INVESTIGATION OF THE SPECIFICITY OF SUBSTANCES IN SOME HUMAN SERA WHICH ARE INHIBITORY IN CERTAIN VIRAL AND RICKETTSIAL COMPLEMENT-FIXING ANTIGEN-ANTIBODY SYSTEMS¹

NATHALIE J. SCHMIDT AND HARRY B. HARDING²

Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois

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In the first paper of this series, we reported our discovery of substances in certain human sera which inhibit complement fixation in the antigen-antibody systems of lymphogranuloma venereum, psittacosis, mumps, Q fever, and lymphocytic choriomeningitis (Schmidt and Harding, 1956). Previous investigators have found that certain avian and mammalian species, or sometimes single individuals of a particular species, respond to antigenic stimuli by producing substances in their serum which are not complement-fixing antibodies, but inhibit complement fixation. These are specific for a given antigen-antibody system and are considered to be a type of antibody.

Goodner and Horsfall (1936) investigated the inhibitory activity of horse antipneumococcus type III sera in an antigen-antibody system consisting of the pneumococcus type III polysaccharide and rabbit antipneumococcus type III serum. They further demonstrated that the specificity of the inhibitory activity of the horse heterologous immune serum would not prevent the fixation of complement by rabbit immune serum and homologous antigen.

The indirect complement fixation technique was employed by Downie and MacDonald (1950) for their study of the antigenic relationships of the viruses of variola, alastrim, vaccinia, cowpox, and mouse pox.

Karrer *et al.* (1950a) tested the specificity of anti-ornithosis inhibitory substances in the sera of chickens, ducks, and turkeys by infecting these birds with the virus and following the inhibitory titers during the course of the disease. These

inhibitory titers were compared with those of normal fowl. It was found that birds not injected with the virus and not exposed to other infected fowl showed negative inhibitory titers, whereas infected birds showed positive inhibitory titers after 7 days.

The question then arises concerning whether the inhibiting substances produced by man (Schmidt and Harding, 1956) may be inhibitory "antibodies." This paper describes experiments conducted to determine whether the inhibitory activity found in these human sera is specific to each antigen-antibody system.

METHODS AND MATERIALS

The reagents and methods for performing the direct and indirect complement fixation tests which were employed for these studies were the same as those described in an earlier paper (Schmidt and Harding, 1956).

EXPERIMENTAL RESULTS

Complement-fixing activity of inhibitory human sera. First, a study was made to ascertain whether sera which were inhibitory in lymphogranuloma venereum (LGV)-psittacosis systems might show complement-fixing activity against other viral or rickettsial antigens. If the inhibitory substances contained in these sera were not specific for LGV-psittacosis systems, they might likewise interfere with the activity of complement-fixing antibodies which might be present in these sera against other viral or rickettsial antigens. One would expect, also, to see a lack of correlation between the inhibitory titers of the sera and their complement-fixing activity with other antigens.

In this study 63 human sera showing inhibitory activity in LGV-psittacosis systems were examined by the direct complement fixation test, using influenza A, influenza B, influenza FM₁,

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² From the Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois.

TABLE 1

Complement fixation titers of sera with a 1:4 inhibitory titer in lymphogranuloma venereum-psittacosis systems

Antigens	Number of Sera with the Following Complement-fixing Titers					
	Neg.	1:2	1:4	1:8	1:16	1:32
Influenza A....	0	0	1	1	2	0
Influenza B....	0	0	2	1	1	0
Influenza FM ₁ ...	3	0	0	1	0	0
Mumps.....	1	0	1	1	1	0
Others.....	0	0	0	0	0	0

Total number of sera, 4.

TABLE 2

Complement fixation titers of sera with a 1:8 inhibitory titer in lymphogranuloma venereum-psittacosis systems

Antigens	Number of Sera with the Following Complement-fixing Titers						
	Neg.	1:2	1:4	1:8	1:16	1:32	1:64
Influenza A.....	8	2	5	6	3	11	1
Influenza B.....	11	1	8	2	3	1	0
Influenza FM ₁	21	1	2	2	0	0	0
Mumps....	17	0	0	3	6	0	0
Q fever....	25	0	0	1	0	0	0

Total number of sera, 26.

TABLE 3

Complement fixation titers of sera with a 1:16 inhibitory titer in lymphogranuloma venereum-psittacosis systems

Antigens	Number of Sera with the Following Complement-Fixing Titers						
	Neg.	1:2	1:4	1:8	1:16	1:32	1:64
Influenza A.....	9	1	6	3	1	0	1
Influenza B.....	14	1	2	1	1	1	1
Influenza FM ₁	19	0	1	1	0	0	0
Mumps....	13	0	4	2	1	1	0
Q fever....	20	0	0	1	0	0	0
LCM....	20	0	0	1	0	0	0

Total number of sera, 21.

TABLE 4

Complement fixation titers of sera with a 1:32 inhibitory titer in lymphogranuloma venereum-psittacosis systems

Antigen	Number of Sera with the Following Complement-Fixing Titers						
	Neg.	1:2	1:4	1:8	1:16	1:32	1:64
Influenza A.....	5	0	2	2	2	1	0
Influenza B.....	5	1	0	2	1	3	0
Influenza FM ₁	9	1	0	2	0	0	0
Mumps....	6	0	1	2	2	0	1
LCM.....	11	0	0	1	0	0	0
WEE.....	11	0	0	1	0	0	0

Total number of sera, 12.

mumps, western equine encephalitis, eastern equine encephalitis, rabies, St. Louis encephalitis, lymphocytic choriomeningitis, rickettsialpox, murine typhus, Q fever, and Japanese B encephalitis antigens. Complement-fixing antibodies for some of these antigens could be demonstrated in these sera. Only 12 of the 63 sera failed to show complement-fixing activity with any of these antigens. The sera which showed a 1:4 inhibitory titer in LGV-psittacosis systems also showed complement-fixing activity with at least 2 of the other antigens. Twenty of the sera with a 1:8 inhibitory titer showed complement-fixing activity with at least 1 of the other antigens. Seventeen of the 21 sera with a 1:16 inhibitory titer showed complement-fixing antibodies against at least 2 of the other antigens, and 10 of the 12 sera with inhibitory titers of 1:32 showed complement-fixing activity with at least 2 of the other antigens. Tables 1, 2, 3, and 4 show the complement-fixing titers of these sera with other antigens.

The inhibitory titers of the sera were compared with the serum dilutions which were necessary to fix complement with each of 4 antigens (tables 5, 6, 7, and 8). The percentages of sera with the given inhibitory titers which will fix complement in each serum dilution are given rather than the complement-fixing titers. For example, a serum which had a complement-fixing titer of 1:16 also fixed complement in a dilution of 1:8, 1:4, and 1:2. The information given in these tables shows that there is no significant correlation between the inhibitory titers of the sera for LGV-

TABLE 5

A comparison of the inhibitory titers of human sera in psittacosis-lymphogranuloma venereum systems and the ability of these sera to fix complement with influenza A antigens

Dilutions of Sera Which Fixed Complement with Influenza A Antigens	Percentage of Sera with Each of the Given Inhibitory Titers Which Fixed Complement with Influenza A Antigens		
	Inhibitory titers		
	1:8	1:16	1:32
0	30.8	43.0	41.5
1:2	69.0	57.0	57.5
1:4	61.5	52.0	57.5
1:8	42.4	23.0	41.5
1:16	19.5	9.5	25.5
1:32	7.7	4.7	8.4
1:64	3.8	4.7	0

TABLE 6

A comparison of the inhibitory titers of human sera in psittacosis-lymphogranuloma venereum systems and the ability of these sera to fix complement with influenza B antigens

Dilutions of Sera Which Fixed Complement with Influenza B Antigens	Percentage of Sera with Each of the Given Inhibitory Titers Which Fixed Complement with Influenza B Antigens		
	Inhibitory titers		
	1:8	1:16	1:32
0	42.4	66.5	41.7
1:2	57.7	33.3	58.4
1:4	54.0	23.8	50.0
1:8	23.1	19.0	50.0
1:16	15.4	14.3	33.3
1:32	3.8	9.5	25.0
1:64	0	4.7	0

psittacosis systems and their ability to fix complement with the 4 other antigens. This would indicate that the inhibitory substance which is active in LGV-psittacosis systems does not prevent the fixation of complement by antibodies in the same sera directed against other complement-fixing antigens.

Results of parallel tests in which the same serum was used in more than one complement fixation inhibition system. Additional investigations concerning the specificity of the inhibitory activity of human sera were made by setting up parallel inhibition tests on sera, using two or more complement-fixing antigen-antibody systems.

Sixty-nine human sera were tested for in-

TABLE 7

A comparison of the inhibitory titers of human sera in psittacosis-lymphogranuloma venereum systems and the ability of these sera to fix complement with influenza FM₁ antigens

Dilutions of Sera Which Fixed Complement with Influenza FM ₁ Antigens	Percentage of Sera with Each of the Given Inhibitory Titers Which Fixed Complement with Influenza FM ₁ Antigens		
	Inhibitory titers		
	1:8	1:16	1:32
0	80.8	90.0	75.0
1:2	19.2	9.5	25.0
1:4	16.0	9.5	16.6
1:8	7.7	4.7	16.6

TABLE 8

A comparison of the inhibitory titers of human sera in psittacosis-lymphogranuloma venereum systems and the ability of these sera to fix complement with mumps antigens

Dilutions of Sera Which Fixed Complement with Mumps Antigens	Percentage of Sera with Each of the Given Inhibitory Titers Which Fixed Complement with Mumps Antigen		
	Inhibitory titers		
	1:8	1:16	1:32
0	61.5	62.0	50.0
1:2	37.5	38.0	50.0
1:4	37.5	38.0	50.0
1:8	34.0	19.0	41.5
1:16	23.0	9.5	25.0
1:32	0	4.7	8.3
1:64	0	0	8.3

hibitory activity in 2, 3, or 4 antigen-antibody systems at the same time, using the same supply of saline, complement, antishoop hemolysin, and sheep erythrocytes for all tests. The only variables in these tests were the antigens and the complement-fixing antisera.

If the inhibitory activity were not specific for a given antigen-antibody system, it would be expected that a serum which was inhibitory in one complement-fixing antigen-antibody system also would be inhibitory in another, and the inhibitory titers in both systems would be approximately the same. However, if the inhibitory activity were specific, a serum might show inhibitory properties in one antigen-antibody system but not in another; or the serum might show inhibitory activity in more than one antigen-antibody system, but the inhibitory

TABLE 9
Reactions of sera in parallel inhibition tests

Parallel Inhibition Test Systems in Which Sera Were Run	No. of Sera	Inhibitory Activity Was Found In:										
		LGV	Mumps	LCM	Qf.	LGV, LCM	Mumps, Qf.	LGV, Qf.	LGV, LCM, mumps	LGV, LCM, Qf.	LGV, IFM, mumps	LGV, mumps
LGV, mumps.....	16	11	4									1*
LGV, mumps, Q fever, LCM.....	7	5	1			1*						
LGV, mumps, Q fever	2						2*					
LGV, mumps, LCM..	4	1		1		1*			1*			
LGV, LCM.....	12	6		2		4†						
LGV, mumps, IFM ₁ , Inf. B.....	4	3									1*	
LGV, mumps, Inf. B.	1	1										
Mumps LCM, Q fever	1		1									
LCM, Q fever.....	3			2	1							
LGV, LCM, Q fever.	19	5		3	1	5*		5*		2*		

* Titers differ.

† Titers differ in 3; titer same in 1.

Key for the abbreviations of the various antigens listed in the above table: LGV = lymphogranuloma venereum; LCM = lymphocytic choriomeningitis; Qf. = Q fever; IFM₁ = the FM₁ strain of influenza type A; Inf. B = influenza type B.

titers in all of the systems would not be the same.

The results of these studies are summarized in table 9. It is seen that a human serum which is inhibitory in one complement-fixing antigen-antibody system is not necessarily inhibitory in another system. A single serum may contain inhibitory substances directed toward more than one antigen-antibody system, but the fact that only 3 of the 69 sera had the same inhibitory titers in 2 inhibitory test systems would indicate that different inhibitory substances are involved in each system.

Removal of the inhibitory activity of human serum by the addition of an excess of antigen to the inhibitory test system. Investigators who have studied the inhibitory activity of certain avian sera in ornithosis complement-fixing antigen-antibody systems have observed that by adding an excess of ornithosis antigen to the test system the inhibitory activity of the sera was overcome. They feel that the excess antigen is sufficient to combine with the inhibitory "antibodies" leaving some additional antigen in the system to combine with the complement-fixing antibodies furnished by the positive complement-fixing serum (Rice, 1948a; Rice, 1948b; Karrer *et al.*, 1950b; Hilleman *et al.*, 1951).

Studies were made of 25 specimens of human sera inhibitory in LGV-psittacosis complement fixation tests to determine if an excess of lymphogranuloma venereum or psittacosis antigen could overcome their inhibitory activity. An antigen dilution containing 2 or more units of antigen was added to inactivated serum in a volume sufficient

TABLE 10
Effect of incubation with an excess of lymphogranuloma venereum antigen upon the inhibitory activity of a human serum in lymphogranuloma venereum systems

Serum Dilution	Control Tests with Untreated Serum		Tests with Serum after Preliminary Incubation with Two Units of Lymphogranuloma Venereum Antigen	
	LGV* complement fixation test	LGV complement fixation inhibition test	LGV complement fixation test	LGV complement fixation inhibition test
1:2	—	—	—	4+†
1:4	—	—	—	4+
1:8	—	1+	—	4+
1:16	—	1+	—	4+
1:32	—	4+	—	4+
1:64	—	4+	—	4+

* LGV = lymphogranuloma venereum.

† 4+ = 4+ complement fixation.

TABLE 11

The effect of incubation with an excess of homologous antigen, heterologous antigen, and antigen controls upon the inhibitory activity of a human serum in lymphogranuloma venereum systems

	Serum Dilution					
	1:2	1:4	1:8	1:16	1:32	1:64
Control tests with untreated serum						
LGV complement fixation test.....	—	—	—	—	—	—
LGV complement fixation inhibition test.....	—	—	—	2+†	4+	4+
LGV complement fixation inhibition test after preliminary incubation with						
3 units LGV* antigen....	4+	4+	4+	4+	4+	4+
3 units LGV antigen control....	—	—	—	2+	4+	4+
4 units Q fever antigen.....	—	—	—	1+	4+	4+
4 units Rocky Mt. spotted fever antigen....	—	—	—	1+	4+	4+

* LGV = lymphogranuloma venereum.

† 4+ = 4+ complement fixation.

to dilute the serum 1:2. These reagents were incubated at 37 C for 30 min to remove the inhibitory activity, after which successive dilutions of the serum were made in saline. The serum dilutions then were run in parallel complement fixation and complement fixation inhibition tests for lymphogranuloma venereum and psittacosis in the usual manner. The total amount of antigen in the inhibitory test systems was the

amount initially incubated with the serum plus the one unit of antigen usually employed in the inhibition tests.

In all 25 cases incubation of the sera with an excess of lymphogranuloma venereum or psittacosis antigens caused partial or complete loss of inhibitory activity. However, incubation of the sera with antigen controls prepared from normal tissues or with heterologous antigens did not overcome the inhibitory activity of the sera in lymphogranuloma venereum or psittacosis complement fixation inhibition tests.

Two examples of these experiments are illustrated in table 10 and 11.

The action of inhibitory sera upon anticomplementary antigens. An additional observation made in our laboratory would lend support to the concept that the inhibitory substances in human sera exert their effect by combining directly with the antigen, thereby depriving the complement-fixing antibodies of antigen: It was found that a group of 12 human sera which were inhibitory in LGV-psittacosis systems overcame the anticomplementary effect of one lot of lymphogranuloma venereum antigen. The dilutions of the sera which reversed the anticomplementary effect of the antigen were the same as those which were inhibitory in LGV-psittacosis systems. The controls employed in the test showed conclusively that it was the antigen, and none of the other reagents, which was anticomplementary.

DISCUSSION AND CONCLUSIONS

The preceding evidence obtained in this laboratory indicates that the inhibitory activity demonstrated by some human sera in complement-fixing antigen-antibody systems is specific although apparently it does not occur in high concentration. First, the inhibitory substances in sera which are active in LGV-psittacosis systems do not interfere with the activity of complement-fixing antibodies for other antigens which may be present in the same sera. Second, sera which are inhibitory in one complement-fixing antigen-antibody system are not necessarily inhibitory in other antigen-antibody systems. The reversal of the inhibitory activity of human sera by the addition of an excess of homologous antigen also supports the concept that inhibitory substances are specific. However, before it may be stated definitely that the inhibitory substances described in human sera actually represent a

form of antibody, biochemical studies should be made to discover which serum fraction contains the inhibitory activity. Also attempts should be made to adsorb the inhibitory substance from sera onto specific particular antigens. In our laboratory repeated attempts were made to adsorb complement-fixing antigens onto collodion particles, and with these to adsorb the inhibitory substances from the human sera. These experiments were unsuccessful, however, as it was impossible to prevent the elution of the complement-fixing antigens from the collodion particles into the serum during the adsorption.

The reversal of the anticomplementary activity of lymphogranuloma venereum antigen by sera which were inhibitory in LGV-psittacosis systems further supports the concept that this substance is an antibody. The latter observation is similar to the effect observed by Caulfield (1911, 1925). He found that the serum from some tubercular human beings would not fix complement with the homologous antigen, but would decrease the anticomplementary activity of antigens consisting of alcohol-ether extracts of the tubercle bacillus. He interpreted this to indicate that the serum contained specific antibodies which combined with the antigen and rendered it less anticomplementary. Perhaps the mechanism of the phenomenon observed by us is similar.

SUMMARY

Certain human sera which fail to fix complement in the presence of viral and rickettsial antigens contain substances which inhibit complement fixation by a known positive complement-fixing serum and its homologous antigen. This communication presents evidence to support the concept that these substances are specific and therefore antibody-like in nature. It was found that sera which are inhibitory in one complement-fixing antigen-antibody system are not necessarily inhibitory in other antigen-antibody systems. Inhibitory substances which are active in psittacosis-lymphogranuloma venereum systems do not interfere with heterologous complement-fixing antibodies present in the same sera. The inhibitory activity of these human sera

was overcome by the addition of an excess of homologous antigen to the test system. Finally it was discovered that sera which are inhibitory in lymphogranuloma venereum-psittacosis systems overcame the anticomplementary activity of a certain lymphogranuloma venereum antigen.

REFERENCES

- CAULFIELD, A. H. 1911 Factors in the interpretation of the inhibitive and fixation serum reactions in pulmonary tuberculosis. *Proc. Roy. Soc. (London)*, **84**, 373.
- CAULFIELD, A. H. 1925 The tuberculo-complement fixation and inhibitive test. *Am. Rev. Tuberc.*, **11**, 508.
- DOWNIE, A. W. AND MACDONALD, A. 1950 A study of the pox viruses by complement fixation and inhibition of complement fixation methods. *J. Pathol. and Bacteriol.*, **62**, 389.
- GOODNER, K. AND HORSFALL, F. 1936 The complement fixation reaction with pneumococcus capsular polysaccharide. *J. Exptl. Med.*, **64**, 201.
- HILLEMANN, M. R., HAIG, D. A., AND HELMHOLD, R. J. 1951 The indirect complement fixation, hemagglutination, and conglutinating complement absorption tests for the viruses of the psittacosis-lymphogranuloma venereum group. *J. Immunol.*, **66**, 115.
- KARRER, H., MEYER, K. F., AND EDDIE, B. 1950a The complement fixation inhibition test and its application to the diagnosis of ornithosis in chickens and ducks. *J. Infectious Diseases*, **87**, 13.
- KARRER, H., MEYER, K. F., AND EDDIE, B. 1950b Complement fixation test in ornithosis. *J. Infectious Diseases*, **87**, 24.
- RICE, C. E. 1948a The nature of the inhibitive reaction in the indirect complement fixation test. *Can. J. Public Health*, **39**, 32.
- RICE, C. E. 1948b Inhibitory effects of certain avian and mammalian antisera in specific complement fixation systems. *J. Immunol.*, **59**, 365.
- SCHMIDT, N. J. AND HARDING, H. B. 1956 The demonstration of substances in human sera which inhibit complement fixation in antigen-antibody systems of lymphogranuloma venereum, psittacosis, mumps, Q fever, and lymphocytic choriomeningitis. *J. Bacteriol.*, **71**, 217-222.