IMMUNOLOGICAL REACTIONS OF PNEUMONIC PLEURAL FLUIDS*

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A study of the pathological and immunological changes that take place in the vicinity of an area of local inflammation may shed some light on the general protective mechanism of the body against infection. During the course of lobar pneumonia the pleura, being contiguous to the lung and sharing, at least in part, in its inflammatory reactions, offers an opportunity for such a study. Furthermore, with the recognition of the value of specific antipneumococcic serum in the treatment of certain types of lobar pneumonia, the problem of whether or not such serum reaches the lung and pleura becomes of practical importance. The present work consists, primarily, of the application of common bacteriological and immunological methods to a study of pleural fluids from patients with pneumococcic pneumonia. The cytological features of such fluids as revealed by observations with a supravital technique are included in a separate communication (1). It was hoped that some correlation between the immunological reactions and the fate of the infectious process might be found.

LITERATURE

Since Andral (2) and Laennec (3) first demonstrated clinically the existence of pleural effusions in lobar pneumonia, much has been written, especially in French literature, on this subject. Netter (4), however, published the first extensive study of the bacteriology of purulent pleural effusions and emphasized the frequency of the pneumococcus as an etiological agent. He showed that each kind of organism imparted to the fluid characteristics which were specific for the par-

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ticular organism involved. From 6 of the 109 fluids that this author studied, no organisms could be recovered either by cultural methods or by animal inoculations, using mice, rabbits, and guinea pigs. Pneumococci had previously been cultured from purulent pleural exudates by Friedländer (5), Netter (6), and Fränkel (7). More recently, Locke (8) collected the bacteriological findings in 478 cases of acute empyema, differentiating the various types among the pneumococci. The frequency of the pneumococcic etiology and the large predominance of Type I among the various types was clearly demonstrated.

Siems (9) quoted a case from Griffon (10) in which agglutination of pneumococci was demonstrated in a pneumonic pleural fluid by growing the organisms in it. Siems believed that the pneumococcic etiology of postpneumonic effusions could be demonstrated not only by culture but also by serological methods applied to the blood serum and to the fluid. In one instance he demonstrated that a pleural fluid had the power to agglutinate the patient's own strain of pneumococcus when it failed to agglutinate the laboratory strain. Cole (11) showed that empyema fluids contained no protective substances or agglutinins but that they inhibited the protective properties of antipneumococcic sera specifically. Floyd (12) obtained "positive precipitin tests" with empyema fluids and noted phagocytosis of pneumococci by the cells in these fluids.

Duyck (13) found fluid containing pneumococci in the pleural cavity of almost every one of his cases during the acute stage of lobar pneumonia, especially in the presence of septicemia. In the latter type of case he found large numbers of organisms in the fluid. In spite of this fact, these fluids, when injected fresh, not only failed to kill mice but were capable of protecting them against lethal doses of pneumococci. This protection he attributed to leucocytic ferments. He treated cases by removing this fluid and injecting it into the same patient subcutaneously. Curphy and Baruch (14) have utilized a similar principle in immunizing horses for the production of antipneumococcic sera.

Pepper (15) demonstrated agglutinins for *B. typhosus* in a pleural effusion accompanying typhoid fever. The agglutinin titer in the fluid was 1:800 and in the blood serum 1:1000. Courmont (quoted by LeDamany (16)) demonstrated agglutination of tubercle bacilli by pleural fluid, but the method was thought by LeDamany not to be infallible. Many other authors have demonstrated antigen and antibodies in tuberculous pleural effusions (17).

General immunological characteristics of exudates and transudates have been studied by a number of workers, some of whom examined pneumonic pleural fluids. Hemolysins and complement have been found in exudates but not in transudates (18), and the cell-free exudates have been shown to be bactericidal for many organisms, including the pneumococcus (19).

Numerous workers have made studies of absorption from the pleural cavity. The literature, however, on the passage of immune substances from the blood into the pleural cavity or other closed cavities is scant. The most significant observations have been made on the cerebrospinal fluid in animals. Mott (20) found that

most drugs and tetanus toxin do not pass from the blood into the cerebrospinal fluid, whereas materials injected into the spinal canal appear rapidly in the blood. Flexner and his coworkers (21) were unable to detect neutralizing substances for the virus of poliomyelitis in the cerebrospinal fluid of actively immunized monkeys. They also failed to demonstrate agglutinins for meningococci in the fluid of actively or passively immunized normal rabbits. If, however, they produced an aseptic meningitis by injecting saline or normal horse serum into the subarachnoid space, they could demonstrate these antibodies. Freund (22) found that the cerebrospinal fluid of animals immunized with killed typhoid bacilli contains agglutinins for these organisms but the titer is very low compared to that of the blood serum. Normal animals passively immunized by the intravenous injection of typhoid immune serum were also shown to have typhoid agglutinins in their cerebrospinal fluid after a lapse of several hours. He explained the negative results previously mentioned by the low titer of antibody in the fluid.

Menkin reviewed the literature on the dissemination of substances from the site of injection (23) and showed that foreign protein introduced in the circulating blood of a rabbit accumulates in an inflamed area in greater concentration than in normal tissues (24).

Materials and Methods

31 pleural fluids from 19 patients with typical lobar pneumonia were studied, and 4 fluids from 3 patients with other diseases were studied for controls. A pneumococcus was obtained in each of the former cases from the sputum or blood culture or both. Type I pneumococci were obtained in 10 cases, Type II in 5 cases, Type III in 3 cases, and Type XVIII in 1 case.[1] Felton's bivalent (Types I and II) concentrated antibody solution was used in the treatment of 5 Type I and 3 Type II cases. 3 patients were treated with sera prepared by the Felton method from normal horse serum (Case 7) and from antimeningococcus serum (Cases 16 and 17). In 1 instance (Case 18) there was an opportunity to study fluid both before and after serum treatment.[2] All of the therapeutic sera were administered intravenously.

The fluids were secured, under aseptic precautions, by thoracentesis, choosing a point in the area of maximum percussion dulness. A solution of 1 or 2 per cent novocaine was injected through a 24 gauge needle to anesthetize the skin and underlying tissues, including the parietal pleura, a total of 1 or 2 cc. being used in the process. The fluid was then aspirated into a dry syringe through an 18 or 20

[1] All of the sera used in typing were obtained through the courtesy of Dr. William H. Park, Laboratories of the New York City Department of Health.

[2] The therapeutic sera were prepared and supplied through the courtesy of Dr. L. D. Felton, Department of Preventive Medicine and Hygiene, Harvard Medical School, and of Dr. Benjamin White, Antitoxin and Vaccine Laboratory, Massachusetts State Department of Public Health.

gauge needle. Some of the fresh fluid was then inoculated into rabbit's blood broth and streaked on an agar plate containing rabbit's blood, and 1 cc. was injected into a mouse for routine pneumococcus typing. The remainder was allowed to clot, then centrifuged and the supernatant fluid stored in the ice box for later studies. In most instances, venous blood for culture and for the determination of circulating antibodies was obtained at about the time of the thoracic puncture.

The hydrogen ion concentration was obtained with the use of indicators by comparison with standard buffer solutions as recommended by Clark (25).

Antibody Determinations.—The *protection* titer of the fluids was determined by a mouse technique similar to that employed by Dochez (26). *Agglutinins* for pneumococci were shown by a technique similar to that employed by Tillett and Francis (27). The meningococcic antigen used in 2 instances was a formalized saline suspension of 1 of the strains employed in the preparation of the therapeutic sera. The agglutinations of this antigen were carried out by heating at 55°C. for 12 to 18 hours. *Precipitinogens* were demonstrated by adding 0.2 cc. of undiluted antipneumococcic typing sera[3] to equal amounts of the fluids, incubating for 2 hours at 37°C. and reading after storage overnight in an ice box. *Precipitins* were demonstrated in a similar manner using 1:1000 solutions of the specific polysaccharides of Types I, II, and III pneumococci. No attempt was made to obtain the exact titer of precipitin and precipitinogen. The presence of *horse serum* was detected in a similar manner by the precipitin reaction except that the anti-horse rabbit serum[4] was used in 1:2 dilution and varying dilutions of the fluids and sera from the patients were used in order to obtain quantitative results. The anti-horse rabbit serum used in this manner gave a precipitate with normal horse serum up to a dilution of 1:80,000 of the latter.

Results of Immunological Studies

The results of the various bacteriological and immunological studies of the pleural fluids and of the corresponding blood sera are given in Table I. Cultures in rabbit's blood broth and on blood agar plates, as well as mouse inoculations, were negative in the controls and in 18 of the pneumonic fluids obtained from 14 patients. The remaining 13 fluids were infected. Pneumococci corresponding in type to the causative agent of the pneumonia were recovered from 11 fluids, whereas hemolytic streptococci were present as secondary invaders in 2 fluids from Case 15.

From a study of Table I it will be seen that neither antigen nor anti-

[3] Obtained through the courtesy of Dr. Augustus B. Wadsworth, Laboratories of the New York State Department of Health.

[4] Prepared by Dr. T. F. Hunnicut.

Explanation of Table I

Day of puncture and *Day of crisis.* The figures represent the number of days after the onset of the pneumonia.

Days under the heading *Serum treatment* represents all the days during which treatment was given (the figures are inclusive).

Horse serum and *Agglutinins.* The figures represent the weakest dilution of fluid or blood sera in which reactions were observed.

Protection. The titer is represented thus:

\+ = protection against 10 or 100 lethal doses.
\++ = protection against 1000 or 10,000 lethal doses.
\+++ = protection against 100,000 or 1,000,000 lethal doses.
\++++ = protection against 10,000,000 or more lethal doses.

Precipitins and *Precipitinogens.* +, + +, etc., represent the intensity of the reaction, from +, indicating a definite but slight precipitate with a cloudy supernatant fluid to ++++, indicating a clear supernatant fluid and a heavy sediment.

Abbreviations

D.	= died.	*Str. h.*	= *Streptococcus hemolyticus.*
P.M.	= post mortem.	Mening.	= meningococcus.
Neg.	= negative.	I	= Pneumococcus Type I.
—	= not done.	II	= Pneumococcus Type II.
Pn.	= pneumococcus.	III	= Pneumococcus Type III.

Case No.	Pneumococcus type	Day of crisis	Day of puncture	Serum treatment		pH	Culture	Mouse inoculation	Pleural fluids							
									Concentration of antig							
									Horse serum	Protection			Agglutinins			—
										I	II	III	I	II	III	
																Sterile
																I. Patients receiving
				cc.	*days*											
3	III	10	3	0	—		Neg.	Neg.	—	—	—	—	0	0	0	
12	I	5	4	0	—	8.0	Neg.	Neg.	—	—	—	—	0	0	0	
2	I	5	5	0	—	7.2	Neg.	Neg.	—	0	—	+	0	0	2	
9	I	10	10	0	—	8.0	Neg.	Neg.	—	+	0	—	0	0	0	
			18			7.7	Neg.	Neg.	—	++	0	—	8	0	0	+
5*	III	7	21	0	—	7.6	Neg.	Neg.	—	0	+++	++	0	2	8	
																II. Patients treated with Felton's con
6	II	4	4	322	2–4	7.8	Neg.	Neg.	400	—	—	—	16	32	0	+
14	I	3	10	70	2	8.0	Neg.	Neg.	100	++	+	—	0	0	0	
11	II	6	9	346	2–5	7.6	Neg.	Neg.	50	++	+	—	0	0	0	
4	I	5	33	174	4–5	—	Neg.	Neg.	—	+	++	—	0	0	0	
15	II	—	9	264	3–5	8.0	Neg.	Neg.	200	—	++	—	16	32	0	+
			13			8.0	Neg.	Neg.	100	0	0	—	2	8	0	+
10	I	18	14	183	13–14	8.2	Neg.	Neg.	400	+++	++	0	0	8	0	
			17			8.0	Neg.	Neg.	400	+	++	0	8	32	0	
																III. Patients treated
7	I	—	15	53	2	8.0	Neg.	Neg.	50	++	0	0	2	0	0	0
16	XVIII	7	5	127	2–3	7.7	Neg.	Neg.	200	—	—	—	Mening. 1:50			—
			7			8.0	Neg.	Neg.	50	—	—	—	Mening. 1:50			—
17	II	3	4	75	2	7.8	Neg.	Neg.	100	—	—	—	0	0	0	0
																Infected
																I. Patients receiving
18	I	D.4	2	0	—	7.4	Pn. I	Pn. I	—	0	0	0	0	0	0	0
13	III	D.8	7	0	—	7.8	Pn. III	Pn. III	—	—	—	0	0	0	0	0
1	II	D.18	17	0	—	6.4	Pn. II	Pn. II	—	—	—	0	0	0	0	0
8	I	—	12	0	—	5.6	Pn. I	Pn. I	—	0	—	—	0	0	0	0
			15			5.6	Pn. I	Pn. I	—	0	—	—	0	0	0	0
																II. Patients treated with Felton's con
18	I	D.4	P.M.	305	3–4	7.2	Pn. I	Pn. I	200	0	0	0	0	8	0	0
15	II	—	22	264	3–5	7.2	*Str. h.*	*Str. h.*	10	0	+	0	0	4	0	+
			23			5.4	*Str. h.*	*Str. h.*	100	0	0	0	0	2	0	0
19	I	—	10	140	4–5	7.2	Pn. I	Pn. I	400	0	++	—	0	8	0	0
			13			7.0	Pn. I	Pn. I	400	0	+	—	0	8	0	0
			14			6.3	Pn. I	Pn. I	200	0	++	—	0	4	0	0
			19			6.3	Pn. I	Pn. I	200	0	++	0	0	2	0	0
																III. Patients treated
7	I	—	26	53	2	6.4	Pn. I	Pn. I	—	—	—	—	—	0	0	
																Non-Pneum
20	Rheumatic pleurisy					7.8	Neg.	Neg.	—	0	0	0	0	0	0	0
21	Cardiac decompensation					7.8	—	—	—	—	—	—	0	0	0	0
22	Pleurisy with effusion					8.2	Neg.	Neg.	—	—	—	0	0	0	0	0
						7.8	Neg.	Neg.	—	—	—	0	0	0	0	0

* This patient received skin tests with the specific polysaccharides of Types I, II, and III pneumococci.

LE I

nic Pleural Fluids and of the Corresponding Blood Sera

en and antibodies						Blood culture	Blood serum									
							Concentration of antigen and antibodies									
Precipitins			Precipitinogens				Horse serum	Protection			Agglutinins			Precipitins		
I	II	III	I	II	III			I	II	III	I	II	III	I	II	III
Fluids																
no serum treatment																
0	0	0	0	0	0	Neg.	–	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	Neg.	–	–	–	–	–	–	–	–	–	–
0	0	+	–	–	–	Neg.	–	+	0	+	0	0	8	0	0	0
0	0	0	0	0	0	Neg.	–	++	0	–	8	0	0	++	0	0
+	0	0	0	0	0	Neg.	–	++	0	0	8	0	0	++	0	0
0	+++	+	0	0	0	Neg.	–	+	+++	+++	0	64	64	0	+++	++
centrated antibodies (Types I and II)																
++	+++	0	0	0	0	Neg.	1600	+++	++	–	16	32	4	+++	+++	0
+	0	0	0	0	0	Neg.	–	–	–	–	–	–	–	–	–	–
0	0	0	0	0	0	Neg.	–	–	–	–	–	–	–	–	–	–
0	0	0	–	–	–	Neg.	–	+	++	–	0	0	0	0	0	0
+	++	0	0	0	0	Neg.	1600	0	0	–	–	–	–	–	–	–
++	+	0	0	0	0	Neg.	1600	0	+	–	4	8	0	+	+	0
0	0	0	0	0	0	Pn. I	1600	++++	++	–	32	64	0	++	+++	0
+	+	0	0	0	0	Neg.	1600	+++	++	–	16	32	0	++	+++	0
with non-specific sera																
	0	0	0	0	0	Neg.	–	+	–	–	4	0	0	–	–	–
	–	–	–	–	–	Neg.	800	–	–	–	Mening. 1:1600			–	–	–
	–	–	–	–	–	Neg.	800	–	–	–	Mening. 1:1600			–	–	–
	0	0	0	0	0	Neg.										
Fluids																
no serum treatment																
	0	0	++++	0	0	Pn. I	–	0	0	0	0	0	0	0	0	0
	0	0	0	0	++++	Pn. III										
	0	0	0	++++	0											
	0	0	++++	0	0											
	0	0	++++	0	0											
centrated antibodies (Types I and II)																
	0	0	++++	0	0	Pn. I	400+	+++	+++	+	32	64	4	0	++++	0
+	+	0	0	0	0	Neg.	1600	0	–	–	2	8	0	+	0	0
	0	0	0	0	0	Neg.	800	–	+	–	2	4	0	0	0	0
	0	0	++++	0	0	Neg.	400	++++	++	–	8	4	0	0	0	0
	0	0	++++	0	0	Neg.	400	+++	+	–	2	4	0	0	0	0
	0	0	++++	0	0	Neg.										
	0	0	++++	0	0	Neg.										
with non-specific sera																
0	0	0	++++	0	0	Neg.										
onic Fluids																
	0	0	0	0	0	Neg.	–	0	0	0	0	0	0	0	0	0
	0	0	0	0	0											
	0	0	0	0	0	Neg.	–	–	–	0	0	0	0	0	0	0
	0	0	0	0	0	–	–	–	–	0	0	0	0	0	0	0

bodies could be demonstrated by precipitin tests in the sterile pneumonic fluids during the acute stage of the disease when no specific sera had previously been given. At this time antibodies could not be found in the blood of these patients. At the time of crisis or later antibodies corresponding to those found in the blood sera were demonstrated in the sterile fluids of the untreated cases. Sterile fluids obtained from patients who had received therapeutic sera were shown to contain horse serum and antibodies corresponding to those of the therapeutic sera. Similar antibodies were found in the blood of these patients.

Fluids infected with pneumococci always showed homologous soluble specific substance, as demonstrated by the specific precipitate with the homologous antipneumococcic serum. In the cases that were given antisera containing the homologous and heterologous antibodies, horse serum and heterologous antibodies could be demonstrated along with homologous antigen. The blood, however, contained both homologous and heterologous antibodies. When infection was due to a secondary invader (Case 15), homologous pneumococcic antibodies could be demonstrated in the pleural fluid. Occasionally no antibodies could be demonstrated in fluid when the serum showed a good titer. In one instance (Case 7), when a fluid, sterile early in convalescence, later became infected, actively acquired antibodies for the homologous type were demonstrated on the former occasion and antigen, but no antibodies, on the latter. The tests on the control fluids in the non-pneumonic patients were entirely negative.

The quantitative relationships between the antibodies in the fluids and those in the corresponding sera were not always constant. The protective titer is difficult to compare, but in half of the instances in which protection for the same organisms was demonstrated in both the serum and fluid the titer was found to be the same. In the remaining instances, with one exception in which more protection was found in the fluid, the serum protected in only 1 or 2 dilutions higher (against 10 to 100 times the number of lethal doses of pneumococci) than the corresponding fluid.

The ratio of the agglutinin titers in the fluids to those in the sera varied from 2:1 to 1:32. In 19 instances where corresponding agglutinins were demonstrated in both fluid and serum, the ratio of the titer

in the fluid to that in the sera was 1:1 or 1:2 in 10 instances; in 4 others, it was 1:4 or 1:8; in 3, it was 1:32; and, in the remaining 2, it was 2:1.

The titer of horse serum, using the same anti-horse rabbit serum throughout, showed similar relations between the fluid and blood. Of 12 instances where a comparison was possible the ratio of the titer in the fluid to that in the serum was 1:1 in 2 instances; 1:4 in 5; 1:8 or 1:16 in 4; and 1:160 in a single instance.

DISCUSSION

Numerous studies have been made on absorption of fluids, fats, particulate matter, and bacteria from the pleural cavity (28). Little mention, however, has been made of the passage of materials from the circulation into the pleural cavity. Furthermore, as far as could be ascertained, the only direct reference to the presence of specific antibodies in pneumonic pleural fluids are those referred to above (9, 10, 13). It has here been demonstrated that antibodies actively produced by the patient or passively introduced into the blood stream appear in the pleuritic effusion of patients with lobar pneumonia, whether such effusions are sterile or infected. As a rule, the concentration of horse serum and of antibodies in the fluids has been either the same or, more commonly, lower than in blood serum. This is not in accord with the findings of Couraux (29) and Paraskevopoulos (30), who each found more antibodies against the tubercle bacillus in pleural fluid than in the blood, but agrees with the finding in Pepper's case (15) where the ratio of the titer of typhoid agglutinins in the fluid to that in the serum was 4:5. Pinner and Moerke (17) found that the blood averaged 27 per cent more globulin than the tuberculous pleural effusions which they studied, although there were individual instances where the fluid contained more globulin than the serum. These findings are significant since the intimate association of antibodies with serum globulin is well recognized.

These observations cannot, of course, be interpreted as indicating that the normal pleura is permeable to immune bodies. That injury to the pleura through the inflammatory processes in the lung renders it more permeable is very likely in view of the findings of Flexner and his coworkers (21) in relation to the passage of immune substances into the cerebrospinal fluid.

It appears that the presence of antibodies in the effusion does not necessarily prevent its subsequent infection by the homologous organisms. When such infection does occur, soluble specific substance appears in the fluid and the homologous antibody can no longer be demonstrated; but such horse serum and heterologous antibodies as may have been introduced can still be found. This agrees with the findings of Cole (11) already mentioned.

SUMMARY AND CONCLUSIONS

Pleuritic exudates from patients with lobar pneumonia may be sterile or infected. Sterile fluids, at or about the time of crisis, contain actively acquired antibodies similar to those in the blood serum. Infected fluids do not contain such antibodies, presumably because of the presence in them of large amounts of soluble specific substance. Sterile fluids from patients treated with immune sera have both horse serum and antibodies similar to those injected. Infected fluids from serum-treated cases contain horse serum and such heterologous antibodies as were contained in the therapeutic sera together with homologous soluble specific substance. The concentration of horse serum and antibodies in pneumonic fluids is usually the same or somewhat less than that of the corresponding blood sera.

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