SPECIFICITY OF THE REACTION BETWEEN RHEUMATOID FACTORS AND GAMMA GLOBULIN*

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The possibility that the rheumatoid factors represent antibodies to γ-globulin has been entertained for a number of years. This concept originated primarily from the observations that the various serological tests for rheumatoid factors require γ-globulin either as antibody or as inert material, in order for agglutination to take place (1). The precipitin reaction with soluble aggregates of γ-globulin and the interaction to give a complex detectable in the ultracentrifuge furnished additional evidence for this point of view (2–4). Recently, the demonstration that these factors in physical, chemical, and antigenic properties closely resembled classical antibodies of the 19S class, considerably strengthened the antibody concept (5–8). The apparent specificity for γ-globulin, however, has not been clearly delineated.

In 1956, Grubb and Laurell noted that certain rare rheumatoid sera could be utilized as test reagents for detecting genetic differences between human γ-globulins (9). These results were confirmed and extended by various investigators (10–12). However, although the genetic differences demonstrable by these tests have been widely utilized by human geneticists (13–19), they have contributed little to an understanding of the rheumatoid factors involved. The possibility arose that a study of the interaction of different rheumatoid factors with a wide variety of γ-globulins from single individuals might reveal specificity for certain types of γ-globulin. Such specificity would considerably strengthen the antibody concept and perhaps throw light on the hypothesis that the rheumatoid factors represent autoantibodies directed against the patient's own type of γ-globulin. Recent work by Oudin, Dray, and others have indicated a wide variety of antigenically different γ-globulins in different individuals of the same species (20–25). A modification (26) of the test system of Waller and Vaughan, utilizing incomplete Rh antibodies from single donors as a test system for rheumatoid factors, has been primarily employed in the present investigation (27).

The results indicate a high degree of specificity for the reaction between individual rheumatoid factors and γ-globulin in the form of incomplete Rh antibodies from single individuals. A large number of different rheumatoid factors are distinguishable according to their reactivity with such individual γ-globu-

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lins. The somewhat surprising observation was made that the rheumatoid factors in many instances react poorly with the patient's own γ-globulin compared to that of certain other individuals. A short report of these findings was presented previously (28).

Materials and Methods

Rheumatoid Sera.—Thirty-six sera were obtained from patients with severe chronic deforming rheumatoid arthritis, most of whom were regularly seen at the Hospital of The Rockefeller Institute. All sera had titers greater than 5120 in the F-11 latex fixation test. Selected sera from patients with chronic liver disease,[1] sarcoidosis, Waldenström's macroglobulinemia, and idiopathic pulmonary fibrosis were also utilized. Highly selected rheumatoid sera, useful as typing reagents for one or another of the hereditary γ-globulin groups in man, were obtained through the kindness of Dr. R. Grubb, Dr. M. Harboe, Dr. C. Ropartz, and Dr. A. Steinberg.

"Coating" Reagents.—Twenty-four high titer "incomplete" anti-Rh_o sera from different persons were obtained from various hospitals and commercial blood banks in the New York City area. This antibody had developed in 16 instances in Rh-negative females during pregnancy, and in 8 instances was produced by deliberate immunization of volunteer Rh-negative males. With two donors, samples were obtained at three different times during a 2-year period of continuing immunization. These six specimens and six additional ones were coded by number so that specimens from the same individual could not be identified as such until conclusion of the experiments. Anti-Rh_o (anti-D) antibodies were produced in selected Rh-negative patients with rheumatoid arthritis by immunization with 5 ml of a mixture of whole blood and Alsever's solution twice weekly for 3 to 6 weeks. Four coating reagents containing incomplete antibodies other than anti-Rh_o were also employed; one sera contained anti-c, one anti-C (anti-rh'), one anti-Duffy, and the fourth anti-Kell. One serum possessed both incomplete anti-C and incomplete anti-D activity; these were separable by absorption. This combination was produced by deliberate immunization, first with Rh_o erythrocytes and later with rh' erythrocytes.

Rabbit amboceptors.—Twelve amboceptors from individual non-litter mate rabbits of different breeds were obtained from the Baltimore Biological Laboratories.

Erythrocytes.—Whole blood was obtained from the same human O R_o donor at 2 week intervals, mixed with an equal volume of Alsever's solution and stored at 4°C. For selected experiments involving antibodies other than anti-Rh_o, donors with red cells of appropriate antigenic composition were employed, *e.g.*, R_2R_2, R_1R_1, Fy^{a+}, $Kell^+$. Sheep whole blood, obtained from Cappel Laboratories, was preserved in a similar manner.

Rheumatoid Factor Activity.—F-II latex fixation (29), F-II tanned cell (30), and sensitized sheep cell (SSC) reactions (31) were performed by standard techniques. The sheep cells were sensitized with rabbit amboceptor in one-fifth the minimum agglutinating dose. Sensitized human red cell reactions (RA Rh_o test) were performed by a modification of the procedures of Grubb (32) and of Waller and Vaughan (27) described elsewhere (26). For such reactions, 2 per cent suspensions of washed red cells were sensitized with coating reagents diluted to contain 128 units of blocking antibody as measured by the indirect Coombs' test. Rheumatoid sera, diluted 1:10 and 1:100, were added to 1.5 per cent suspensions of coated and control noncoated red cells.

Gm Typing.—Typing of normal human sera for the hereditary γ-globulin groups Gm(*a*) (9), Gm(*b*) (10), and Gm(*x*) (11), was performed according to the procedure of Grubb and Laurell (9), except that 1.5 per cent suspensions of red cells were employed and the tubes read

[1] One hundred sera kindly furnished by Dr. Howard Ticktin, Liver Clinic, District of Columbia General Hospital, Washington.

with the aid of a hand lens after 30 seconds centrifugation in a serological centrifuge (serofuge, Clay-Adams, Inc.). Sera containing anti-Rh antibodies were typed after absorption of most of the anti-Rh activity by human red cells of appropriate antigenic composition. Gamma globulins isolated from normal human sera were typed in an identical fashion after the protein solutions had been concentrated to 10 mg per ml. Typing of the 7S γ-globulin from sera containing rheumatoid factor activity was performed after removing all rheumatoid agglutinating activity by density gradient ultracentrifugation; γ-globulin from normal sera, treated in an identical fashion, was included as a control in such experiments.

Block Titration.—These were performed with serial dilutions of rheumatoid sera and with red cells sensitized with serial dilutions of coating reagent. Separate pipettes were used for each dilution to prevent carry-over.

Absorption Experiments.—Red cell stromata were prepared from human R_o cells by the method described elsewhere (33). Threefold volumes of undiluted anti-Rh reagent were added to the washed stromata. After sensitization for 2 hours at 27°C the stromata were washed thrice with 50- to 100-fold volumes of saline. Rheumatoid sera were absorbed with the sensitized stromata (4 hours at room temperature) until serologic reactions with coated human red cells were no longer positive. Other aliquots of rheumatoid sera were repeatedly absorbed with half-fold volume of peaked sensitized sheep cells until serologic reactions in SSC test were negative.

Fractionation Procedures.—Starch block electrophoresis (34) and density gradient ultracentrifugation (8) were performed as described previously. Fractions thus obtained were titered for agglutinating or inhibitory activity; protein content of the fractions was measured by a modified Folin-Ciocalteu reaction. When necessary, fractions were concentrated by evaporation under negative pressure.

Analytical ultracentrifugation was performed in a Spinco model E ultracentrifuge.

RESULTS

Relation to Specificity to Human Hereditary γ-Globulin Groups.—With the sensitized human red cell test system it was possible to readily study the efficacy of γ-globulin from a single individual as a "coating reagent" in tests with various rheumatoid sera. The basis for the reaction is shown in Fig. 1. 0 Rh_o (D)-positive erythrocytes are sensitized by an "incomplete" anti-Rh_o (anti-D) serum. With a given anti-Rh coat, for example anti-D (*a*), addition of certain rheumatoid sera such as rheumatoid serum (*a*) produces agglutination. The use of 24 individual anti-Rh_o sera with a panel of 36 rheumatoid sera produced many different patterns of reactivity. A summary of some of these differences is presented in Table I, which shows selected examples from the broader study. It is evident that rheumatoid serum III reacted with cells sensitized by all four of the anti-Rh coats, whereas rheumatoid serum IV reacted only with coat 4. Other rheumatoid sera such as I and II reacted with some coats but not with others. Such differences in reactivity were not due to differences in titer of either the rheumatoid serum or of the anti-Rh coat, for rheumatoid sera I and II gave diametrically opposed reactions with coats 1 and 2. Table II depicts the results of checkerboard titrations of rheumatoid serum I (Co) and II (Pe) with anti-Rh coats 1 (K 2722) and 2 (Sw). It is evident that differences in titer cannot explain the differing reactivities.

Table III lists the agglutination patterns of 13 selected rheumatoid sera in reactions with red cells sensitized by 9 selected anti-Rh sera. Cells coated with Mu were agglutinated by all of the 36 rheumatoid sera in the test panel.[2] A second coat, Ri, kindly furnished by Dr. Marion Waller, also reacted with all of the rheumatoid sera. Several other coats, such as Ki, CD, and Ga, were ag-

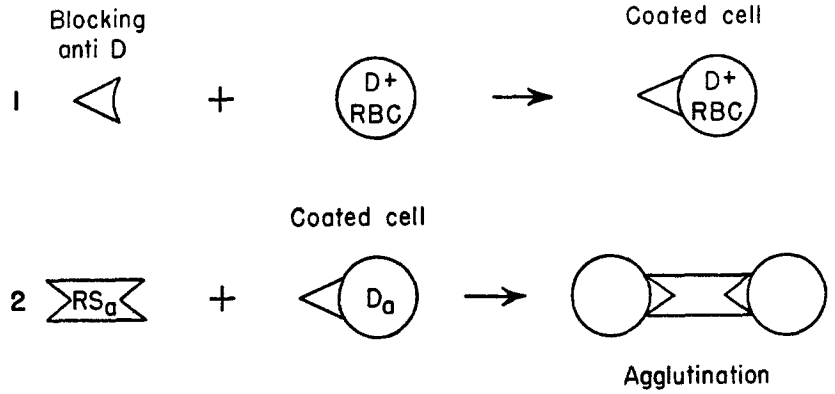

FIG. 1. Schematic drawing of the interaction of incomplete Rh antibodies with red blood cells and their agglutination by rheumatoid factors of complementary specificity. The Da refers to an anti-Rh_0 antibody of a given genetic globulin group, *e.g.* Gm(*a*) which reacts with RSa (a rheumatoid serum containing the complementary rheumatoid factor), *e.g.*, anti-Gm^a.

TABLE I

Selected Agglutination Patterns of 4 Rheumatoid Arthritis Sera with 4 Anti-Rh_0 Coats.

R. A. serum	Anti-Rh_0 (anti-D) coat			
	1	2	3	4
I	+++	0	+++	+++
II	0	+++	+++	+++
III	+++	+++	+++	++++
IV	0	0	0	+++

glutinated by most but not all of the rheumatoid sera, whereas some coats, *e.g.* Vik, were agglutinated by very few of the rheumatoid sera. Coats giving intermediate reactions, *i.e.* agglutination by 15 to 50 per cent of the rheumatoid

[2] Anti-D serum Mu is supplied by Knickerbocker Blood Bank, N. Y. Some problems of exact reproducibility of results with different specimens obtained over a 5 year period have been encountered. This appears to be partially due to variation in the titer of saline anti-Rh agglutinins present.

TABLE II

Block Titration of 2 Rheumatoid Arthritis Sera with 3 Special Anti-D Coats

R. A. serum	Coat K 2722 dilution						Coat Sw dilution						Coat Mu						
	4	8	16	64	256	Sal	4	8	16	64	256	Sal	4	8	16	64	256	1027	Sal
1. Co. dilution																			
4	+++	++++	++++	++	0	0	0	0	0	0	0	0	+++	+++++	+++++	+++	+	0	0
8	+++	++++	++	+	0	0	±	0	0	0	0	0	++++	++++	++++	++	0	0	0
16	+++	++	++	±	0	0	0	0	0	0	0	0	++++	++++	++++	++	0	0	0
64	+++	++	++	±	0	0	0	0	0	0	0	0	+++	+++	+++	+	0	0	0
256	0	0	0	0	0	0	0	0	0	0	0	0	++	++	+	0	0	0	0
1024	0	0	0	0	0	0	0	0	0	0	0	0	++	+	±	0	0	0	0
2. Pe dilution																			
4	0	0	0	0	0	−	++++	+++	++	0	0	0	+++	++++	++++	+++	0	0	0
8	0	0	0	0	0	−	++++	++++	+++	+	0	0	++++	+++++	+++++	++++	0	0	0
16	+	0	0	0	0	−	+++++	+++++	++++	+	0	0	+++++	+++++	+++++	++++	0	0	0
64	+	0	0	0	0	−	++++	++++	++++	++	0	0	+++++	+++++	+++++	++++	0	0	0
256	0	0	0	0	0	−	+++	++++	++++	+	0	0	++++	+++++	++++	++++	0	0	0
1024	0	0	0	0	0	0	0	0	0	0	0	0	+++	++	0	0	0	0	0
3. Saline control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4. Coombs' reagent	+++++	+++++	+++++	++++	++++	0	++++	++++	++++	+++	+++	0	++++	++++	++++	++++	+++	+++	0

sera, are not shown in this table but comprised the vast majority of the anti-Rh sera. None of the rheumatoid sera reacted with all 24 of the anti-Rh coats. However, one serum, from a normal blood donor, kindly furnished by Dr. F. Milgrom, did react with cells sensitized by all 24 coats.

In general, all sera active with a given coat at 1:10 dilution were also reactive at a 1:100 dilution; hence reactions negative at 1:10 dilution are listed as 0 in the table and those positive at 1:100 as ++ to ++++, depending upon the degree of agglutination. Only a very few sera, *e.g.* Ga with coat Sw, were positive with a given coat at 1:10

TABLE III

Agglutination Patterns of 13 R.A. Sera with Various Selected Anti-D Coats

R. A. serum	Anti-D source								
	Mu	Ri	Ki	CD	Ro	Ga	*ax* Ask	*a* Sw	*b* Vik
1. Du	+++	+++	+++	+++	+++	+++	+++	+++	+++
2. Ch	+++	+++	+++	0	0	+++	+++	+++	+++
3. Co	+++	+++	+++	+++	+++	+++	+++	0	+++
4. Ga.	+++	+++	+++	+++	+++	0	+++	+	0
5. Do	+++	+++	+++	+++	0	+++	++	++	0
6. So	+++	+++	+++	+++	+++	+++	+++	0	0
7. Gr	+++	+++	++	++	++	++	++	++	0
8. He	+++	+++	+++	+++	+++	+++	+++	+++	0
9. Kv	+++	+++	++	++	++	++	0	0	++
10. Pa	+++	+++	+++	+++	0	+++	0	0	0
11. To	+++	+++	+++	+++	+++	0	0	0	0
12. Si	+++	+++	+++	0	0	0	0	+++	0
13. Ha	+++	+++	0	0	0	0	+++	0	0

and negative at 1:100; these are listed as +. These results are in keeping with those of Grubb (9), who found that the inhibitory capacities of Gm(a^+) compared to Gm(a^-) sera were not all or none, but rather about tenfold.

Many different agglutination patterns were demonstrable and some were shown to be correlated with the presence or absence of the known, genetically determined γ-globulin groups (Gm groups) in the anti-Rh sera. The sera containing the anti-Rh antibodies were themselves typed for their own genetic γ-globulin groups in the standard inhibition test systems (9–11) after absorption of anti-Rh activity. In Table III the six coats on the left are all heterozygous for the genetic alleles Gm(a) and Gm(b). The three anti-Rh coats at the right serve as reagents in the inhibition test for typing normal sera for the genetic γ-globulin groups Gm(a) and Gm(x). Because our clinic population included no Negro patients with rheumatoid arthritis, Gm-like, which is present only in Negroes (12, 19), was not studied. Further, genetically determined

erythrocyte antigens previously assumed to be identical in Negroes and whites have recently been demonstrated to be qualitatively different in Negroes and whites (35). Since similar factors may complicate the genetically determined γ-globulin groups, the present studies avoided use of serologic reagents obtained from Negroes.

Note that rheumatoid sera 7 (Gr) and 8 (He) gave identical agglutination patterns; they reacted only with the coats containing the hereditary γ-globulin group Gm(a) and failed to react with the only coats lacking the Gm(a) property; namely, Vik.[3] These two rheumatoid sera, He and Gr, are useful for typing normal human sera as Gm(a+) or Gm(a−) in the standard typing system. Three additional rheumatoid sera obtained from Dr. Grubb, also useful as Gm(a) typing reagents, gave agglutination patterns identical with those of He and Gr with the panel of anti-Rh coats. Similarly, rheumatoid serum 9 (Kv), useful as a typing reagent for the Gm(b) factor, failed to agglutinate the two coats lacking this factor, namely Sw and Ask, but agglutinated all the other coats, all Gm(b) positive. Rheumatoid serum 6 (So), useful for Gm(x) typing, failed to react with standard reagents Sw and Vik, both lacking the Gm(x) property, but reacted with standard reagent Ask which possess the Gm(x) factor.

Table III also illustrates eleven different agglutination patterns demonstrable with the panel of rheumatoid sera. Rheumatoid serum 13 only agglutinated cells coated with one anti-Rh reagent (Ask) other than the universally reactive Mu and Ri coats; these were the only coats in the 24 tested with which serum 13 reacted. Serum 12 showed a different pattern of reactivity, indicating the presence of at least a second agglutinating factor of different specificity. Serum 11 reacted with two additional coats, CD and Ro, not agglutinated by either serum 13 or 12, indicating an additional agglutinating factor, either a separate factor or one present in the same serum in addition to the others. Serum 10 reacted with still another coat, Ga, adding a fourth factor. Proceeding in this fashion with further sera, a total of eight factors of differing specificity are demonstrable and, undoubtedly, several more exist. Similar agglutination patterns revealing at least eight specificities were duplicated with various of the other 36 rheumatoid sera in the test panel.

With rare exceptions, several specific factors were present in combination in each rheumatoid serum. This could be demonstrated directly by differential absorption of the rheumatoid sera with selected coats. With such absorption, reactivity with some coats vanished, leaving reactivity for other coats behind, as is illustrated in Table IV. Similar multiple specificities of rheumatoid factor in the same serum have previously been demonstrated in a few sera thoroughly studied by other methods (36, 37).

[3] Agglutination patterns identical to Vik were obtained with D25, another anti-Rh serum useful in the Gm(b) typing system.

TABLE IV

Effect of Absorption of Rheumatoid Sera by Individual Human "Coats"

R. A. serum	Titers			
	Test coat		CD	Latex fixation
	Mu	Sw		
GR (anti-Gm(a))				
Absorbed with Mu	0	0		
Absorbed with Sw	160	0		
Control non-absorbed	160	80		
Cd.				
Absorbed with Mu	0	0	0	20
Absorbed with CD	80	0	0	200
Absorbed with Sw	80	0	10	400
Control non-absorbed	160	40	80	12,800

TABLE V

Comparison of the Agglutination Patterns of Various R.A. Sera with Cells Coated with Anti-C and Anti-D Antibodies from the Same Serum

R. A. serum	rh'(Cde) cells	Ro(cDe)cells
Du	++	++
Ga	++	++
Co	++	+++
Ha	0	0
Mc	+++	+++
Do	++	+
Si	±	+
Pe	++++	++++
T.Ca	++	++
To	+++	+++
R.Ca.	+++	+++
He	+++	+++
Bo	0	0
Gr	0	0
So	++++	++++
Kv	++++	++++
Pa	+++	++
St.	++++	++++
Fa	+++	+++
We	+++	++++
So	0	0
Wa	0	0
Fo	0	0
Hu	0	0
Be	+++	+++

When coating reagents containing antibodies of different specificities against red cells were used to coat the corresponding red cells, identical results were obtained in agglutination reactions with the panel of rheumatoid sera. Table V demonstrates the results of such agglutination tests with anti-rh′ (anti-C) and anti-Rh_o (anti-D) blocking antibodies present in the same serum, but separable by differential absorption. When this serum was used to coat C+D−(rh′) and C−D+(R_o) cells, respectively, with equivalent doses of blocking antibody, the two sets of coated cells gave identical reactions with the rheumatoid panel, as is evident from Table V. Similar results were obtained with another antisera containing two separable incomplete antibodies. Identical agglutination patterns with the panel of rheumatoid sera were also obtained when serial specimens of anti-Rh antibodies from the same individual were used as coats. In two donors, three specimens were obtained during a 2 year period during which immunization continued; coded specimens from a given donor produced identical results in double-blind tests.

Relation of the "Human γ-Globulin Factor" to the "Rabbit γ-Globulin Factor." The sera of eight normal rabbits of different rabbit γ-globulin groups[4] were tested in standard Gm test systems for the Gm(*a*), Gm(*b*), Gm(*x*), and Gm-like inhibitory properties after prior absorption of the rabbit sera with uncoated human red cells. All of the rabbit sera were found to lack the inhibitory properties corresponding to the human genetic γ-globulin groups.

Attempts to inhibit the sensitized sheep cell reactions of two rheumatoid sera of anti-Gm(*a*) specificity with Gm(a^+) and Gm(a^-) human γ-globulin were uniformly unsuccessful under conditions where clear inhibition would be obtained in the Rh system. Further, use of individual rabbit amboceptors, rather than the pool commonly employed, failed to distinguish differences in specificities of rheumatoid factors to individual rabbit γ-globulins (Table VI). A given rheumatoid serum invariably gave equivalent titers (differences within two tubes) with the various individual rabbit amboceptors employed. In these experiments, rheumatoid sera which reacted with a relatively small number of human Rh coats were purposely utilized. However, these too reacted with all the rabbit antibody coats. Previous experiments by Vaughan (38–40) demonstrated that absorption of rheumatoid sera with sensitized sheep cells failed to remove all the activity for cells coated with human incomplete Rh antibodies. A similar experiment is illustrated in Table VII. All serologic activity directed against rabbit γ-globulin was removed, while only moderate reduction of serologic activity against human γ-globulin, as measured by the sensitized human cell, F-II latex fixation, and F-II tanned cell methods, was obtained.

Specificity of "Rheumatoid Factors" in Non-Rheumatoid Serum.—Sera from patients with various forms of chronic hepatic disease, sarcoidosis, Waldenström's macroglobulinemia, idiopathic pulmonary fibrosis, and other conditions characterized by the frequent occurrence of positive tests for rheumatoid factor

[4] These typed sera were furnished by Dr. Sheldon Dray.

with human γ-globulin as the reactant, were tested by F-II latex fixation, F-II tanned cell, sensitized sheep cell, and Mu-coated human cell reactions. Selected positive sera were also tested against the panel of individual Rh coats. As is illustrated by the examples in Table VIII, such non-rheumatoid sera showed a

TABLE VI

Endpoints of Sensitized Sheep Cell Reactions of Various Sera with Individual Amboceptors

Numbers represent twofold serial dilutions of rheumatoid serum beginning at 1:9 dilution.

Amboceptor / Serum	a	b	c	d	e	f	g	h	i	j	Saline
Co	9	10	7	8	9	—	—	9	8	9	0
Ga	9	9	7	7	8	—	—	9	8	8	0
Pe	11	11	10	9	10	—	—	10	9	10	0
Ha	10	9	10	9	9	8	10	—	8	10	0
Do	11	11	11	11	10	11	12	—	10	12	0
To	9	10	9	9	9	7	10	—	8	9	0
Normal	1	1	0	0	0	1	2	1	0	0	0

TABLE VII

Effect of Absorption of Rabbit Antibody Factor from Rheumatoid Arthritis Sera

R. A. Serum	Titers				
	Sensitized sheep cell	Human cells sensitized with			Latex fixation
		+Mu	Ri	Sw	
Ga					
Absorbed with sensitized sheep cell	0	200	400	100	4,000
Non-absorbed control	10,240	800	800	200	16,000
Du					
Absorbed with sensitized sheep cell	0	400	400	100	8,000
Non-absorbed control	128,000	1,600	1,600	200	256,000

limited range of specificity with the anti-Rh coats. The majority of these sera were negative in the sensitized sheep cell reaction despite high titers in the tests using human γ-globulin on particulate materials. Case Fe (Table VIII) where the sensitized sheep cell test was positive showed a broader reactivity with the various Rh coats resembling that seen in the rheumatoid arthritis sera.

One serum from a normal blood donor (kindly furnished by Dr. Felix Milgrom) reacted with all of 24 different Rh coats with which it was tested. This

serum showed an even broader reactivity than the rheumatoid arthritis sera. These observations confirm those of Milgrom (41) and indicate that the Milgrom factor differs from ordinary rheumatoid factors as well as those usually observed in other disorders.

Autospecificity versus IsoSpecificity.—The possibility of preferential specificity of the patient's own rheumatoid factor for his own genetic type of γ-globulin was investigated by two methods. The first, illustrated in Table III, involves reaction of the patient's serum with his own Rh antibody. Rheumatoid serum 4

TABLE VIII

Agglutination Patterns of Various Non-Rheumatoid Sera, Strongly Positive by Latex Fixation, with Various Anti-D Coats

serum	Mu	Ri	7397	Bre	Ax Ask	a Sw	bc Wa	b Vik	Sensitized sheep cell
Sarcoid									
Wa	++	+++	0	0	0	0	0	0	0
Fo	++	++	0	0	0	0	0	0	0
Re	++	0	0	0	0	0	0	0	0
Liver Disease									
Blu	++	0	0	0	0	0	0	0	0
War	++	0	0	0	0	0	0	0	0
DC 4	++	0	0	0	0	0	0	0	0
Macroglobulinemia									
Hu	++	0	0	0	0	0	0	0	0
Be	++	+++	0	0	0	0	0	0	0
Miscellaneous									
So	++	0	0	0	0	0	0	0	0
Ca	++	0	0	0	0	0	0	0	0
Fe	++	±	+	+	0	++	0	0	+++

(Ga) was obtained from an elderly patient with rheumatoid arthritis who was Rh-negative. Following immunization with Rh_o-positive erythrocytes, a potent anti-Rh_o antiserum was produced. The pre-immunization rheumatoid serum, while reactive with red cells sensitized by most other anti-Rh coats, failed to react with red cells coated with the patient's own anti-Rh antibody, although as is evident from Table III, most other rheumatoid sera reacted well with this anti-Rh coat. Similar results have been obtained in one other patient so immunized.

The anti-Rh antibody portion of the γ-globulin cannot be distinguished from the remainder of the 7S γ-globulin carrying the genetic (Gm) groups by current physical and chemical methods (42). However, the possibility exists that this antibody does not carry all the antigenic determinants found in the total 7S

γ-globulin, although the finding of the same antigenic (Gm) specificity in two different antibodies (as described above) is somewhat against this hypothesis.

In order to test whether the non-reactivity of the patient's own rheumatoid factor involves not only his own anti-Rh antibody but all of his 7S γ-globulin, the following experiments were carried out. The 7S γ-globulin, in which the Gm group specificities wholly reside (42), was separated from the 19S γ-globulin of various rheumatoid sera by density gradient ultracentrifugation in order to free it from rheumatoid factor agglutinating activity. When a rheumatoid serum containing only one specificity of rheumatoid factor, namely anti-Gm(*a*), was thus separated and the 7S γ-globulin typed for its genetic groups in standard Gm-typing systems, it was found that the 7S γ-globulin lacked the Gm(*a*) antigen, but instead contained the genetic allele, Gm(*b*). This is shown in Table IX. Similarly, the 7S γ-globulin of a rheumatoid serum of anti-Gm(*b*) specificity was itself Gm(*b*−) but possessed the genetic allele Gm(*a*) and the 7S γ-globulin of a rheumatoid serum of anti-Gm(*x*) specificity and was itself Gm(*x*−). Results obtained with 7S γ-globulins of such specific sera isolated by density gradient ultracentrifugation are depicted in Table X.

Similar results were also obtained in one experiment in which the 7S and 19S γ-globulins were separated by DEAE column chromatography rather than by density gradient ultracentrifugation. Hence the rheumatoid factors obtained from certain rheumatoid sera appeared to be specifically directed toward genetic groups not present in the γ-globulin of the given serum. Although only relatively few rheumatoid factors were studied, these findings were invariably true for those rheumatoid factors useful as typing reagents for one or another of the Gm groups. However, among other sera, not useful as typing sera, evidence of inhibition by the patient's own 7S γ-globulins was obtained. This was most evident from strong prozone reactions which disappeared when the rheumatoid factors were isolated by ultracentrifugation and thus separated from the patient's own γ-globulin. In addition, observations with isolated rheumatoid factors indicated occasional instances in which these were clearly positive with a given Rh coat while the whole serum was negative. Here the γ-globulin in the serum was of the same type as the rheumatoid factor specificity and autoinhibition occurred. These results will be published in detail separately (43).

Reactivity of Different γ-Globulins with Rheumatoid Factors to Give "22S Complexes."—Previous studies have indicated that the rheumatoid factors combine with γ-globulin to give what has been termed the 22S complex (2, 3). This is observed in whole sera of patients with large amounts of rheumatoid factors. Recently, it has been demonstrated that isolated preparations of rheumatoid factors with an *s* rate of approximately 19S acquire an *s* rate close to 22S and an increase in area on the addition of ordinary γ-globulin (4). This complex formation also is readily seen on the addition of isolated rheumatoid factor to normal serum; a 22S component similar to those observed in arthritis sera is obtained in the ultracentrifuge.

In view of the differences in reactivity of various rheumatoid factors with different genetic types of γ-globulin noted above, it seemed of interest to determine whether these differences would also be apparent in respect to complex formation. Initial experiments demonstrated that the addition of isolated rheumatoid factor to a Gm(a+) serum and to a Gm(a−) serum resulted in each instance in formation of a 22S component. However, in view of the findings of many more types of γ-globulin through the reactivity of different rheumatoid factors with various Rh coats, this experiment did not furnish a conclusive answer. Therefore, rheumatoid factor was isolated from serum Ga by methods described previously. One of these preparations is illustrated in the upper por-

TABLE IX

Inhibitory Activity of Isolated 7S γ-globulin from Anti-Gm(a) Rheumatoid Arthritis Serum

Mg/ml 7S γ-globulin of serum 19N added.

System	2.0	0.5	0.125	0.03	Saline
Anti-Gm(a)	+++	+++	+++	+++	+++
Anti-Gm(b)	0	0	0	++	+++

TABLE X

Genetic γ-Globulin Groups of Individuals with Rheumatoid Factor of a Single Specificity

Rheumatoid factor specificity	No. tested	Genetic type of isolated 7S γ-globulin
Anti-Gm(a)	4	Gm($a-b+$)
Anti-Gm(b)	1	Gm($a+b-$)
Anti-Gm(x)	2	Gm($a+b+x-$)

tion of Fig. 2. This preparation was added to four different sera which contained anti-Rh antibodies utilized in the above agglutination studies. Two of these sera contained Rh antibodies that reacted strongly with this rheumatoid factor; the other two contained non-reactive Rh antibodies. On the addition of rheumatoid factor Ga, all the added material complexed with the γ-globulin in the serum to give equal amounts of 22S complex in all four sera. Part of this experiment is illustrated in Fig. 2. The lower portion of this figure shows a pattern of one of the nonreactive sera before (a) and after (b) the addition of rheumatoid factor Ga. The new peak is the 22S complex sedimenting slightly faster than the normal 19S; the pattern (c) shows the effect of addition of an equal concentration of this rheumatoid factor on a reactive serum. The amount of 22S complex is very similar in the two types of sera. As controls for this experiment it was determined that isolated rheumatoid factor Ga sedimented exactly the same as a standard Waldenström macroglobulin (equal mixtures of the two

gave one peak with an *s* rate of 18.7S). Addition of the standard macroglobulin to these two sera caused accentuation of the normal 19S peak only, without producing a 22S complex as always occurred with rheumatoid factor.

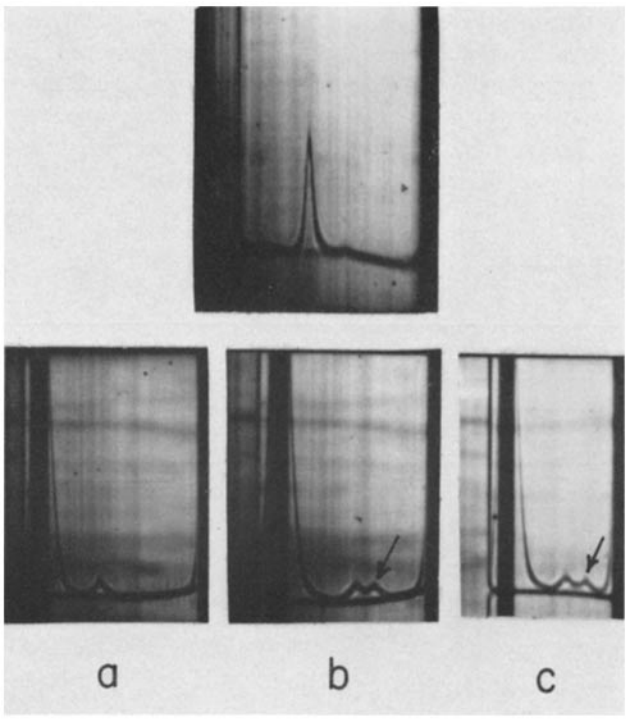

FIG. 2. Ultracentrifuge patterns of isolated rheumatoid factor (upper) and a human serum containing a "non-reactive" anti-Rh antibody before (*a*) and after (*b*) addition of isolated rheumatoid factor. The effect of an equal concentration of this rheumatoid factor on serum containing a reactive anti-Rh antibody is illustrated in (*c*). 22S complexes (arrows) formed equally well with both types of sera.

Similar results were obtained with rheumatoid factor isolated from a different serum. Serum Bomb. (kindly furnished by Dr. Arthur Steinberg) is known to be a powerful anti-Gm (*b*) serum. A highly purified preparation of 19S rheumatoid factor was isolated by precipitation and ultracentrifuge techniques. The addition of Gm(*b*) negative γ-globulin to this isolated material caused complete conversion to 22S complex to the same degree as the addition of Gm(*b*) positive γ-globulin. The reactivity of γ-globulin with rheumatoid factor to give a 22S complex appeared to be a universal property of different γ-globulins unrelated to the specificity in the Rh system.

Precipitin Activity of Soluble Aggregates from Different Types of γ-Globulin.—

It also seemed of interest to determine whether the specificity of the sensitized Rh cell system was reflected in the precipitating action of rheumatoid factors with soluble γ-globulin aggregates. Pooled F-II γ-globulin has usually been employed in this reaction following aggregation by heating (2–4). Five preparations of γ-globulin separated from normal serum by zone electrophoresis were concentrated to 10 mg per ml and aggregated by heating at 63°C for 10 minutes. All preparations failed to react with rheumatoid factor prior to heating. Table XI shows the results of precipitin tests for these five preparations with four different rheumatoid sera. Each preparation was active with all of the different sera. Of particular interest is serum Hu which reacted strongly only with Rh coat Mu out of approximately 35 different anti-Rh sera tested, but still gave a strong precipitin reaction with all five of the γ-globulin preparations. Gamma

TABLE XI

Reactivity of Different Aggregated γ-globulin Preparations from Different Individuals with Various Rheumatoid Sera

γ-Globulin preparation	Rheumatoid serum				Normal serum
	Ga	Ha	Hu	Fa	
I	++++	++++	++++	++++	0
II	++	++	+++	++	0
III	+++	+++	++++	+++	0
IV	+++	+++	+++	+++	0
V	+	++	++	+	0
Ga	+++	+++	+++	+++	0

globulin was also prepared from rheumatoid serum Ga and the rheumatoid factor removed by ultracentrifugation. This γ-globulin, when heated, reacted with all the rheumatoid sera. It also reacted well with rheumatoid factor in the serum from which it was obtained. The anti-Rh coat from this same serum failed to react with the homologous rheumatoid factor. In these systems no selectivity comparable to that in the anti-Rh system could be demonstrated for the precipitin reaction.

The above observations were carried out primarily with systems where the Gm specificity of the rheumatoid factors and Rh coats were not clearly identified. In view of the recent observations of Steinberg and Stauffer (44), that heating of sera alters their specificity in the Gm inhibition system in some instances and not in others, this problem was investigated with isolated γ-globulin aggregates from γ-globulin of known genetic type. Gm($a-$) γ-globulin became strongly inhibitory in the Gm(a) system when aggregates were formed and the isolated aggregates tested. This is in line with the above observations. However, Gm($b-$) aggregates completely failed to inhibit the Gm(b) system, indicating

a striking difference between these two systems. These results are continuing and will be published in detail elsewhere (45).

DISCUSSION

It seems highly likely that the eight different specificities of rheumatoid factor demonstrated by the sensitized human cell technique in the present studies will define at least eight genetically determined γ-globulin groups in man. Four such hereditary groups have already been delineated by employing the inhibition system of Grubb and Laurell in family studies (9, 10). Such genetic differences in human γ-globulin, analogous to the genetic differences in the antigens in human red cells, and the differing specificities of their complementary rheumatoid factors suggest that the latter are antibodies directed against the genetically determined "antigens" in human γ-globulin. Physical, chemical, and immunochemical evidence to support the antibody concept of "rheumatoid factor" has been accumulated during the past few years (2–5), but evidence of such marked specificity has not hitherto been brought forward.

The specificity has thus far been demonstrated only through the use of incomplete antibodies. However, the same specificity was obtained with anti-rh′ (anti-C) and anti-Rh_o (anti-D) antibodies when these were present in the same serum. The results of inhibition studies with the γ-globulin of normal serum indicate that similar specificities are present in the γ-globulin even when such antibodies are not detectable. Absorption experiments with Rh antisera have demonstrated that the γ-globulin lacking the anti-Rh antibody corresponds in genetic type to the incomplete antibodies of each individual serum. This was demonstrated by removing all anti-Rh activity of selected sera by absorption with Rh-positive erythrocyte stromata. The sera were then typed for the various Gm groups in standard inhibition systems. However, the question of whether all γ-globulin molecules possess this specificity, still remains unanswered. It seems possible that certain other molecules in the population of γ-globulins are responsible for precipitin reactions with aggregates and for complex formation. These reactions fail in most instances to show specificity, but other explanations appear more reasonable.

Attempts to reproduce agglutination and agglutination-inhibition reactivity for latex particles and tanned red cells coated with γ-globulin from selected individuals failed to demonstrate specificity and all Gm types were agglutinated by different rheumatoid factors, and no differential inhibitory capacity was demonstrable in sera of differing Gm types. These results might be in part explainable on the basis of aggregates of γ-globulin coating the particles. However, special efforts were made to utilize 7S γ-globulin freed from aggregates by centrifugation as coating for the latex particles. It was evident that 7S γ-globulin functioned well as coating material but no specificity could be demonstrated. Seven different individual γ-globulins were utilized and they reacted with all of six rheumatoid sera. Both the coats and the rheumatoid sera were selected be-

cause of strong specificity in the anti-Rh system. Efforts were also directed toward showing specificity in a streptococcal antibody system similar to that described by Ling and Gibson (46) in which streptococcal antigens are applied to red blood cells, followed by subagglutinating concentrations of antibody from certain normal sera. Again, no specificity for agglutination by certain rheumatoid sera could be demonstrated, although a completely satisfactory system was not obtained because of technical difficulties.

Special mention should be made of the uniformly positive reactions of rheumatoid sera, irrespective of specificity, with coats Mu and Ri. None of the other 22 coats in the test panel, and none of the additional 27 anti-Rh sera used in screening tests for this purpose showed similar universal reactivity. Presumably sera Mu and Ri are heterozygotic for every pair of genetic γ-globulin groups, and thus are analogous to the rare human erythrocytes heterozygous for and thus possessing all the various erythrocyte antigens. Such red cells[5] provide extremely useful tools for screening human sera for the presence of any antibody directed against a genetically determined antigen in human erythrocytes.

The failure of Gm(a+) human γ-globulin to inhibit the agglutination of sensitized sheep cells by rheumatoid sera of anti-Gm(a) specificity strongly implies that the specificity of the rheumatoid factors directed against human γ-globulin is completely divorced from the specificity of the factors directed against rabbit γ-globulin. In addition, inhibitory properties corresponding to those delineated for the known human γ-globulin groups were not found in the γ-globulin of rabbits when tested in the human Rh systems. The panel of rheumatoid factors also displayed no differences in agglutination patterns when individual rabbit amboceptors were employed in the sensitized sheep cell test instead of the pooled commercial amboceptor commonly utilized.

Previous observations (2, 4), as well as those presented in this study, demonstrated that the rheumatoid factors were capable of reacting with the patient's own γ-globulin to form the 22S complex. In addition, soluble aggregates of the patient's own γ-globulin gave precipitates with his rheumatoid factors. Thus there appears little doubt that some affinity exists between the rheumatoid factors and the patient's own γ-globulin. This is compatible with the concept that these factors represent autoantibodies to the patient's own γ-globulin. However, the striking finding that certain rheumatoid factors failed to react with the patient's γ-globulin in the highly specific incomplete Rh system, while reacting well with that of other persons of different genetic types raises the possibility that these proteins actually are isoantibodies analogous to the isohemagglutinins. In the latter case, persons of blood group A, for example, whose red cells contain the A but lack the B antigen produce anti-B isoagglutinins. Current evidence on the mechanism of production of these isoagglutinins sug-

[5] Hemantigen, Knickerbocker Blood Bank, New York.

gests that they are produced from bacterial antigens which cross-react with A and B substances on the red cell (47). In the situation mentioned, anti-B hemagglutinins are produced but anti-A isoagglutinins are not detectable in the presence of A substance on the individual's cells, either because of tolerance or because of self-absorption mechanisms. It is possible that a similar mechanism applies to the production of rheumatoid factors. A foreign antigen which cross-reacts with γ-globulin may produce rheumatoid factor "antibodies" which likewise fail to react with the patient's own γ-globulin because of tolerance or self-absorption mechanisms, but react with the γ-globulin antigens of other persons. The universal autospecificity demonstrated in the 22S complex formation is not so readily explained by this concept.

Another explanation for the apparent isoantibody nature of certain of the rheumatoid factors, which is still compatible with the autoantibody hypothesis, is a concept envisaging the rheumatoid factors as antibodies to minor determinants on the patient's γ-globulin, which nonetheless represent major determinants in γ-globulin of a different genetic type. Antibodies against the patient's major determinants would either fail to form or would be rapidly absorbed. Some precedence for such a concept is seen in the case of the autoantibodies to red cells in acquired hemolytic anemia of the warm variety. Here, the antibodies are rarely to the dominant Rh_o antigens but are usually directed against relative weak (rh″) antigen (48). Also, in the work of Mackay and associates, in certain instances complement-fixing antibodies, measured with whole tissue extracts reacted poorly with the extracts from the patient's own tissues (49) compared to their reactions with extracts from tissues of other individuals. The difficulty with such a concept for the γ-globulins is that it presupposes the presence of small amounts of the foreign genetic determinants in the patient's γ-globulin, either in the buried state on the dominant molecules or on minor constituents of the γ-globulin. This has not as yet been demonstrated. Current evidence does not permit complete evaluation of these hypotheses. In any event, it would appear that the patient's own genetically determined γ-globulin type frequently restricts the specificity of his circulating rheumatoid factor, so that "iso-specificity" exists.

One explanation for the failure of certain rheumatoid arthritis sera to react with their own anti-Rh coats is that the anti-Rh antibodies obtained, despite their high titer, represented only a limited number of specificities arising early in immunization. More prolonged immunization might lead to broader antigenic specificities for the Rh antibodies, including some which would react with the patient's own rheumatoid factors. This hypothesis requires further investigation, especially since "broadening of specificity" with eventual autoreactivity following prolonged isoimmunization, has been observed before with other antigen-antibody systems (50, 51). However, anti-Rh coat Ga, which was studied in greatest detail, reacted well with the majority of rheumatoid arthritis sera, and the failure to react with the rheumatoid factors of this same serum was

in striking contrast to the broad reactivity of this Rh antibody coat. In addition, the results of the inhibition studies with the 7S γ-globulin of rheumatoid arthritis sera containing rheumatoid factors of defined specificities clearly indicated a general absence of the γ-globulin specificity toward which the rheumatoid factors were directed. This apparent isospecificity however in certain instances does broaden into autospecificity, even in the Rh system, as was demonstrated above with isolated rheumatoid factors. It is most clearly and constantly observed in the 22S complex formation. Additional evidence for the occurrence of both auto- and isospecificity for rheumatoid factors has been obtained recently by Harboe (52). The situation may be analogous to that recently described for pancreatic antibodies (53). Although these are frequently isoantibodies, autoantibodies have been observed.

Well delineated isoantibodies have been produced by Oudin (20–22) and others (23–25) by deliberately immunizing individual rabbits with γ-globulin obtained from rabbits of differing γ-globulin "allotype" (20). The serum of such isoimmunized rabbits bears a strong similarity to that of patients with rheumatoid factor; namely, specificity for a genetically determined γ-globulin not present in the immunized rabbit. However, there are also differences. The rabbit antibodies are typical low molecular types (54) in contradistinction to the high molecular weight rheumatoid factors. In addition, the rheumatoid factors form a complex with the patient's own γ-globulin, hence demonstrating a general affinity for γ-globulin that extends beyond the specificity demonstrated in the Rh system. This does not appear to be true of the antibodies in the rabbit. A situation perhaps resembling that in the human is found in the recent work of Milgrom and associates (55): Reinjection of the rabbits' own γ-globulin produced antibodies which reacted with human γ-globulin. The authors postulate that the rabbit γ-globulin is modified in preparation and that antibodies produced to such altered determinants react better with unmodified human γ-globulin than with unmodified rabbit γ-globulin. These results have been confirmed in this laboratory and the question of isospecificity in regard to the reactivity with γ-globulin of other rabbits has been investigated. Thus far a reaction has only been obtained with foreign γ-globulin and no such isospecificity has been demonstrated in this rabbit system (54).

SUMMARY

Rheumatoid factors in the sera of patients with rheumatoid arthritis appear to be specifically directed against genetically determined "antigens" in human γ-globulin. At least eight rheumatoid factors of differing specificity exist; usually several are present in combination in the same serum. The different rheumatoid factors can be readily detected through their pattern of reactivity with anti-Rh antibodies from different individuals. Rheumatoid factors in diseases other than rheumatoid arthritis were found to have a more restricted specificity, contrasted to the broader reactivity of the factors in most rheumatoid arthritis

sera. A specificity similar to that for incomplete antibodies was not demonstrated for the reaction of rheumatoid factors with aggregated γ-globulin or with γ-globulin to form the "22S complex."

In certain instances, using the anti-Rh system, rheumatoid factors were found to react poorly with the patient's own γ-globulin, compared to that of other individuals of different genetic γ-globulin types. These results, as well as additional indirect evidence, indicate that the rheumatoid factors can possess isospecificity. However, a certain degree of autospecificity was also found which was most clearly evident through complex formation with the patients own γ-globulin and in the reaction with aggregates. The relevance of these findings to possible isoantibody as well as autoantibody concepts is discussed.

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