution of serum R1 to:				
10	+++	+++	+++	—
20	+++	+++	+	—
40	+++	+++	+	—
80	+++	+++	+	—
Dilution of anti-globulin serum 1 to:				
50	—	—	—	—
150	—	—	—	—
Saline	—	—	—	—

serum produced a reaction with serum T considerably weaker than but similar to those produced by sera G and R.

The detection of circulating immune complexes in pathologic human sera is of considerable interest and importance. Several procedures used to this end have been employed, and they have been reviewed in previous papers from this laboratory^{1, 3, 6}. Thus far, all the procedures used are neither simple nor well reproducible. When anti-antibody was employed as a reagent for the detection of circulating immune complexes, the rather laborious procedure used

involved the neutralization of anti-antibody by the tested serum, which was assessed by the disappearance of the reaction of anti-antibody with sensitized Rh-positive erythrocytes.

In the present study we observed, for the first time, precipitating anti-antibody, which was quite an interesting finding for us. Such an anti-antibody could serve as a reagent in a very simple method for the detection of circulating immune complexes in sera. Therefore, observations reported in this paper warrant further research which, however, cannot be conducted by these authors.

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