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Research Article

ANTIBODY RESPONSE TO INFECTIONS WITH THE NEWLY CLASSIFIED TYPES OF PNEUMOCOCCI (COOPER) [1]

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The separation of the pneumococci previously included in Group IV into serologically specific types (1) has made possible the accurate identification of nearly all pneumococci. In this paper will be presented the result of studies on the specific antibody response of human subjects to infections associated with these newly classified types of pneumococci. Similar studies in patients with 2 important pairs of immunologically related types of pneumococci, namely, Types III and VIII and Types II and V, have been reported in the preceding communications (2, 3) and only a summary of these results are included here for comparison.

The sera of 190 patients with infections associated with pneumococci other than Type I were studied. The number of patients and sera in each of 3 kinds of cases studied are listed in Table I according to the type of pneumococcus obtained. The "non-pneumonias" include patients with acute and chronic respiratory infections, but without pulmonary consolidation, and those with purulent focal infections. The methods employed were the same as those used in the previous studies (2).

Agglutinins for pneumococci of the homologous type

Agglutinins for the homologous type of pneumococcus were demonstrated in the sera of two-thirds of the patients with lobar or bronchopneumonia associated with Types II and III and with 9 of the newly classified types (see Table II). The data for the individual patients in whose sera such antibodies were demonstrated are shown in Table III and in the corresponding tables in the previous papers (2, 3). Also, 6 of the 22 fatal cases showed agglutinins for the homologous type in the serum before death.

The titer of the agglutinins for the newly-classified types was comparable with that found in similar patients with Types I, II and III pneumococcus pneumonia (4). Sera which agglutinated one strain of a given type also agglutinated all other strains of the same type to approximately the

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TABLE I

Material examined

Patients' type	Number of strains	Total number of patients	Lobar pneumonias				Bronchopneumonias				Non-pneumonias*	
			Recovered		Died		Recovered		Died			
			Number of patients	Number of sera	Number of patients	Number of sera	Number of patients	Number of sera	Number of patients	Number of sera	Number of patients	Number of sera
I	1	4†	3	8	—	—	—	—	—	—	1	1
II	1	11	8	19	2	2	0	—	0	—	1	1
V	4	25	19	68	4	4	1	2	0	—	1	1
III	1	40	19	44	3	5	5	14	3	3	10	17
VIII	8	33	18	65	4	11	4	12	2	3	6	8
IV	3	8	6	16	0	—	0	—	0	—	2	2
VII	4	14	10	30	2	4	2	5	0	—	0	—
IX	2	4	0	—	1	1	0	—	1	2	2	3
XII	2	4	4	11	0	—	0	—	0	—	0	—
XIV	4	8	2	6	1	1	2	3	0	—	3	5
XVII	2	3	2	4	1	1	0	—	0	—	0	—
XVIII	1	4	0	—	0	—	2	6	0	—	2	2
XIX	2	7	3	9	0	—	2	7	0	—	2	2
VI	5	8	2	5	2	3	0	—	0	—	4	9
X	2	4	0	—	1	4	0	—	0	—	3	4
XI	2	2	2	5	0	—	0	—	0	—	0	—
XIII	1	5	1	3	1	1	0	—	0	—	3	3
XV	1	1	1	3	0	—	0	—	0	—	0	—
XX	2	9	5	9	0	—	1	3	0	—	3	3
XXII	1	2	1	2	0	—	1	4	0	—	0	—
XXIX	1	1	0	—	0	—	0	—	0	—	1	1
XXXI	1	1	0	—	0	—	0	—	0	—	1	1
All	51	194‡	106	307	22	37	20	56	6	8	45	63

* Includes acute and chronic respiratory infections without pneumonia, the pneumococcus being obtained from the sputum, and focal pneumococcic infections, where the pneumococcus was obtained from the lesion.

† Each of these had other types of pneumococci in addition to the Type I.

‡ Four patients are listed under 2 separate types.

same titer. In a number of instances, different methods were used to prepare the antigens and carry out the tests, but the results were practically the same in each instance.

In 2 cases, a recovered Type XII and a fatal Type XIV, the same type of pneumococcus was obtained from sputum and blood culture, but agglutinins for the homologous type could not be demonstrated in several sera. No instances were found of homologous type-specific agglutinins for the 9 types shown in the lower portion of Table I, although there were included 14 recovered pneumonia patients from whom these types were obtained.

TABLE II

Results of agglutination tests with homologous type pneumococcus antigens in the sera of pneumonia patients †

Patients' type*	Lobar pneumonia				Bronchopneumonia			
	Recovered		Died		Recovered		Died	
	Number tested	Number positive	Number tested	Number positive	Number tested	Number positive	Number tested	Number positive
II	8	6	2	0	0	—	0	—
III	19	11	3	0	5	2	3	2
V	19	17	4	0	1	1	0	—
VIII	18	11	4	0	4	3	2	1
IV	6	5	0	—	0	—	0	—
VII	10	8	2	2	2	2	0	—
XII	4	1	0	—	0	—	0	—
XIV	2	1	1	0	2	1	0	—
XVII	2	0	1	1	0	—	0	—
XVIII	0	—	0	—	2	1	0	—
XIX	3	2	0	—	2	2	0	—
All	91	62	17	3	18	12	5	3

* Only types against which agglutinins were demonstrated in the sera of 1 or more pneumonic patients are given in this table.

† The following abbreviations and notations apply to this and subsequent tables of this paper:

Pn. IV, Pn. VII, etc. = *Pneumococcus Type IV*, *Pneumococcus Type VII*, etc.

"Day" = The numbers represent the number of days after the onset of the disease.

"Agglutinins" = The numbers represent the highest dilution of serum in which floccular agglutination was observed. More than one number in these columns are recorded when different titers were obtained with different antigens or on repeated tests.

"Mouse protection" = The figures represent the highest number of lethal doses against which mice were protected.

+ = end point not made out.

x = irregular survivals.

— = indeterminate or test not done.

Only 2 patients without pneumonia had homologous type agglutinins. One of these had an acute upper respiratory infection and Type III and the other was a cardiac patient with chronic bronchitis and Type IX pneumococci in the sputum. Others had protective antibodies without agglutinins (2, 3).

Agglutinins for pneumococci of heterologous types

The serum of every patient was tested for agglutinins with Types I, II and III and usually with strains of about 15 heterologous types of

TABLE III

Immune response to infections with the newly classified types of pneumococci (Only cases with antibodies are listed; Types V and VIII excluded)

Case number	Patient	Age	Patient's type	Termination		Day of serum	Homologous antibodies		Remarks
				Mode	Day		Agglutinins	Protection	
		years							
106	A. F.	34	IV	Crisis	8	7	0	0	Blood culture negative 6th day. Pn. IV 8th day
						12	8–16	10^3	
						19	4–16	—	
						26	4–16	10^4x	
107	D. P.	58	IV	Lysis	7	11	8	10^4	
						18	4–8	10^4	
108	J. A.	28	IV	Lysis	7	4	8	10^3	
						16	4	10^3	
						25	4	10^3	
109	M. S.	48	IV	Lysis	8	8	8	—	
110	J. P.	25	IV	Crisis	9	7	0	0	
						11	0	0	
						13	2	10^2	
111	C. C.	65	VII	Lysis	7–13	9	0	—	Bronchopneumonia
						11	0	10^3	
						15	16	10^5	
						24	8	10^7	
112	A. G.	18	VII	Lysis	8	9	16	—	
						11	64	—	
						18	64	10^6	
113	A. F.	19	VII	Crisis	8	34	512	10^7	Pn. I pneumonia 3 years previously
114	J. B.	19	VII	Lysis	11	9	0	10^4	
						19	16–64	10^7	
115	J. Z.	37	VII	Crisis	12	14	64	10^5	
116	S. J.	20	VII	Crisis	6	5	0	10	
						10	8–16	10^5	
						15	4	10^3	
						24	0	10^4	
117	A. McD.	43	VII	Lysis	7	4	0	0	
						10	512	10^6	
						15	256	10^7	
						29	32–64	10^6	
						36	32	—	
						43	32	—	
						49	32	10^6	

TABLE III (*continued*)

Case number	Patient	Age	Patient's type	Termination		Day of serum	Homologous antibodies		Remarks
				Mode	Day		Agglutinins	Protection	
		years							
118	M. C.	14	VII	Crisis	9	8	8	—	
						11	8	10^6	
						16	32	—	
						22	16–32	10^{6+}	
119	H. L.	37	VII	Crisis	7	10	64	10^{6+}	Bronchopneumonia
6	D. Van F.	39	VII	Lysis.	23?	25	0	—	(See Table of Type III cases in (2))
				Recurrence	26–34	31	0	0	
						40	4	10^6	
120	P. W.	37	VII	Lysis?	18–28?	22	0	10	Prolonged low-grade fever
						23	0	—	
						25	0	—	
						43	0	10^4x	
						64	0	10^2	
121	H. P.	54	VII	Died	10	9	8–16	10^4	Pseudocrisis 7th day
122	A. McN.	44	VII	Died	32	4	0	10	Pn. VII in blood culture repeatedly to 28th day
						11	2–4	10^4	
						15	16	10^6	
						25	64	10^7	
123	J. O.	44	XII	Crisis	8	5	0	—	
						10	4	—	
						13	8	—	
124	L. A.	62	XIV	Lysis	14	10	0	—	Bronchopneumonia
						21	4–8	—	
125	L. C.	50	XIV	Crisis	8	11	64	—	
						15	256	—	
126	J. L.	42	XVII	Died	13	10	64	—	
127	B. McL.	18	XVIII	Lysis?	5–10	7	4	—	Paratyphoid B infection with positive blood culture on 6th and 7th days
						9	4	—	
						24	8	10^3	
						45	2	—	
						60	0	10	
128	J. F.	53	XVIII	Crisis	16	24	0	10	Bronchopneumonia
129	M. B.	39	XIX	Lysis	7	7	8	—	Bronchopneumonia
						15	0	—	
						23	2	—	
						30	0	—	

TABLE III (*continued*)

Case number	Patient	Age	Patient's type	Termination		Day of serum	Homologous antibodies		Remarks
				Mode	Day		Agglutinins	Protection	
		years							
130	E. McD.	26	XIX	Crisis	4	3	4	—	Bronchopneumonia
						6	8	—	
						10	8	—	
131	J. K.	39	XIX	Crisis	8	10	16	—	
						16	8	—	
132	G. F.	38	XIX	Crisis	12	5	24	—	
						8	4	—	
						20	0	—	
						35	0	—	
133	J. M.	54	IX	—	—	—	8	—	Chronic bronchitis; died 3 months later

pneumococci. There were 18 patients in whom agglutinins were found for types other than those found in the sputum (Table IV). In one-half of these cases, the heterologous agglutinins may be considered to be cross-agglutinations in antisera of types which, from other evidence, appear to be immunologically related. These included 8 instances of cross-agglutination between Types III and VIII and one between Types II and VI.

Results of absorption tests

A number of sera showing agglutinins for the homologous types were absorbed with strains of the same type and with heterologous types of pneumococci. In each instance, the agglutinins were successfully absorbed with pneumococci of the homologous type but were not materially affected by those of heterologous types. These results are shown in Table V and in the corresponding tables in the previous communications (2, 3).

Results of mouse protection tests

Attempts to enhance sufficiently the virulence of a number of strains of the new types of pneumococci for use in protection tests proved unsuccessful, except with Types V and VIII. Virulent strains of Types IV, VII and XVIII pneumococci, however, were obtained through the kindness of Miss Georgia Cooper. Protection tests were carried out on the sera of most of the patients with pneumonia from whom these types were

obtained. The results of similar tests with Types III and VIII and Types II and V have already been reported (2, 3).

The results of the protection tests paralleled, in general, the findings of agglutinins (Table III). Occasional sera showed mouse-protection in the absence of agglutinins for the same type. The titer of antibodies was similar to that observed with the common types.

Mixed infections

In Table VI are listed 12 patients with pneumonia from whom 2 or more organisms, chiefly pneumococci, were isolated. The presence or

TABLE IV

Cases with agglutinins for heterologous type-specific pneumococci

Case number	Patient	Types from sputum	Agglutinins with homologous type	Heterologous type	Heterologous titer	Remarks*
134	C. McD.	XV	0	I	4	Protection against 10^4 L.D., Pn I
135	G. B.	XVII	0	I	16	Pn. XVII in first 2 sputa, B. Friedländer and no pneumococci in a later one
136	H. G.	XX	0	I	4	First sputum showed Hem. Strep. and no pneumococci. Protection for 10^3 L.D., Pn. I
137	A. C.	XXII	0	I	16	Pn. XXII in 2 sputa. No pneumococci in a third
27	D. McC.	III	0	V	4	Fatal case. No protection against Pn. II, III or V. Agglutinins (1 : 2 only) for Pn. II and VIII. Protection 10^2 L.D., Pn. VIII. Blood culture Pn. III
77	C. R.	II	0	III	4	Protected only 1 L.D., Pn. III. No protection Pn. II or V
50	C. L.	VIII	32	III	4	Protection 10^4 L.D., Pn. III and 10^4 L.D., Pn. VIII
43	R. S.	VIII	4	III	4	Protection 10^3 L.D., Pn. VIII; 10^2 L.D., Pn. III
53	E. S.	VIII	8	III	4	Protection 10^5 L.D., Ph. VIII; 10^4 L.D., Pn. III
54	F. H.	VIII	0	III	16	Protection 10^3 L.D., Pn. VIII; 10^5 L.D., Pn. III

TABLE IV (*continued*)

Case number	Patient	Types from sputum	Agglutinins with homologous type	Heterologous type	Heterologous titer	Remarks*
138	L. K.	XII	0	III	8	Protection 10^2 L.D., Pn. III. Also agglutinins (1 : 2) and protection (10 L.D.) Pn. VIII
14	T. A.	III	0	V	8	Protection 10^5 L.D., Pn. V
16	J. S.	III	0	VIII	4	No pneumococci in first sputum. Pn. III in second. Protection 10^5 L.D., Pn. VIII. None for Pn. III
6	D. Van F.	III VII	8 8	VIII	8	First sputum Pn. III. Pn. VII during relapse. Protection 10^3 L.D., Pn. III; 10^6 L.D. for Pn. VII; 10 L.D. for Pn. VIII
18	M. K.	III	2	VIII	16	Protection 10^3 L.D., Pn. VIII; none for Pn. III
3	A. S.	III	64	VII	4	Pn. VII agglutinins in 4th month
73	J. J.	II	16	VI	8	
9	W. L.	III X XVII	4 0 0	VIII	8	Pn. III first, Pn. X and XVII late in convalescence. Protection 10th day 10^4 L.D., Pn. III; O, Pn. VIII; 2 months later O, Pn. III; 10^4 L.D., Pn. VIII

* The titer is given as the greatest dilution in which floccular agglutination was observed.

L.D. = Lethal doses; Hem. Strep. = Hemolytic streptococcus.

absence of serum antibodies for each of these organisms is noted. Antibodies, if present, were usually demonstrated for only one of the organisms. The patients without pneumonia, from whose sputum more than one type of pneumococcus was obtained, are not shown, inasmuch as antibodies were not demonstrated in any of their sera.

Agglutinins and mouse protection in normal subjects

The sera of 26 hospital patients without recent infection or previous history of pneumonia and laboratory workers were tested for agglutinins with all of the types of pneumococci encountered in this investigation. Tests for mouse protection were carried out with Types I, II, III, IV, V, VII and VIII pneumococci. Agglutinins were found in only 2 in-

TABLE V

Absorption of type-specific agglutinins with homologous and heterologous type-specific strains of pneumococci *

Case number	Patient	Patient's type	Day of serum	Titer of homologous type agglutinins				
				Unabsorbed	After absorption with			
					Homologous types	Type I	Other types	Titer
107	D. P.	IV	18	16	0	8	XIV	16
106	A. F.	IV	26	8	0	8	XIX	4
108	J. A.	IV	25	4	0	4	XIX	4
118	M. C.	VII	22	32	0	16	IV	16
							XIV	32
111	C. C.	VII	15	16	0	8	XIV	16
114	J. B.	VII	19	64	0†	—	XIV	32
122	A. McN.	VII	24	64	8–0‡	64	XIX	64
117	A. McD.	VII	10	256	0	128	IV	256
115	J. Z.	VII	14	64	4	64	IV	32
113	A. F.	VII	27	512	64–0‡	512	XIX	256
112	A. G.	VII	11	128	0	128	XI	128
116	S. J.	VII	10	16	0†	64	—	—
123	J. O.	XII	13	8	0	8	VII	8
125	L. C.	XIV	11	32	4–0‡	8	VII	8
131	J. K.	XIX	10	16	0	16	VII	16
129	M. B.	XIX	30	4	0	4	IV	4
130	E. McD.	XIX	10	8	0	8	IV	8

* For absorption of Types II, III, V, and VIII agglutinins see (2) and (3).
† Same result with autogenous and one other strain.
‡ Negative result only after second absorption.

stances: one serum agglutinated Type VII and another Type XIX, each to 1:4 dilution only. Protection against 100 or more lethal doses of the various types occurred as follows: against Type I in one serum; Type II in 2; Type III in 3; Type IV in 5; Type V in 3; Type VII in 19 and Type VIII in 3 subjects. In 6 of the subjects the serum protected against as much as 100,000 lethal doses of Type VII pneumococci. There was no correlation between the presence of protection for any one type and that against any other. These results correspond to those previously obtained with some of these types (5).

DISCUSSION

Strains of pneumococci formerly included in Group IV are found in the nose and throat of most normal individuals (6) and may be obtained, with proper methods, from some patients with pneumonia in whom the Type I or II pneumococcus is the probable invader (7). They are usually less virulent for animals than are strains of Type I and II. Their relationship to lobar pneumonia, therefore, has been doubted (7), especially

TABLE VI

Antibodies in cases of pneumonia with mixed infections *

Case number	Patient	Patient's types	Days isolated	Corresponding antibodies	
				Present or absent	Days
24	J. O'B.	III, VIII	4	Absent	8, 10, 12, 23
		II	7	Present	10, 12
21	P. Ci.	III	11	Absent	12, 17, 24, 31
		V	16	Present	12, 17, 24, 31
90	L. N.	XI	3	Absent	4, 11, 31, 51
		V	21	Present	11, 31, 51
6	D. Van F.	III	22	Present	25, 31, 40
		VII	30	Present	40
9	W. L.‡	III	5	Present	3, 12
		X	82	Absent	3, 12, 83, 94
		XVII	86	Absent	3, 12, 83, 94
58	R. J.	VIII	6 (B.C.)†	Absent	7, 9, 15, 27
		XVIII	12	Absent	7, 9, 15, 27
109	M. S.	IV	1	Present	8
		B. mucosus capsulatus, Type A	1	Absent	8
127	B. McL.§	XVIII	9	Present	7, 9
		B. paratyphosus B	6, 7 (B.C.)	Present	7, 9, 24, 45, 60
139	H. D.	XIX	6, 8	Absent	7, 10, 18
		XX	6	Absent	7, 10, 18
		I	8	Present	7, 10, 18
140	G. O.‖	X	5, 11	Absent	5, 9, 10, 12
		I	8, 13 (B.C.)	Absent	5, 9, 10, 12
141	C. R.	XX	6	Absent	16, 24, 32
		I	15	Absent	16, 24, 32
62	E. H.¶	VIII	4	Present	9
		Strep. hem.; Staph. aur.	10 (P.M.)⁂	——	No tests

* Patients with 2 types of pneumococci or with other significant organisms. See also Table IV.

† (B.C.) = organism from blood culture only; all others are from sputum.

‡ Only *Streptococcus viridans* from sputum on 6th day. No significant organisms from sputum on 19th and 33d days.

§ Only *Streptococcus viridans* from sputum on 6th day.

‖ No pneumococci from sputum on 5th day. Died 13th day.

¶ Agglutinins and protection for Pn. VIII absent 5th day. Blood cultures negative 5th and 9th day. Autopsy cultures: Heart's blood: *Strep. hem.;* lungs: *Strep. hem.* and *Staph. aureus.*

⁂ (P.M.) = Cultured at postmortem.

in the favorable cases, since bacteremia in such cases is rare and lung punctures usually yield negative results (8).

In the present study, the sera of patients with infections associated with the newly classified types of pneumococci were tested for antibodies reactive with pneumococci of the homologous type and with most of the other types commonly encountered. The objects of these tests were (1)

to verify the antigenic, and, therefore, also the probable etiologic relationship of the newly classified types of pneumococci to the diseases with which they are associated, (2) to determine the specificity of this relationship, (3) to determine whether, in cases where the serum fails to react with the homologous type, another pneumococcus, not recovered from the patient by the ordinary methods, was responsible for the infection, and (4) to shed some light on the significance of the finding of more than one type in the same patient.

The results of these tests and of the absorption experiments indicate that, in most instances, these newly classified types of pneumococci are comparable to Types I and II in their antigenic relationship to the diseases in which they are found. The rarity with which Types I and II antibodies were demonstrated in these cases is worthy of note, since every serum was tested with these types. These types cannot be considered the exclusive causes of lobar pneumonia.

Protective antibodies, frequently of high titer, against some types of pneumococci, were demonstrated in many normal individuals. These findings, however, do not vitiate the results obtained in the pneumonia patients, since only the latter showed agglutinins and since the tests early in the disease were usually negative.

SUMMARY AND CONCLUSIONS

Two-thirds of the recovered pneumonia patients with 11 different types of pneumococci, including 9 of the newly classified types (Cooper), were found to have antibodies for the homologous type of pneumococcus. The results were similar in the patients with bronchopneumonia and those with lobar pneumonia. A number of fatal patients also had such antibodies late in the disease. Occasional patients with acute respiratory infections, without demonstrable pneumonia, also showed antibodies for the type of pneumococcus recovered from their sputa.

The specificity of the immune response was demonstrated by the failure, in such instances, to find antibodies for the other common types and by the specific absorption of the antibodies with pneumococci of the homologous and not the heterologous type.

A few cases were found to have antibodies against types other than the one isolated from the patient's sputum. Most of these represented cross-agglutinations with related types, particularly Types III and VIII.

In the patients in whom more than one type of pneumococcus was isolated, immunity was usually present against only one of these.

These findings tend to confirm the biological identity of the newly classified types of pneumococci. They lend further support to the etiological relationship of these types to the acute pulmonary infections with which they are associated.

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