SPECIFIC ANTIBODY RESPONSE OF HUMAN SUBJECTS TO INTRACUTANEOUS INJECTION OF PNEUMOCOCCUS PRODUCTS*

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Recent observations by Francis and Tillett (1) and by the present writers (2) have indicated that in patients ill with or recovering from lobar pneumonia the intracutaneous injection of small amounts of the protein-free type-specific carbohydrate of the pneumococcus is followed by the appearance of circulating antibodies specific for the type of carbohydrate injected. These findings contrast with previous failures to stimulate antibody production in animals with the same materials (3). It was, therefore, of interest to determine whether such specific antibody response could be elicited in the absence of pneumococcic infection or by the use of other pneumococcus products.

In the present investigation, human subjects without recent pneumococcic or other infections were studied with respect to their specific humoral antibody response to the intracutaneous injection of various pneumococcus products. Type-specific carbohydrates, acetic acid-precipitable proteins, and autolysates were used, simultaneously in some subjects and separately in others. The whole defibrinated blood of the subjects was studied for pneumococcidal power and their serum tested for agglutinins and for passive protection in mice.

The subjects chosen for the present study, the materials used for the intracutaneous injections, and the immunological methods employed were, in almost all respects, identical with those described elsewhere (4), except that no children under 15 years of age were included. Throughout this work pneumococcidal power was said to be present only when *more than 10 organisms* were killed and

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protection only when it was demonstrated against *more than 10 lethal doses*. Increases or decreases of less than 100-fold in these two properties were not considered significant.

Multiple Simultaneous Injections of Various Products of Three Types of Pneumococci

The first tests were done on 10 persons over 60 years of age. Old individuals were chosen because of the well known high fatality of lobar pneumonia among them and because of the finding of a somewhat lowered incidence of pneumococcidal power in their blood (4). Blood was taken for immunological studies and, subsequently, 16 intracutaneous injections were arranged on the flexor surfaces of the forearms. The solutions injected were in 0.1 cc. amounts, containing the following substances:—

0.01 mg.	S.S.S.	of Pneumococcus I
0.01 "	"	" " II
0.01 "	"	" " III
0.1 "	nucleoprotein	" *Streptococcus scarlatinae*
0.01 "	"	" " "
0.01 "	"	" Pneumococcus I S
0.01 "	"	" " I R
0.01 "	"	" " II S
0.01 "	"	" " II R
0.01 "	autolysate	" " I S
0.1 "	"	" " I S
0.01 "	"	" " II S
0.1 "	"	" " II S
0.01 "	"	" " I R
0.1 "	"	" " I R
Physiological saline		

Blood was again obtained for immunological studies at various intervals after the intracutaneous injections.

Antibodies appeared or an increase was demonstrated in previously existing antibodies in 9 of the 10 subjects. The results of the immunological tests performed on the whole blood and serum of 4 individuals, representing the various kinds of response, are shown in Table I.

Some individuals who had a high pneumococcidal titer before the intracutaneous injections, later had either lowered or absent pneumococcidal action for some pneumococcus types against which specific mouse-protective antibodies and agglutinins appeared or increased. This is illustrated in Subject L. M., Table I. Among these 10 sub-

jects there were 4 instances where mouse-protective antibodies against one type of pneumococcus were found before the intracutaneous inoculations. The titer of protection against this type in later tests was the same in 2 of these subjects and was increased in the other 2 (see Table I, Subject M. B.)

The aggregate results in the 10 persons receiving multiple simultaneous injections of several pneumococcus products are shown graph-

TABLE I

Subject, age, and diagnosis	Date of tests*	Pneumococcidal titer (diplococci killed)			Protection titer (lethal doses)			Agglutinin titer (serum dilution)		
		Type I	Type II	Type III	Type I	Type II	Type III	Type I	Type II	Type III
M. D., 70 yrs., fractured hip	Mar. 3	0	0	0	0	0	0	0	0	0
	" 19	10^4	10^3	0	10^2	10^3	0	1:1	1:2	1:1
	Apr. 3	10^5	10^5	0	10^2	10^3	0	1:1	1:1	1:2
	" 27	0	10^4	0	10	0	0	0	0	0
M. B., 65 yrs., arteriosclerosis	Jan. 27	0	10^2	10^3	0	10^2	0	0	0	0
	Feb. 24	10^4	10^3	10^4	0	10^{5+}	10^4	0	1:4	0
R. R., 60 yrs., hypertension	Mar. 5	10^2	10^3	10^4	0	0	0	0	0	0
	" 13	10^6	10^6	10^4	10^2	10^2	0	0	0	0
	" 19	10^5	10^5	10^3	10^2	10^4	0	0	1:4	0
L. M., 66 yrs., fractured femur	" 4	10^4	10^5	10^4	0	0	10	0	0	0
	" 19	0	10^4	10^2	10^3	10^{5+}	10^{4+}	1:2	1:8	1:8

* The intracutaneous injections were done on the day of the first tests.

ically in Fig. 1. A study of this figure shows that pneumococcidal power, mouse-protective antibodies, and agglutinins were more frequent following the intracutaneous injections than before. This was true for each of the three pneumococcus types.

The time of appearance or increase of the various antibodies and their duration were not accurately determined. In general, the increase appeared in 1 week and was demonstrable 1 month after the inoculations. The only person in this group on whom observations were made after more than 4 weeks (M. D., Table I) showed a loss of most of the newly demonstrated antibodies after about 8 weeks.

It is thus seen that the simultaneous intradermal injection of many pneumococcus products in human subjects without recent pneumo-

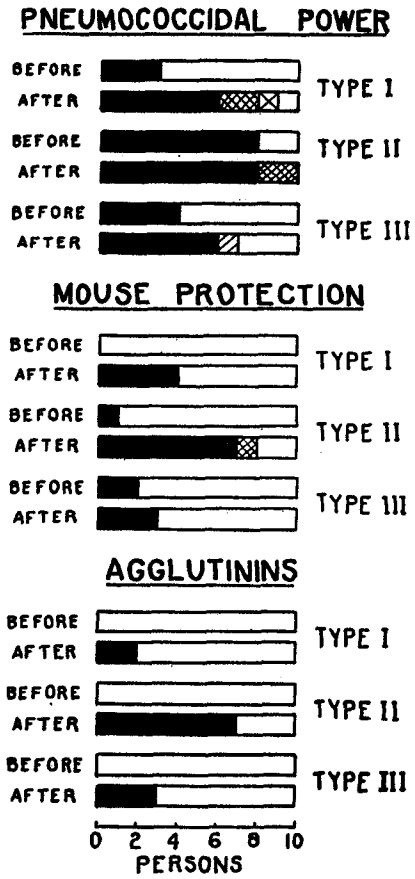

FIG. 1. Antibody response to the simultaneous intradermal injection of several pneumococcus products.

The *solid* portions in Figs. 1 to 4 represent subjects with antibodies and the *light* portions those without antibodies. *Single crosses* represent subjects in whom previously existing antibodies could not be demonstrated after the intracutaneous injections. The areas with *single hatchings* represent subjects in whom antibodies were diminished and the *cross-hatched* portions subjects in whom antibodies were increased after the injections.

coccic infection, was followed by the appearance or increase in antibodies for all three types of pneumococci. In the experiments which

follow, the response of such subjects was studied after injections of individual pneumococcus materials.

Single and Repeated Injections of Type-Specific Polysaccharide

A single intracutaneous injection of 0.01 mg. of the protein-free type-specific carbohydrate was given to 19 individuals (Type I, 7 cases; Type II, 6; and Type III, 6) and similar injections on 4 successive days were given to 10 others (Type I, 3 cases; Type II, 3; and Type III, 4). The subjects ranged in age from 15 to 56 years. Antibodies for all three types were studied in each subject before and at intervals after the intracutaneous injections. Inasmuch as no apparent differences were observed between the results of the single and of the 4 daily injections, they are considered together.

The results obtained with each of the polysaccharides were similar. Among the 29 subjects, 11 had pneumococcidal power for the homologous type of pneumococcus before the injection. 12 of the 18 persons having no demonstrable pneumococcidal action for the type corresponding to the polysaccharide injected showed, in subsequent tests, pneumococcidal power for the homologous type of pneumococcus, either alone or along with mouse protection or agglutination for the same type. Of the 11 persons having pneumococcidal power for the homologous organism before the intracutaneous injection, 8 showed diminished or unchanged titer for this organism along with the appearance or increase in the serum of mouse-protective antibodies or agglutinins, or both. In 9 persons no change was demonstrated; 3 of these had pneumococcidal power in their blood before the intracutaneous injections. The homologous pneumococcidal titer acquired was usually moderate, 0.5 cc. of blood acquiring the capacity to kill 1000 diplococci in 2 instances, 10,000 in 8, and 100,000 in 2.

Mouse protection was acquired against 100 lethal doses of the homologous organism in 7 subjects, against 1000 lethal doses in 5, and against 100,000 lethal doses in 2. Agglutinins were found in 10 individuals in final dilutions of 1:4 or 1:8 of the serum.

Heterologous antibodies did not, as a rule, appear following the injection of one of the polysaccharides. In several subjects pneumococcidal power, that had been present for heterologous types before the inoculations, was significantly lower or entirely absent after the intracutaneous injection. Unlike the lowering of pneumococcidal power

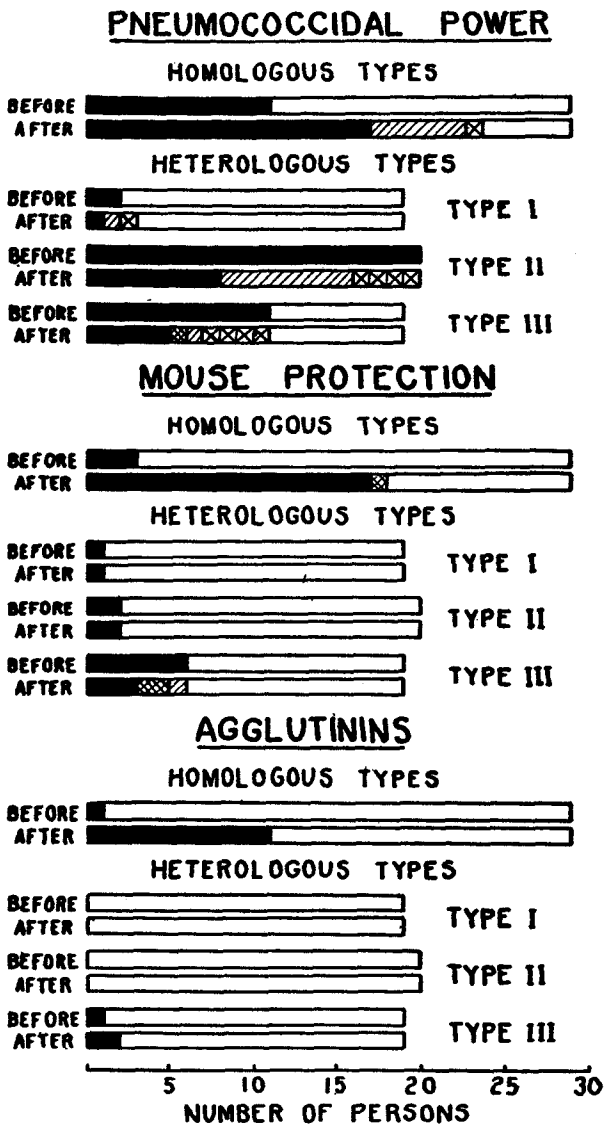

FIG. 2. Antibody response to the intracutaneous injection of the purified type-specific polysaccharides of Type I, II, or III pneumococci.

for the homologous type, this was not associated with the appearance of protective antibodies or agglutinins. In isolated instances, heterologous antibodies were demonstrated following the injections of one of the specific carbohydrates. Pneumococcidal power for Type I was demonstrated in 1 instance after the injection of Type II S.S.S. Agglutinins for Type III pneumococci were found in 1 person after he had received Type I S.S.S. This person, however, had high Type III pneumococcidal and mouse protection titers before the injection of the Type I material. In 2 instances there was a definite increase in the protection titer against Type III pneumococci after Type I and Type II S.S.S. respectively had been given.

The cases showing significant changes in their circulating homologous and heterologous antibodies following intracutaneous injections of one of the type-specific polysaccharides are shown graphically in Fig. 2.

The specific antibodies were demonstrated in almost every instance from 7 to 10 days after the intracutaneous inoculations. They were again found throughout the period of observation which was about 3 weeks in most cases. In a single instance, homologous protective antibodies and agglutinins acquired following an injection of Type II S.S.S. were demonstrated after 2 months.

These observations, therefore, show that a single intracutaneous injection of 0.01 mg. of the specific polysaccharide of Type I, Type II, or Type III pneumococci or 4 similar daily injections may be followed by the appearance of pneumococcidal power, mouse protection, and agglutinins for the homologous organism in human adults whose blood previously lacked these properties. In subjects originally having pneumococcidal power there may be a decrease in this power with a simultaneous appearance of protective antibodies and agglutinins for the same organism. The pneumococcidal power for heterologous types of pneumococci may be depressed in some individuals without changes in the mouse-protective or agglutinating properties for these types. Heterologous antibodies are not usually acquired following the intracutaneous injection of the type-specific polysaccharides.

Single Injections of Pneumococcus Nucleoprotein

13 subjects were given a single intracutaneous injection of 0.1 mg. of nucleoprotein and their humoral antibodies were studied before and at intervals up to 5

weeks after this injection. The proteins used in 7 instances were obtained from highly virulent strains of Type I (5 cases) and Type II (2 cases) pneumococci. Those used in the remaining subjects were derived from degraded, avirulent strains obtained from Type I (4 cases) and Type II (2 cases) organisms. No significant differences were observed in the response to the various proteins used, and the results are considered collectively.

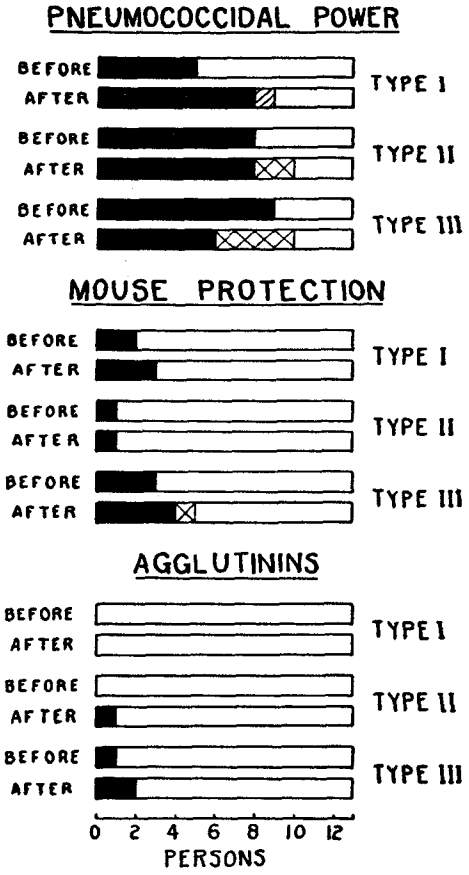

FIG. 3. Antibody response to single intracutaneous injection of pneumococcus protein.

The results of the antibody studies in the 13 subjects are summarized graphically in Fig. 3. A study of this figure shows that no uniform changes were exhibited in this group of individuals following the protein injections. 4 persons apparently acquired pneumococcidal

power, but this was only of low titer; whereas, lowering or loss of pneumococcidal power was more often encountered. Agglutinins were irregular. Mouse-protective antibodies did not develop.

From the findings in this small group of subjects, therefore, it appears that no definite or constant changes in the humoral type-specific antibodies follow a single intracutaneous injection of 0.1 mg. of pneumococcus nucleoprotein. This is in accord with previous observations in animals by Avery and Morgan (3).

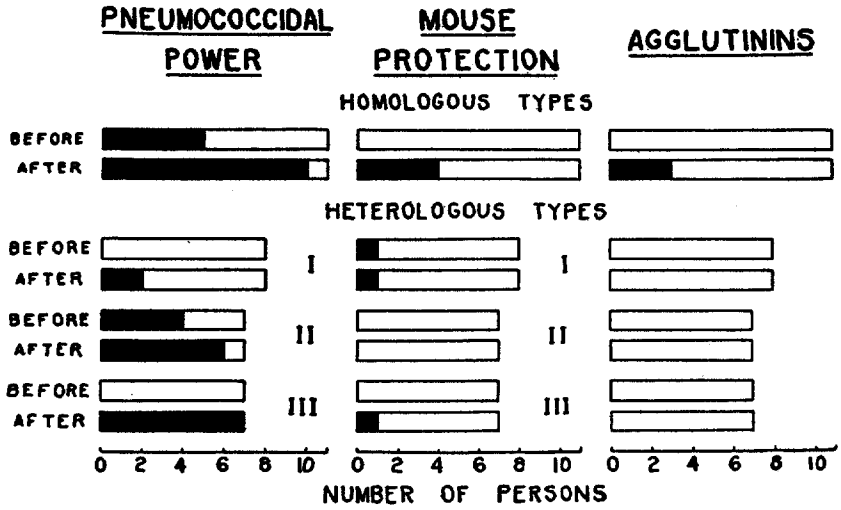

FIG. 4. Antibody response to single intracutaneous injection of autolysate of virulent strains of Type I, II, or III pneumococci.

Single Injections of Pneumococcus Autolysate

The antibody response to a single intracutaneous injection of small amounts of autolysates prepared from highly virulent strains of Type I, II, or III pneumococci was studied in 11 individuals, ranging from 17 to 50 years of age. The Type I autolysate was given to 3 subjects and the Type II and Type III autolysates were each given to 4 subjects. The quantity injected in each instance contained 0.1 mg. of protein. The responses to the autolysates of the various types were uniform and are considered together.

There was a general increase in pneumococcidal power for both the homologous and heterologous types of pneumococci in most instances. Mouse-protective antibodies (against 1000 lethal doses in 2 subjects,

10,000 in 1, and 100,000 in another) and agglutinins (in 1:4 dilutions of sera), however, appeared for the homologous organism only. Type I pneumococcidal power appeared in only 2 of the 8 subjects that received Type II or Type III autolysate, whereas Type II and Type III pneumococcidal power appeared or increased in all of the subjects without respect to the type of autolysate injected. The results of these findings are summarized graphically in Fig. 4.

A single injection of a small amount of autolysate derived from a virulent strain of either Type I, Type II, or Type III pneumococci was, thus, followed in most of the subjects studied, by an appearance or increase in the pneumococcidal power of the blood for all three types. Mouse-protective antibodies and agglutinins, however, appeared for the homologous types only.

DISCUSSION

In the first group of subjects various products of virulent strains of pneumococci, including their type-specific carbohydrates, were injected simultaneously. Following these injections there was a general rise in the circulating antibodies for the types of pneumococci from which these products were derived. This is not in agreement with the findings of Julianelle (5), who failed to demonstrate, in rabbits, a type-specific response to the intracutaneous injection of materials derived from Type I pneumococci.

The findings in the other groups of subjects suggest that the intradermal injection in normal human adults of a protein-free, type-specific carbohydrate, prepared according to the methods of Heidelberger, Goebel, and Avery (6), is frequently followed by the appearance in the circulating blood of antibodies specific for the type of pneumococcus from which the carbohydrate was derived. Heterologous antibodies could not be demonstrated. It would thus appear that the carbohydrate was responsible for the type-specific response that followed the simultaneous injection of a variety of pneumococcus products. Furthermore, the circulating antibodies to heterologous types of pneumococci demonstrated, following intracutaneous test injections of carbohydrate, in cases recovering from lobar pneumonia (1, 2) need not be considered dependent on processes associated with recovery from the pneumococcic infection. They would appear rather to be the

usual reaction of at least a large percentage of normal human adults following such injections.

It is necessary to emphasize that the studies here presented refer, primarily, to the results of single intracutaneous injections of small doses. The findings of Schiemann and Caspar (7) suggest that dosage may be an important factor. These workers demonstrated specific protection in mice following the intraperitoneal injection of small amounts of their soluble protein-free, specifically precipitable substance, whereas, an increase of 100-fold in the dose of the same material failed to stimulate such immunity.

It would appear from the present data that 4 daily injections of the same amount of the polysaccharide do not materially alter the response. From the results, however, in pneumonic patients reported elsewhere (2), it would appear that repeated skin tests with polysaccharides at longer intervals may result in the maintenance of a fairly high degree of specific immunity for long periods, for 2 of the pneumonic subjects who received repeated injections were shown to have antibodies at the end of 13 and 14 months after the polysaccharide injections were begun.

In the case of the pneumococcidal power of the whole defibrinated blood, the response to the intracutaneously injected polysaccharides was different in those subjects originally possessing or lacking this power. Among the former a significant percentage acquired this power with respect to the homologous type after the injections. This property was not, however, demonstrated for a type of pneumococcus heterologous to that of the polysaccharide injected. In the subjects originally having pneumococcidal power, the intracutaneous injections were frequently followed by a decrease in titer or even complete loss of pneumococcidal power for both the homologous and heterologous types. This decrease was accompanied by the appearance, in the serum, of mouse-protective antibodies or agglutinins for the homologous type. No such antibodies appeared for the heterologous types. The explanation of these phenomena is not entirely clear and may depend on a more exact understanding of the factors involved in the pneumococcidal test.

The specific humoral response to single injections of acetic acid-precipitable proteins derived from virulent and avirulent pneumococci was quite irregular and can hardly be considered significant. On the

other hand, the antibody response to the autolysate used in these experiments was distinctly different from that obtained with the purified carbohydrates or with the nucleoprotein solutions. The autolysate of each of three types of virulent pneumococci used gave a general rise in pneumococcidal power for all three types but such specific agglutinins and mouse-protective antibodies as were demonstrated appeared only for the homologous type organism. No lowering of the pneumococcidal titer was observed in this group. The number of subjects studied was, however, small and none originally had any high degree of pneumococcidal power.

SUMMARY

The blood of 63 human subjects selected because of the absence of recent infections, was studied for its content of specific antibodies against virulent strains of Types I, II, and III pneumococci before and after intracutaneous injections of minute amounts of pneumococcus products.

The simultaneous injection of the specific polysaccharides of all three types of pneumococci and of proteins and autolysates derived from Types I and II pneumococci was followed by the appearance or increase of pneumococcidal power in the whole defibrinated blood and, in most instances, by the appearance of mouse-protective antibodies and agglutinins for one or more types.

A single intracutaneous injection of 0.01 mg. of the protein-free type-specific polysaccharide of either Type I, Type II, or Type III pneumococci or 4 similar daily injections was followed, in most of 29 subjects, by the appearance of antibodies against the homologous, but not against the heterologous type pneumococci. Some subjects showed a simultaneous lowering of a preexisting pneumococcidal power for heterologous or homologous types.

A single intracutaneous injection of 0.1 mg. of pneumococcus protein in 13 individuals was not followed by the appearance of specific antibodies to any appreciable degree.

Single intracutaneous injections of small amounts of autolysates derived from virulent strains of Type I, II, or III pneumococci were followed in 11 subjects by a more or less general rise in the pneumococ-

cidal power with the appearance of homologous type agglutinins and protective antibodies in about one-third of the subjects.

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