STUDIES ON HOST FACTORS IN PNEUMOCOCCUS INFECTIONS

I. Certain Factors Involved in the Curative Action of Specific Antipneumococcus Serum in Type I Pneumococcus Dermal Infection in Rabbits

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There is a growing impression among many investigators in the field of immunology that the protective or curative action of any antibacterial serum, such as antipneumococcus serum, is definitely conditioned by factors intrinsic to the animal body. For several decades the emphasis in immunology has been placed on the highly specific elements involved in resistance to infection, with the result that the physiological factors which make possible the proper functioning of these specific elements have been largely neglected.

The antibody content of antipneumococcus serum can be accurately estimated by *in vitro* methods such as that of Heidelberger, Sia, and Kendall (1), but the determined titers are frequently not quantitatively reflected in the results obtained by protective or therapeutic tests in mice or rabbits. There remains, of course, the possibility that the antiserum contains important, but as yet unrecognized elements, other than the anticarbohydrate antibody. On the other hand, it is a common observation that these *in vivo* methods of evaluation give confusing results because of a lack of sharpness as to end-point. Thus in any series of apparently similar animals treated in exactly the same way some individuals may die whereas others survive. Investigators in this field have been much influenced by the experience obtained in the use of the neutralization method in evaluating antitoxic serum, the efficacy of any lot of serum being determined by the amount required to save a guinea pig from death following the administration of a given amount of toxin. Experience has shown that, in general, there is a definite ratio between the amount of toxin and the protective dose of the serum. In the matter of antipneumococcus serum every effort has been made to show that a similar ratio exists between the amount of serum and the number of infecting organisms, but in general the work in animals has not demonstrated any accurate or consistent quantitative relationship.

In another type of experiment it has been shown that, in rabbits immunized with heat-killed pneumococci, the degree of resistance to subsequent infection was not necessarily correlated with the specific antibody titer in the serum of the individual animal (2).

It is obvious that these facts can be variously explained, but the most rational assumption is that some individual host factors may decisively modify or qualify the function of the highly specific antibody. In other words, the result in any individual animal must depend roughly on algebraic sum of all specific and constitutional factors. This problem has been approached from many sides, most successfully perhaps by the genetic studies of Webster (3), but there still remains the probability that individual animals differ other than in the genetic characters. Some workers have suggested the importance of so called physiological maturation, others have mentioned the state of physiological reactivity, but the problem has remained unsettled because of the difficulties of devising a suitable experimental approach.

In order to acquire some knowledge of host factors we have employed several types of experimentation each of which involved the pneumococcus as the infecting organism. The first experiments to be presented deal exclusively with Type I pneumococcus infections in rabbits in which antipneumococcus serum (Type I) has been used as a specific curative or protective agent. The general plan has been to arrange controllable conditions so that the situation presented is near a threshold; the ideal experiment being one in which the number of infecting organisms and the amount of specific antiserum are constant in a large number of animals and yet the balance between them so sharply determined as to permit the survival of certain individuals and the death of others. Then by analysis of other individual variables, both physiological and immunological, one can determine if the result is correlated therewith. This ideal type of experiment has not been entirely achieved in the course of the present work, but the results of the experiments tend to be mutually supportive and furnish evidence of the existence and nature of certain host factors.

The intradermal injection in rabbits of Type I pneumococci leads to the development of a characteristic lesion in the skin and to a definite symptom-complex marked by a high fever and accompanied by an invasion of the microorganisms

into the blood stream (4). With this type of infection the result is fatal in the great majority of instances if no curative measures are employed. When, however, type-specific antipneumococcus serum is injected intravenously in large amounts the disease process may be abruptly terminated as judged by a fall in temperature, by the disappearance of pneumococci from the blood stream and from the local lesion, and by the healing of the local lesion. In the earlier work this prompt alleviation of symptoms was emphasized, but Sabin (5), who used survival and death as criteria, called attention to the fact that with certain small amounts of the specific serum some animals died whereas others survived. This quantity of serum was designated the "subeffective amount" in contrast to the "effective amount," which is the amount required to bring about prompt recovery. The subeffective amount of specific serum has no relationship to prompt termination of the infectious process but concerns only the ultimate survival or death of the animal (6). In almost every instance in which the administration of the subeffective amount of serum leads eventually to survival, the effect seems to be that of providing conditions which prolong the life of the animal until its own immunity mechanism can be developed and the disease thus autoterminated. The use of subeffective amounts of specific serum therefore provides experimental conditions favorable for the study of certain factors which might influence the outcome of the infection.

EXPERIMENTAL

The general technical procedures are those described in a previous paper on the dermal pneumococcus infection in rabbits (4).

Culture.—Pneumococcus Type I, original Neufeld strain; virulence such that 0.000,000,01 cc. produces a fatal infection in rabbits following intradermal inoculation.

Infection.—Healthy male rabbits weighing from 1,600 to 2,500 gm. were used. Animals were injected intradermally at a site midway on the flank area with 0.2 cc. of a 1–200 dilution of an 18 hour blood broth culture.

Serum Treatment.—Each rabbit received a single intravenous injection of Type I antipneumococcus serum 24 hours after infective inoculation.

Blood Cultures.—The number of organisms per cubic centimeter of blood was determined by a procedure previously described in detail (4). In brief, the method consists in plating 0.4 cc. of blood withdrawn from the marginal ear vein with an accurately graduated syringe.

The general characteristics of the pneumococcus dermal infection in rabbits have been so frequently and extensively described that they need not be here repeated (4).

Survival Rate after Treatment with Various Amounts of Specific Antiserum

In order to determine the subeffective ranges of certain lots of Type I antipneumococcus sera four series of rabbits were treated with

varying amounts under the conditions described above. The results of one typical series are shown in Table I.

The 78 rabbits included in this series received amounts of the specific antiserum (Lot 1) ranging from 1 to 16 cc. Of the 3 animals which received 1 cc. each, none survived, while on the other extreme the 4 rabbits which received 16 cc. all survived the infection. Of 42 rabbits which were given 8 cc., 20 or 48 per cent survived. 36 per cent of those which received 4 cc. recovered while only 25 per cent of the animals treated with 2 cc. of serum survived.

This experiment demonstrates that between certain limits of serum dosage the administration of the specific therapeutic agent results in the survival of certain animals although others succumb. It is perhaps noteworthy that the fourfold increase in amount of serum from 2 to 8 cc. resulted in only a twofold increase in the survival rate. In other series the quantitative effect of serum dosage in this subeffective range has been less marked.

TABLE I

Survival Rate after Treatment with Various Amounts of Specific Antiserum

Each rabbit infected intradermally with Pneumococcus Type I. Designated amount of antipneumococcus serum (Lot 1) administered intravenously 24 hour after infective inoculation.

Group	Amount of serum given to each animal in group	No. of animals in group	Rabbits surviving	
			No.	Per cent
	cc.			
A	16	4	4	100
B	8	42	20	48
C	4	22	8	36
D	2	8	2	25
E	1	3	0	0

Previous experiments have shown that in any series of rabbits, even though each individual has received the same infective inoculum, the experimental disease may progress more rapidly in some animals than in others. This is clearly demonstrated by differences in the rate of blood invasion and, in untreated animals, by differences in the time of death. The first analyses to be presented deal with the severity of the infection as a factor in the determination of the outcome.

Results of Serum Therapy in Relation to the Severity of the Infection at the Time of Treatment

In order to determine if the severity of the infection might be correlated with the results in terms of survival and death an analysis was made of the data obtained from four series of animals similar to that shown in Table I. If the severity of the infection as indicated by the number of viable pneumococci per cubic centimeter of circulating blood were the only factor which determined the result it might be expected that with a given serum dosage all animals with severe infections would die whereas those with mild infections would survive, and further, that increasing amounts of serum would compensate for increasingly severe infections. The analyses, too detailed to reproduce in full here, showed that none of these assumptions was entirely true. Thus among rabbits treated with the same amounts of the same serum some animals with bacteriemias of low degree died whereas others with extremely high bacteriemias survived. A division of the animals into groups showed, however, that the larger percentage of animals with low bacteriemias responded favorably after the injection of a subeffective amount of serum, while the larger proportion of those with high bacteriemias eventually died. Two analyses of this type are shown in Table II. For the purpose of correlation two end-points have been used. The first is that of death, 14 days being reckoned as the limit of experimental observation. The second end-point is that of death within 4 days. This time period was arbitrarily chosen as including the larger number of the animals which died and yet sharply separating others in which the result was much less critical. Sabin (5) has already described the phenomenon of delayed death, and the present work confirms his observation that these delayed deaths are frequently associated with negative blood cultures and hence decidedly of a class differing from that which includes the earlier deaths for the latter are invariably associated with overwhelming bacteriemias.

By reference to Table II it will be seen that of the 42 rabbits, each of which received 8 cc. of Serum 1, 22 animals, or 52 per cent, died. The rate fell well below this average in the case of the animals having the lowest bacteriemias while in the group of highest bacteriemias the average rate was much higher. The same type of result is also apparent in the column dealing with the deaths occurring within the

first 4 days. Similarly in the second series of 43 animals each of which received 4 cc. of Lot 2 the death rate was 65 per cent but the larger number of deaths occurred in the group of highest bacteriemias while the rate among those with the lower bacteriemias was much smaller. The same trend is shown among the animals which died early.

The data indicate that there is no absolute correlation between the severity of the infection as judged by the degree of the bacteriemia

TABLE II

Results of Serum Therapy in Relation to the Bacteriemia at the Time of Treatment

Each rabbit infected intradermally with Pneumococcus Type I. Designated amount of antipneumococcus serum administered intravenuosly 24 hours after infective inoculation. Degree of bacteriemia determined just before the administration of the specific serum.

Serum lot and amount given to each rabbit	Bacteriemia group	No. of pneumococci per cc. of blood	No. of animals in group	Total deaths		Deaths within 4 days	
				No.	Per cent	No.	Per cent
Lot 1, 8 cc.	A	0–200	5	1	20	0	0
	B	200–2,000	9	4	44	2	22
	C	2,000–20,000	15	6	40	5	33
	D	Over 20,000	13	11	85	8	69
	Entire group.....		42	22	52	15	36
Lot 2, 4 cc.	A	0–200	9	3	33	1	11
	B	200–2,000	8	4	50	3	38
	C	2,000–20,000	11	7	63	6	55
	D	Over 20,000	15	14	93	12	80
	Entire group.....		43	28	65	22	51

and the result of subeffective serum therapy beyond the fact that those individuals with the more severe bacteriemias usually respond less well to the treatment. Perhaps certain of the discrepancies may be related to the technical difficulties in the exact determination of the number of pneumococci in the blood but even after due allowance is made for this possibility it remains a fact that all animals with infections of equal severity do not respond in the same way.

As these studies progressed it became increasingly apparent that some other factor or factors were involved in the results. In succeed-

ing series, determinations were made of the following variables: the white cell count at the time of infective inoculation and at the time of serum administration; body temperatures at all phases of the infection; the appearance of the local lesion as regards the area involved, the amount of edema, the intensity of the inflammatory color, and the occurrence of purpura; body weight; the coagulation time of the blood both at the time of infective inoculation and at the time of serum administration; the capacity of the rabbits' sera to agglutinate rough pneumococci; the heterophile antibody titer of the normal rabbit sera. In the case of only one of these was a suggestive correlation obtained, this being the white blood cell count at the time of serum administration.

Results of Serum Therapy in Relation to the White Cell Counts at the Time of Treatment

Although it was seldom possible to correlate the white cell count at the time of serum administration with the outcome of the infection it is possible by a system of averages to show that animals which had a low white count had much less chance for recovery than did those rabbits which had a high white count. Such an analysis is presented in Table III.

The normal white cell count of rabbits varies within wide ranges, the average in our series being 11,920. As a rule this is considerably depressed during the earlier phases of the infectious process so that at 24 hours after infective inoculation the average is near 6,600 although here again there are many wide deviations from the mean. The animals have been divided into four groups on the basis of the white counts made at the time of serum administration so that the first group includes those animals with very low counts and the fourth group those with the very high counts. When the death rate in each of these groups is considered it is found that those animals with the very low counts died whereas those with high counts more frequently survived. Thus with Serum Lot 1, the 3 animals with very low white counts died while 4 of the 5 with counts over 9,000 survived. The results are even more striking when the early deaths are considered. Similarly with Serum Lot 2, 4 animals with counts below 2,000 died whereas the death rate of those with high white cell counts was relatively low. Intermediate between these two extremes a definite difference is found when the comparative averages are considered but with the individual animal of any given group no significance can be attached to the count.

Since by the use of averages there seemed to be a definite significance attached to both the severity of the infection and to the white blood cell count at the time of serum administration a comparison was next made of the relation between the number of bacteria in the blood stream and the white cell count. Outside of the fact that animals with a very low white count generally also showed a very high bacteriemia no direct correlation exists between these two factors. How-

TABLE III

Results of Serum Therapy in Relation to the White Cell Count at the Time of Treatmen

Each rabbit infected intradermally with Pneumococcus Type I. Designated amount of antipneumococcus serum administered intravenously 24 hours after infective inoculation. White cell counts determined just before the administration of the specific serum.

Serum lot and amount given to each rabbit	White count group	White cell counts	No. of animals in group	Total deaths		Deaths within 4 days	
				No.	Per cent	No.	Per cent
Lot 1, 8 cc.	*a*	Less than 2,000	3	3	100	3	100
	b	2,000–5,000	8	5	63	4	50
	c	5,000–9,000	6	1	17	1	17
	d	Over 9,000	5	1	20	0	0
	Entire group.....		22	10	45	8	36
Lot 2, 4 cc.	*a*	Less than 2,000	4	4	100	4	100
	b	2,000–5,000	19	15	79	13	68
	c	5,000–9,000	12	6	50	3	25
	d	Over 9,000	8	3	38	2	25
	Entire group.....		43	28	65	22	51

ever, if these two elements are considered in terms of the outcome of the infection a definite result is obtained. Such an analysis is shown in Table IV.

Table IV is compiled from the results obtained with subeffective amounts of three different lots of antipneumococcus sera of almost equivalent antibody content. Altogether 115 animals have been classified. This combination of results has been made in order to build up groups of significant numbers. It can be stated that the combined results are in every way similar to those of any one series.

The correlation between the white cell counts and height of bacteriemia with the result of the infection is shown in two parts, the first dealing with the total number of deaths, the second with only the early deaths. The significance of the two factors is quite apparent in the first part but is even more striking in the second.

TABLE IV

Combined Summary of the Results of Serum Therapy in Relation to Bacteriemia and to White Cell Count at the Time of Treatment

This analysis includes the results of seven series of animals all of which were infected intradermally with Type I pneumococci. Each animal received a sub-effective amount of specific antipneumococcus serum intravenously 24 hours after infective inoculation. Height of bacteriemia and white cell counts at the time of serum administration.

(*A*) *Analysis Based on Total Deaths*

White cell counts	Bacteriemias											
	Over 20,000			20,000–2,000			2,000–200			200–0		
Less than 2,000	**9**	9	*100*		—		**2**	2	*100*		—	
2,000–5,000	**14**	13	*93*	**16**	9	*56*	**13**	8	*61*	**5**	3	*60*
5,000–9,000	**10**	10	*100*	**10**	7	*70*	**10**	4	*40*	**7**	1	*14*
Over 9,000	**6**	5	*83*	**6**	1	*17*	**1**	0	*0*	**6**	1	*17*

Figures in bold face type indicate total number of animals in group. Figures in ordinary type indicate number of deaths in group. Figures in italics indicate the percentage of animals which died.

(*B*) *Analysis Based on Deaths within 4 Days*

White cell counts	Bacteriemias											
	Over 20,000			20,000–2,000			2,000–200			200–0		
Less than 2,000	**9**	9	*100*		—		**2**	1	*50*		—	
2,000–5,000	**14**	13	*93*	**16**	8	*50*	**13**	6	*46*	**5**	2	*40*
5,000–9,000	**10**	8	*80*	**10**	5	*50*	**10**	3	*30*	**7**	0	*0*
Over 9,000	**6**	4	*67*	**6**	1	*17*	**1**	0	*0*	**6**	0	*0*

Figures in bold face type indicate total number of animals in group. Figures in ordinary type indicate number of deaths within 4 days. Figures in italics indicate percentage of animals which died within 4 days.

All animals with very high bacteriemias and very low white counts died promptly whereas the opposite is true of those with low bacteriemias and high white counts.

In spite of the apparent significance of both of these factors no absolute prediction of the outcome in the individual case was possible.

DISCUSSION

These results appear to show that in the experimental dermal pneumococcus infection in rabbits both the number of organisms in the blood stream and the white blood cell count have a definite bearing on the curative action of specific antipneumococcus serum. Thus animals with low bacteriemias and high white cell counts responded well to specific serum therapy whereas those rabbits with low cell counts and high bacteriemias were apparently not benefited by the administration of the serum under the conditions of these experiments. Nevertheless it was impossible to predict with certainty the outcome in any individual instance. Certain factors of obvious importance were not controlled, first amongst them the breed of animals and thus indirectly the entire system of genetic host factors. Furthermore, rabbits of different weights were used.

SUMMARY

In the experimental disease brought about by infecting rabbits intradermally with Type I Pneumococcus the use of relatively small or subeffective amounts of specific antipneumococcus serum leads to the survival of some individuals and the death of others. It has been shown that both the severity of the infection and the white blood cell count are factors in the determination of the outcome of the disease but that there remain other host factors which have to do with the utilization or functioning of the specific antisera.

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