

TESTS FOR PNEUMOCOCCUS HYPERSENSITIVENESS IN
DOGS AFTER RECOVERY FROM EXPERIMENTAL
PNEUMOCOCCUS LOBAR PNEUMONIA

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There are certain clinical and experimental data indicating that allergy or hypersensitiveness may play an important rôle in the production of pneumococcus lobar pneumonia.

Lauche (1) has pointed out that infants under 4 months of age seldom contract lobar pneumonia. This apparent insusceptibility he attributes to their not yet having had opportunity to become sensitized to the pneumococcus. He contends that as individuals grow older they come into contact with the pneumococcus or even contract mild unrecognizable infections until an occasion may occur when the microorganisms invade the lung tissue and produce a marked infection. The basis of this infection in a sensitized individual is the localization of pneumococci in the hilum lymph nodes or hilum, producing an inflammation which obstructs the lymph flow and reverses its direction toward the periphery thereby carrying the organisms into the lung parenchyma. Lausche believes that the rapid onset of the pneumonic infection is an allergic phenomenon occurring in a sensitized individual.

Wadsworth (2) was the first to note that after intratracheal inoculation with pneumococcus cultures a localized inflammation was produced only in animals previously immunized by means of killed cultures of pneumococci. Recently Sharp and Blake (3) have shown that pneumococcus autolysates produce an exudative inflammatory pulmonary lesion in sensitized rabbits, and that there is a close parallelism between cutaneous and pulmonary hypersensitiveness.

From the observations of Tillett and Francis (4) it was shown that a protein-free, type-specific polysaccharide of the homologous pneumococcus elicits an immediate wheal type of reaction when injected into the skin of patients recovering from pneumococcus lobar pneumonia. However, Finland and Sutliff (5) obtained skin reactions with the specific pneumococcus polysaccharides in a number of hospital patients who had no recent history of pneumonia.

Mackenzie (6) showed that intraperitoneal injections of killed and living broth cultures of virulent pneumococci produce in guinea pigs a high degree of active immunity and a serum with strong protective power. But guinea pig immunity

to the pneumococcus by the above described method was not attended by cutaneous allergy to derivatives of the pneumococcus used for immunization. At the same time Mackenzie and Woo (7) injected guinea pigs with an alkaline pneumococcus extract. In about two-thirds of their animals an allergy was produced similar to the allergic response of the tubercle bacillus, but the animals showed no significant alteration in susceptibility to pneumococcus infection by intraperitoneal inoculation. Zinsser and Mallory (8) proved that guinea pigs can be artificially sensitized to bacterial products. Furthermore, they believe that an actual infection is a better means of sensitizing an animal than is the artificial administration of bacterial antigens. Bull and McKee (9) definitely showed that rabbits having recovered from an acute pneumococcus infection were hypersensitive to an autolysate of the homologous organism. This hypersensitive state was observed within 48 hours and lasted as long as 4 months. Also rabbits immunized with killed and living organisms were found to be hypersensitive to the pneumococcus autolysate. However, immunization by the above means did not produce as high a degree of sensitivity as did infection. Reimann (10) has recently published an excellent critique of the literature relating to allergy and pneumococcus lobar pneumonia.

We have made an attempt to throw further light on this subject by observing the pulmonary and cutaneous reactions to pneumococcus autolysate in dogs recovering from experimental pneumococcus lobar pneumonia. As far as we can find there have been no studies of pneumococcus hypersensitiveness in this animal. The experimental disease produced in the dog, which we have described elsewhere (11), resembles human lobar pneumonia more closely than that so far induced in any other animal with the exception of the monkey (12).

Methods and Materials

Male dogs were used exclusively. Their weights ranged from 10 to 15 kilos. They were healthy in external appearance and very active. All were isolated for a preliminary period of at least 1 week and temperatures taken; any animal with an elevated temperature was discarded. They were fed a normal diet of meat, carrots and bread ground into a hash, plus cod liver oil, the latter given three times weekly.

Fourteen dogs, as shown in Table I, were infected with Type I and Type II virulent pneumococci. Dosages ranging from 0.02 to 1 cc. of culture suspended in a broth-starch mixture were placed directly into a terminal bronchiole of the lung by means of a No. 11 F. ureteral radio-opaque catheter with the dog under the fluoroscope (11). The animals were infected on twenty-three different occasions; three of them, Nos. 6B, 15B and 23B, received four or more infections each, at various time intervals. The character of the initial and subsequent attacks in

these animals has been described in the preceding study of which this series of dogs formed a part (13).

At various intervals, from 2 days to 2 months following recovery from the initial or recurrent attacks, Type I pneumococcus autolysate was injected directly into the previously affected lobe by the use of the radio-opaque catheter. The initial dose was 0.5 cc. Later 1.0 cc. was administered. The autolysate was also injected intracutaneously in amounts of 0.2 cc. to determine the presence or absence of skin sensitivity. Temperature and pulse rates, white blood cell counts, total and differential, X-rays of the lungs and reinfection were utilized to detect increased sensitivity. Tests for the pneumococcal-promoting activity of inactivated serum were also carried out with a view to determining the possible relationship of acquired immune bodies to allergy. The method used for determining the presence of this serum property was that devised by Robertson and Sia (14). Mixtures of heated diluted dog serum and washed rabbit leucocytes and serum seeded with varying numbers of pneumococci were agitated in the incubator and tested for sterility at the end of 72 hours.

Preparation of Autolysates.—The autolysates were prepared according to the method used by Sharp and Blake (3). The preparation was standardized on the basis of nitrogen content. In the various lots the content of nitrogen ranged from 134 to 160 mg. per 100 cc. of autolysate. The potency of the autolysate was ascertained by testing rabbits sensitized to the pneumococcus. Intracutaneous injection of these animals was followed in 24 hours by the characteristic urticarial reaction.

EXPERIMENTAL

Pulmonary Reactions.—Seven dogs received intrapulmonary injections of the pneumococcus autolysate at varying time intervals of 1 week to 2 months following an attack of the experimental pneumonia. Four of the seven dogs, Nos. 6B, 15B, 22B, and 23B, showed a definite shadow by X-ray at the site of the injection. The three remaining dogs, Nos. 2B, 3B and 10B, which had received similar previous infections, showed no pulmonary lesion by X-ray. The pulmonary lesions when present were best seen 24 hours after injection. At the end of 48 hours they were clearing up or had disappeared entirely. The X-ray shadows averaged 4 cm. in diameter and in no single instance were they large enough to conform to the boundaries of any one lobe. There was no difference in the size of the lesion whether the dogs were injected within 1 week after an infection or if they were allowed to rest as long as 2 months. Also, dogs with repeated autolysate injections reacted in the same manner on each occasion. Likewise, there was

TABLE I

Dog No.	Date	Infection or auto-lytate	Type of pneumococcus	Amount of culture injected	Route of inoculation	Leucocytosis	Elevation of temperature	Increase in pulse rate	Skin reaction	Titers of acquired immune substance ^a	Definite X-ray lesion	Additional data
2B	10/21/30	Inf.	II	0.25 cc.	I. T.	+	+	+	-	-	+	Moderately ill. Recovered 4th day
	10/27/30	Aut.	II	0.5	I. T.	+	+	+	0	-	0	
3B	12/ 5/30	Inf.	II	0.25	I. D.	-	+	+	-	-	+	Recovered 3rd day
	12/22/30	Aut.	II	0.5	I. T.	0	+	+	0	-	+	Recovered 3rd day
	12/29/30	Inf.	II	0.25	I. D.	+	+	+		-	+	Recovered 3rd day
	11/ 4/30	Inf.	II	0.3	I. T.	+	+	+	0	-	+	Recovered 3rd day. Mild infection
6B	12/ 5/30	Aut.	II	0.5	I. T.	0	+	+		-	+	Recovered 3rd day
	12/29/30	Inf.	II	0.2	I. D.	+	+	+	-	-	+	Dog very ill. Spread of lesion. Recovered 5th day
	12/ 1/30	Inf.	I	0.25	I. T.	+	+	+	0	-	+	X-ray shadow 3 cm. diameter
	12/15/30	Aut.	I	0.5	I. T.	0	0	0	0	-	+	X-ray shadow 5 cm. diameter
	1/19/31	Aut.	I	0.5	I. D.	+	0	0	0	-	+	Recovered 3rd day
	1/26/31	Inf.	II	0.2	I. T.	+	+	+	0	-	+	
3/ 2/31	Aut.	II	1.0	I. T.	+	+	+	0	-	0		
					I. D.							

9B	3/ 9/31	Inf.	I	0.2	I. T.	+	+	+	+	+	+	+	+	+	+	+	+	+	Recovered 3rd day. Infected in R.L. lobe
	3/12/31	Inf.	I	0.2	I. T.	+	+	+	+	+	+	+	+	+	+	+	+	+	Recovered 3rd day. Infected in L.L. lobe
	3/16/31	Inf.	I	0.2	I. T.	+	+	+	+	+	+	+	+	+	+	+	+	+	Recovered 3rd day. Infected in R.L. lobe. Consolidation of R.L. lobe
10B	12/16/30	Aut.	I	0.5	I. T.	+	0	+	+	+	+	+	+	+	+	+	+	+	Mild infection. Recovered 3rd day. Even consolidation of R.L. lobe
	1/19/31	Aut.	I	0.5	I. D.	+	0	0	0	0	0	0	0	0	0	0	0	0	
	1/26/31	Inf.	I	0.02	I. T.	+	+	+	+	+	+	+	+	+	+	+	+	+	
15B	12/20/30	Aut.	I	0.5	I. T.	+	0	0	0	0	0	0	0	0	0	0	0	0	Mild infection. Recovered 3rd day
	12/29/30	Inf.	I	0.25	I. D.	+	+	+	+	+	+	+	+	+	+	+	+	+	
	1/19/31	Aut.	I	0.50	I. T.	0	0	0	0	0	0	0	0	0	0	0	0	0	
15B	1/ 9/31	Inf.	I	0.05	I. T.	+	+	+	+	+	+	+	+	+	+	+	+	+	Moderately severe infection. Recovered 5th day
	3/ 2/31	Aut.	I	1.0	I. T.	+	0	0	0	0	0	0	0	0	0	0	0	0	Shadow 2 cm. diameter R.L. lobe
	3/ 7/31	Inf.	I	0.02	I. D.	+	+	+	+	+	+	+	+	+	+	+	+	+	Mild infection R.L. lobe
15B	3/12/31	Inf.	I	0.02	I. T.	+	+	+	+	+	+	+	+	+	+	+	+	+	Recovered 4th day. L.L. lobe
	3/16/31	Inf.	I	0.02	I. T.	+	+	+	+	+	+	+	+	+	+	+	+	+	Recovered 3rd day. R.L. lobe

+ = present. 0 = absent. - = not done. I.T. = intratracheal. I.D. = intradermal.
 * Acquired immune substances in terms of pneumococcal-promoting activity of the serum. The figures indicate that dilution of the heated dog serum which when added to 0.2 cc. of fresh rabbit serum + rabbit leucocytes is capable of destroying 10^{-6} of the standard suspension of pneumococci (approximately 1000 microorganisms).

TABLE I—Concluded

Dog No.	Date	Infection or auto-lysate	Type of pneumococcus	Amount of culture injected	Route of inoculation	Leucocytosis	Elevation of temperature	Increase in pulse rate	Skin reaction	Titer of acquired immune substance*	Definite X-ray lesion	Additional data
22B	2/ 2/31	Inf.	I	cc. 0.04	I. T.	+	+	+	-	0	+	Recovered 8th day. Very ill. Entire left side involved
	2/24/31	Aut.	I	1.0 0.2	I. T. I. D.	+	0	+	0	0	+	Lesion in R.L. lobe 2 cm. diameter at 24 hrs.
	3/ 3/31	Inf.	I	0.02	I. T.	+	+	+	-	0	+	Moderately ill. Recovered 4th day. R.L. lobe consolidated
	3/16/31	Inf.	I	0.25 1.0 0.2	I. T. I. T. I. D.	+	+	+	-	0	+	Recovered 3rd day. R.L. lobe consolidated
23B	4/ 7/31	Aut.	I	0.2	I. D.	0	0	+	0	0	+	Lesion in R.L. lobe 3 cm. diameter
	2/ 2/31	Inf.	I	0.02	I. T.	+	+	+	-	0	+	Recovered 4th day. Mild infection R.L. lobe
	2/24/31	Aut.	I	1.0 0.2	I. T. I. D.	+	0	+	0	0	+	Lesion R.L. lobe 3 cm. diameter
	3/ 3/31	Inf.	I	0.02	I. T.	+	+	+		-	+	Recovered 3rd day. R.L. lobe
36B	3/16/31	Inf.	I	0.25	I. T.	+	+	+		-	+	Recovered 4th day. R.L. lobe
	7/27/31	Inf.	I	1.0	I. T.	+	0	+		-	+	Recovered 3rd day. R.L. lobe
	4/ 7/31	Aut.	I	1.0 0.2	I. T. I. D.	0	0	0	0	-	+	Control dog
37B	4/ 7/31	Aut.	I	1.0 0.2	I. T. I. D.	0	0	0	0	-	+	Control dog

25D	3/ 2/32	Aut.	I	1.0 0.2	I. T. I. D.	0	0	0	0	0	0	+	Last infection 6 months previously. Killed after 24 hrs.
34D	3/ 2/32	Aut.	I	1.0 0.2	I. T. I. D.	+	0	0	0	0	0	+	Recovered 10 days previously. Killed after 24 hrs.
42D	3/ 2/32	Aut.	I	1.0 0.2	I. T. I. D.	0	0	0	0	0	0	+	Given Type I antipneumococcus serum (20,000 units). Killed after 24 hrs.
3X	3/ 2/32	Aut.	I	1.0 0.2	I. T. I. D.	0	0	0	0	0	0	+	Normal dog. Killed after 24 hrs.

no relationship between the severity of the preceding infection and the presence or extent of the autolysate lesion in the lung.

In the control series, three normal dogs, Nos. 36B, 37B and 3X, showed a definite pulmonary lesion by X-ray after autolysate injection. The appearance of these lesions was indistinguishable from those seen in the recovered dogs. In two other normal dogs, Nos. 9B and 10B, no reaction to the autolysate could be detected.

Cutaneous Response to the Autolysate.—Observations on the skin tests were recorded every 2 hours for the 1st day and then twice daily for 1 week after intradermal injections of the autolysate. A positive response was not elicited in any of the animals. In one dog (No. 15B) there was a small hemorrhagic area at the site of injection at 12 hours which, however, had none of the characteristics or appearance of a wheal reaction.

Reinfection.—It has been shown in this series of dogs and in the preceding report (13) that the second and subsequent attacks of experimentally induced lobar pneumonia are not only no more severe but are definitely milder than the first infection. Two dogs, Nos. 22B and 23B, were infected on three different occasions, each inoculation being done as soon as the previous infection had cleared up. On the last inoculation the dose was 0.25 cc. of culture, one that was usually lethal. However, each infection was very mild and the course of the disease after the large dosage was also mild, terminating by crisis on the 4th day. An inoculation of 1 cc. of a culture, known to be lethal in this amount under ordinary circumstances, was then given to Dog 23B, which likewise promptly recovered on the 4th day.

Two dogs, Nos. 9B and 10B, were given an injection of autolysate before the first inoculation. All of the remaining animals were inoculated previous to autolysate administration. Subsequent infections in both groups were similar in intensity and in duration. There was no evidence to suggest that administration of the pneumococcus autolysate had altered the disease in any manner.

Acquired Humoral Immunity.—In five of the animals repeated tests of the pneumococcal-promoting activity of the dogs' serum were made before and after infection and intrabronchial autolysate injection. Two of the five dogs tested (Table I) showed the presence of acquired humoral immune substances when the autolysate was given.

In both these animals immune substances appeared in the blood after the initial autolysate injection, which was made before the first infection. Subsequent infections did not increase the titer of these immune bodies. In the third dog, No. 15B, acquired humoral immunity did not appear until after the third attack of the experimental disease. Thus the presence or absence of humoral immune substances could in no way be associated with the lack of detectable hypersensitivity to the pneumococcus.

Temperature, Pulse, White Blood Counts.—No febrile reaction was observed in any of the animals after intratracheal or intradermal administration of autolysate, but in many instances the pulse rate increased for a short period of time. Leucocytosis as noted in the protocol was usually present. However, it has been shown in a later experiment that normal dogs after an intramuscular injection of morphine develop an elevated white count. The explanation of this is probably that most of the morphinized dogs salivate, vomit and have several loose stools, thereby becoming dehydrated. Eosinophilia was not observed in any of the animals.

Pathology.—Four dogs were given intrapulmonary injections of 1 cc. of pneumococcus autolysate and were killed at the end of 24 hours for the purpose of microscopical examination (Table I). The time interval of 24 hours was selected because fluoroscopy revealed that the pulmonary lesion was of the greatest extent at that time. In this experiment the same lot of Type I autolysate was used in all four animals. Dog 3X was presumably a normal dog; No. 25D was a dog that had recovered from a Type I pneumococcus pneumonia 6 months previously; No. 34D had recovered from a similar infection 10 days before; and Dog 42D was given 25 cc. of unconcentrated Type I antipneumococcus serum 6 hours before administration of the autolysate.

The lesions in all four dogs were of a deep mottled red color with irregular edges. They were approximately 3 cm. in diameter and on palpation slightly crepitant.

On microscopical examination certain differences between the lesions of the two recovered dogs on the one hand, and the normal and immune serum-treated dogs on the other, were noted. The inflammatory exudate, of rather a diffuse nature in all four animals, was

definitely more cellular in the two dogs recovered from previous infection and resembled a lobular pneumonia, partly coalescent. In the areas of intense cellular infiltration polymorphonuclear leucocytes predominated. In the less infiltrated parts a considerable number of mononuclear cells were present—many of the macrophage type. Some of these were observed to be arising locally but there was no definite thickening of the alveolar walls such as is observed regularly when macrophages appear in the exudate at the time of recovery from experimental lobar pneumonia (15). However, the lesions in both these animals showed marked perivascular accumulations of large mononuclear cells, characteristic of the macrophage reaction, which we have described as occurring early in the evolution of the secondarily induced (recurrent) infections (13).

The exudative process in the normal dog and the dog previously injected with type-specific antipneumococcus serum was more edematous and hemorrhagic than that above described. Large mononuclear cells were fairly frequent in the cellular exudate but a smaller percentage of these appeared to be typical macrophages. While the lung of the normal dog showed slight, if any, increase in the number of perivascular mononuclear cells, this reaction was present in the lesion of the serum-treated animal although not nearly as marked as that observed in the recovered dogs.

An adequate estimate of the significance of these differences would require considerably more data than we have obtained. However, the results of this and previous studies suggest that the fixed cells of the lung tissue which have been subjected to a previous pneumococcus infection, react much more quickly to the presence of the pneumococcus or its products than do the cells of the normal lung.

SUMMARY AND CONCLUSIONS

The data derived from the above described investigations indicate that dogs do not develop hypersensitivity to the pneumococcus as the result of experimental lobar pneumonia. This inference is based on the following findings:

1. Fifteen dogs were given Type I and Type II pneumococcus lobar pneumonia and following recovery were tested for hypersensi-

tiveness by means of intrabronchial and intracutaneous injections of the autolysate made from the homologous pneumococcus.

2. Seven dogs showed a pulmonary lesion discernible with the X-ray at site of the autolysate inoculation; three of these dogs were normal controls.

3. No evidence of a positive skin reaction was found in any of the fifteen dogs, many of which received repeated infections and intradermal autolysate injections.

4. Subsequent infections in the same animals were definitely milder than the initial infection.

5. The infections following the administration of intrapulmonary and cutaneous autolysate were practically of the same intensity as the initial infection.

6. Temperature, pulse rates, white blood counts and differential blood pictures showed no significant variations following intrapulmonary injection of autolysate.

7. Tests for the acquisition of humoral immune bodies following autolysate injection and recovery from the experimental disease showed the presence of these substances in some of the dogs and their absence in others.

8. Study of the pathology of the pulmonary lesions produced by the autolysate failed to reveal histological changes characteristic of an allergic reaction. However, the presence of perivascular accumulations of large mononuclear cells observed in the lesions of the recovered dogs does suggest a locally accelerated reactivity of the fixed tissue cells to the products of the pneumococcus.

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