EXPERIMENTAL PNEUMONIA IN GUINEA PIGS

II. Effect of Anti-autolysate Sera on Pneumococcus Pneumonia in Guinea Pigs

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In a previous communication (1) it was shown that extensive pneumonia associated with unrestrained multiplication of the organisms may be produced regularly in guinea pigs by the intratracheal injection of sublethal doses of certain toxic autolysates (pneumotoxin) mixed with sublethal doses of living pneumococci. Neither the pneumococci alone nor the toxic autolysate alone produced a comparable condition.

In the present paper, experiments are reported which demonstrate (a) that under certain conditions, anti-autolysate sera prepared in rabbits or horses by immunization with sterile filtrates of pneumotoxin prevent the development of pneumonia in animals inoculated with mixtures of pneumococci and autolysate; (b) that the protection against the development of pneumonia exhibited by these sera is heterologous, at least as regards Types I and II; (c) and that certain anti-pneumococcus horse sera used in the treatment of pneumonia in man contain either no heterologous pneumonia-preventing antibodies or slight amounts only.

EXPERIMENTAL

Methods

Immunization of Animals.—Rabbits were immunized over a period of 2 or 3 months by intracutaneous, subcutaneous or intravenous injections of sterile Berkefeld filtrates of the toxic autolysates from either Type I or Type II pneumococci, each rabbit being inoculated with the autolysate of one type only. The horses were immunized with subcutaneous injections of either mixed autolysate filtrates from Pneumococcus I, II and III or with mixed autolysates and the living organisms of all three types. The anti-autolysate sera from the horses were concentrated and refined by the usual methods and in this form tested for their

pneumonia-preventing properties. Normal rabbit or normal horse sera were used as serum controls in the work.

The pneumococcus strains—Types I and II—were the same as those used in our previous work. A strain of Pneumococcus III was also used. This was isolated from a patient with pneumonia in the Presbyterian Hospital in May, 1928. The method of preparing the toxic autolysates has been described in previous papers (1, 2). Only those filtrates from Types I, II and III were used which would kill guinea pigs within 24 hours in doses of 0.2 cc. when injected intratracheally.

Young guinea pigs weighing from 190 to 250 gm. were used, and owing to the fact that the susceptibility to the autolysate usually varies inversely with the weight of the pig, it was found important to have the animal in each experiment as nearly the same weight as possible.

The injections of serum were given intraperitoneally 14 to 24 hours before the intratracheal infecting dose of pneumococci and autolysate. At first, the rabbit immune sera were given intraperitoneally 24 hours previous to the intratracheal dose of organisms and pneumotoxin, but some of these sera caused the development of anaphylactoid reactions immediately after the intratracheal inoculations. In later experiments the serum injections were made 14 to 18 hours before the intratracheal dose and in this way the anaphylactoid reactions were avoided. Our anti-pneumotoxic horse sera have never given anaphylactic sensitization, even when the sera were given 24 hours before the infecting dose of toxin and pneumococcus (3).

Intratracheal Injections.—In carrying out the pneumonia preventing tests, the proper adjustment of the doses of pneumotoxin and pneumococcus is essential. The dose of pneumococcus used was about one half that which would cause death from septicemia when injected intratracheally without mixture with autolysate. The same principle governs the dose of autolysate used in these experiments. The amount employed was one half the quantity necessary to kill a pig of approximately the same weight in 24 hours. With experience as to the virulence of the culture and the strength of the autolysate, it is comparatively easy to obtain the proper amounts of autolysate and pneumococci for these experiments.

The intratracheal injections were carried out as follows:—An 18–24 hour broth culture of the pneumococcus was diluted with broth to the strength desired. This tube, containing the diluted pneumococcus culture, was kept in ice water throughout the experiment.

A tube containing the toxic filtrate was chilled, the vaseline seal opened, and the filtrate pipetted into a narrow, chilled tube. This tube, containing the toxic autolysate, was also kept in ice water throughout the experiment. Just before the injection, 0.2 cc. of the diluted pneumococcus culture and 0.2 cc. of the autolysate were pipetted into another iced tube, mixed, and 0.2 cc. of the mixture was drawn into an iced syringe and immediately injected intratracheally into a guinea pig. A fresh mixture was always prepared for each injection and the same precautions as to chilling, etc., were carried out with both control preparations consisting of pneumococcus and broth, and autolysate and broth.

Protective Action of Anti-autolysate Sera Against the Pneumonia Caused by Pneumococci Plus Toxic Filtrates of Homologous Type

Table I demonstrates the protective action of two preparations of concentrated anti-autolysate horse sera against pneumonia caused by mixtures of toxic autolysate Type I and living pneumococcus Type I.

This experiment shows that two pigs injected intraperitoneally with 1 cc. each of normal horse serum 18 hours previous to the inoculation of

TABLE I

Action of Anti-autolysate Sera in the Prevention of the Pneumonia Caused by Pneumococci and Toxic Filtrates of Homologous Type

Pneumococcus culture Type I; very slight growth. Autolysate from Type I. 0.2 cc. pneumococcus, Type I culture + 0.2 cc. autolysate. 0.2 cc. inoculated intratracheally; Concentrated autolysate horse 30336. 9/24/28. Concentrated autolysate horse 30337. Normal horse serum.

No.	Wt.	Intraperitoneal injection 10/1	Intratracheal injection 10/2	Symptoms	Died or survived	Cultures		Extent of lung consolidation
						Heart	Lung	
55	238	1 cc. normal horse serum	Pn. + autolysate	+++	D. 3 days	+++	+++	++
58	230	1 cc. 30336	Pn. + autolysate	0	S.	−	−	−
69	232	1 cc. 30337	Pn. + autolysate	0	S.	−	−	−
61	226	—	Broth + autolysate	+	S.	−	−	−
64	224	—	Pn. + broth	0	S.	−	−	−
54	230	1 cc. normal horse serum	Pn. + autolysate	+++	D. 2 days	+++	+++	+++
56	232	1 cc. 30336	Pn. + autolysate	0	S.	−	−	−
65	238	1 cc. 30337	Pn. + autolysate	0	S.	−	−	−
57	222	—	Broth + autolysate	+++	S.	−	−	−
60	222	—	Pn. + broth	0	S.	−	−	−

pneumococci and toxic autolysate died in 2 and 3 days respectively, with pneumonia and septicemia; while four pigs previously prepared with 1 cc. each of two preparations of concentrated anti-autolysate sera remained well. The difference in the appearance of the protected and unprotected animals was striking. 18 hours after the intratracheal injection of pneumococcus and autolysate the two pigs which had received the normal serum were severely dyspnoeic and appeared extremely sick, while the pigs which had received the anti-autolysate

serum had normal respiration and appeared well. The two pigs which were injected with the autolysate and broth were dyspnoeic, one being desperately sick for 8 days but finally recovering; while the two which were injected with the pneumococcus broth culture remained well.

Serum preparation 30336 was obtained from two horses immunized with toxic autolysate filtrates and contained no specific protective substances. Serum preparation 30337 was obtained from two horses immunized with toxic autolysates and living pneumococci and protected Pn. $1,10^{-4}$. The question of the importance of pneumococcus specific protective properties in these experiments will be considered later.

Protective Action of Anti-autolysate Sera on the Pneumonia Caused by Pneumococci Plus Toxic Filtrates of a Heterologous Type

That the pneumonia preventing action of our anti-autolysate sera is heterologous as regards pneumococcus Types I and II has been demonstrated conclusively many times. These experiments were carried out with anti-autolysate sera produced in rabbits by the immunization with autolysates of one type only (Type I or Type II). The experimental pneumonia in each instance was caused by the autolysate and pneumococci of heterologous strains (Type I or II). Table II demonstrates the preventive action of Type I anti-autolysate serum against pneumonia caused by pneumococcus and autolysate Type II, and is self explanatory. The same pneumonia-preventing action has been demonstrated for rabbit anti-autolysate Type II sera against the pneumonia caused by injections of Pneumococcus I toxin and living pneumococcus I.

These experiments therefore demonstrate that our anti-pneumotoxic sera are definitely protective against pneumonias caused by mixtures of pneumococci and autolysates of a heterologous strain.

Action of the Anti-pneumococcus Sera Used in the Treatment of Human Pneumonia for the Pneumonia-preventing Heterologous Substances

In testing the anti-bacterial pneumococcus sera for the presence of the pneumonia-preventing substances which have been shown to be present in our anti-autolysate sera, it was important to rule out the

effect of the specific protective substances which all these anti-bacterial sera contain. To accomplish this, it was only necessary to test out the efficacy of these sera against the pneumonia produced by a pneumococcus and autolysate from a heterologous strain.

Table III gives one of our experiments of this kind. It is seen that the serum concentrated by the Felton method shows no heterologous pneumonia-protective powers; the serum produced by the Zinsser

TABLE II

Action of an Anti-autolysate Serum in Preventing Pneumonia Caused by Pneumococci Plus Toxic Filtrates of a Heterologous Type

Pneumococcus II—good growth. Diluted 1–10 with broth Autolysate II. 0.3 cc. Pn. II dilution + 0.1 cc. autolysate. 0.2 cc. mixture injected. Controls: (a) 0.3 cc. Pn. + 0.1 cc. broth—0.2 cc. injected. (b) 0.3 cc. broth + 0.1 cc. autolysate—0.2 cc. injected. Rabbit serum 7–54, Anti-autolysate I.

No.	Wt.	Intraperitoneal injection 5/9	Intratracheal injection 5/10	Symptoms	Died or survived	Cultures		Extent of lung consolidation
						Heart	Lung	
10-11	204	2 cc. normal rab.	Pneumococcus + autolysate	+++	D. 40 hrs.	+++	+++	+++
8-74	202	2 cc. 7–54	Pneumococcus + autolysate	0	S.	−	−	−
8-88	204	2 cc. 7–54	Pneumococcus + autolysate	0	S.	−	−	−
8-89	210	—	Pneumococcus + autolysate	+++	D. 40 hrs.	+++	+++	+++
10-09	206	—	Pneumococcus + autolysate	+++	D. 18 hrs.	+	+++	+++
8-87	200	—	Broth + autolysate	0	S.	−	−	−
10-06	206	—	Pneumococcus and broth	0	S.	−	−	−

method shows a slight amount (protected one of the two pigs injected), while the rabbit anti-autolysate serum gives perfect protection. The same results were obtained in another experiment carried out in a similar way. Still another anti-pneumococcus serum used in treating human pneumonia was tested for heterologous pneumonia-preventing substances, and proved to be even weaker in these properties than the serum produced by the Zinsser method.

These experiments demonstrate how poor in heterologous anti-pneumonia antibodies the ordinary anti-bacterial pneumococcus sera are. The explanation for the complete absence of these antibodies in the serum produced by the Felton method is probably to be found in its method of preparation, which concentrates the specific protective

TABLE III

Action of Certain Anti-pneumococcus Sera Used in the Treatment of Human Pneumonia in Preventing the Pneumonia Caused by Autolysate and Pneumococci of a Heterologous Type

Pneumococcus Type II; slight growth on broth. Autolysate Type II. 0.2 cc. Pn. culture + 0.2 cc. autolysate. 0.2 cc. injected. Controls: Pn. + broth and autolysate and broth. Sera: Felton's antibodies I; Zinsser anti-pneumococcus serum; Rabbit 7–54; Anti-autolysate I.

No.	Wt.	Intraperitoneal injection 3/2	Intratracheal injection 3/3	Symptoms	Died or survived	Cultures		Extent of lung consolidation
						Heart	Lung	
7-59	200	2 cc. Felton antibodies	Pn. + autolysate	+++	D. 2 hrs.	0	+	++
7-77	200	Same	Pn. + autolysate	+++	D. 2 days	++	+++	+++
7-67	200	2 cc. Zinsser serum	Pn. + autolysate	0	S.	−	−	−
7-78	205	Same	Pn. + autolysate	+++	D. 40 hrs.	++	+++	+++
7-73	200	2 cc. 7–54	Pn. + autolysate	++	S.	−	−	−
7-66	200	2 cc. 7–54	Pn. + autolysate	+	S.	−	−	−
7-69	195	2 cc. normal horse	Pn. + autolysate	+++	D. 18 hrs.	+++	+++	+++
7-75	200	2 cc. normal rabbit	Pn. + autolysate	+++	D. 5 hrs.	0	+	++
7-71	190	—	Pn. + autolysate	+++	D. 40 hrs.	+++	+++	+++
7-80	195	—	Broth + autolysate	+	S.	−	−	−
7-74	190	—	Pn. + broth	0	S.	−	−	−

bodies in the globulin fraction of the serum and discards that part of the serum which contains neutralizing antibodies (the pseudoglobulin fraction.)

It is logical to suppose that a serum containing specific protective antibodies, would have a preventive effect by its antibacterial properties, on the pneumonia caused by the homologous type of organism and autolysate; and that this preventive effect would correpond closely to

the amounts of specific protective substances which these sera contain. This was found to be the case. The serum produced by the Felton method, containing 1000 protective units, protected 75 per cent of the animals injected; the Zinsser serum protected 50 per cent, and the other Anti-pneumococcus I serum only delayed death in one instance. To save space, a table giving the details of these experiments is omitted.

Protective Effect of Normal Rabbit or Horse Serum

Occasionally normal rabbit serum protects against the pneumonia produced by a mixture of living pneumococcus and autolysate. We believe this to be due to a non-specific reaction induced by the foreign serum in a small percentage of the injected pigs (about 8 per cent). Horse serum occasionally shows this non-specific protective action. Stillman had the same experience in his pneumococcus work in mice. He found that normal rabbit serum injected intraperitoneally in mice sometimes protected them from pneumococcus infections. These rabbit sera contained no pneumococcus specific protective substances (4).

The Content of Pneumococcus Specific Protective Substances in the Anti-autolysate Sera

That the pneumonia-preventing properties of anti-autolysate sera are not related to the pneumococcus specific protective substances was demonstrated conclusively by the fact that the anti-autolysate sera are equally effective against a pneumonia due to the toxin and pneumococci of either a heterologous or homologous strain. We also titrated all our rabbit anti-autolysate sera and in no instance did they show the presence of specific protective substances for mice. Neither were there specific protective substances in horses immunized with the autolysate filtrates, but protective substances for pneumococci Types I and II were present in small amounts in the sera obtained from the horses which had been injected with living pneumococcus Types I, II and III, in addition to the autolysates.

DISCUSSION

The experiments reported in this paper appear to indicate that under certain conditions the sera produced in rabbits or horses by immuniza-

tion with Berkefeld filtrates of certain anaerobically produced autolysates of pneumococcus Type I or II protect against the pneumonia caused in guinea pigs by the intratracheal injection of mixtures of living pneumococci and toxic autolysates. Since the sera contain no specific anti-bacterial antibodies, it is evident that their protective action must be due to some other form of immune body. The immune body present differs also from the specific anti-bacterial bodies in that it is apparently identical for both Types I and II of the pneumococci and is therefore not type specific.

Whether this lung-toxic substance is produced in spontaneous pneumonia, or in the experimental pneumonia produced by intratracheal injections of pneumococci alone—as in the dog or monkey—is a question which cannot be answered at present. The demonstration that a toxic substance of this nature is present in these conditions would help to explain some of the obscure features of pneumococcus pneumonia in animals and man.

In the production of curative sera for lobar pneumonia, most of the efforts have been devoted to obtaining sera with a high content of specific protective antibodies against living organisms, but Cecil and Blake (5) have found that the serum of a monkey may be completely free of any specific protective substance and yet be highly immune to pneumonia. The reverse was also found to occur. A monkey whose serum may protect mice against 100 to 1000 M.L.D.'s of pneumococci may still be susceptible to the homologous pneumococcus pneumonia.

In connection with this work, it is also of interest to note that Cecil and Blake demonstrated that resistance to experimental pneumonia in monkeys is to some degree species specific as regards pneumococci Types I, II and III (6). Stillman (7) has come to the same conclusion in his work on the production of pneumococcus pneumonia in mice by the inhalation method. He found that mice which had been rendered immune to one type of pneumococcus (Type I) by the inhalation method, may show an increased resistance locally when exposed to infection by the same method with organisms of another type (Type II).

CONCLUSIONS

1. Anti-pneumotoxic sera prepared in rabbits or horses by immunization with sterile filtrates of the pneumotoxin, under certain condi-

tions protect against the pneumonia caused by the intratracheal injections of mixtures of living pneumococci and toxic autolysates.

2. The protection against the development of pneumonia is heterologous, at least as regards Type I, Type II, *viz.:* an anti-autolysate serum prepared by the immunization with a pneumotoxin from one type of pneumococcus will prevent the development of pneumonia caused by the injection of pneumococci and autolysate from another type.

3. Certain anti-pneumococcus horse sera used in the treatment of pneumonia in man, either contain no heterologous pneumonia-preventing antibodies or slight amounts only. These sera, however, protect against the pneumonia produced by injections of pneumococci and pneumotoxin of the homologous strain, the degree of protection depending on the amount of specific protective substances such sera contain.

4. Anti-pneumotoxic sera produced in rabbits or horses by the injection of sterile Berkefeld filtrates of the toxic autolysates contain no pneumococcus specific protective substances.

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