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J Clin Invest. 1926;**3**(2):423-433. <https://doi.org/10.1172/JCI100089>.

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STUDIES IN SCARLET FEVER

IV. POST-SCARLATINAL IMMUNITY IN PATIENTS TREATED WITH ANTITOXIN¹

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(Received for publication July 22, 1926)

INTRODUCTION

The present investigation was undertaken to determine, if possible, whether the treatment of scarlet fever with antitoxin has any effect on the degree of permanent active immunity which ordinarily follows an attack of the disease in persons who have not received antitoxin.

The immunity developed by the vast majority of patients convalescent from scarlet fever presumably comes as a response to the presence in the circulating blood during the first week of the disease of the specific toxin produced by the etiological agent, *Streptococcus scarlatinae*. Inasmuch as scarlatinal antitoxic serum, recently introduced for the treatment of scarlet fever (Dochez, 1924), rapidly neutralizes this circulating toxin (Blake, 1924) and thereby removes the stimulus which induces a permanent active immunity, it has seemed possible that patients promptly cured by antitoxin might become susceptible again, after the relatively transient passive immunity conferred by the administration of antitoxic serum had terminated.

Trask and Blake (1924) have demonstrated that the specific toxin of scarlet fever appears in the circulating blood of patients very soon after the onset of symptoms. The greatest number of patients have demonstrable amounts of toxin in the blood on the second and third days of the disease, the incidence declining from this point to the

¹ This work was done with the aid of the Goodhart scarlet fever fund.

² This paper is in part a thesis presented to the Graduate School of Yale University in candidacy for the degree of Master of Science.

eighth day after which relatively few patients show toxin in the blood (Blake and Trask, 1926). During this period, in the natural course of events, the immunity mechanism of the body responds to the toxemia by producing autogenous antitoxin which eventually becomes sufficient to neutralize the toxin formed at the site of infection. An excess of antitoxin and permanent immunity results in almost all cases not treated with serum. Theoretically, if this toxin were artificially neutralized very early by treatment with the specific antitoxin for scarlet fever, it is conceivable that the natural defensive mechanism of the patient would not be sufficiently stimulated to establish an effective permanent immunity.

Immunity to scarlet fever is in all probability a relative matter. It may conceivably vary with the following factors and possibly with others (1) fluctuations in the amount of immune bodies present at the time of exposure, (2) the site of the area involved in the infection, (3) the local conditions promoting the spread of the infection and rapid absorption of the toxin, such as a concurrent rhinopharyngitis, and (4) the intensity of the "seeding" with *Streptococcus scarlatinae*. Bearing this in mind it is evident that a study of the degree of immunity to scarlet fever developed by individuals treated several months previously with scarlatinal antitoxic serum, as contrasted with the immunity developed by those who did not have the benefit of this serum during the stage of specific toxemia, will give only relative and not absolute results.

SOURCE OF MATERIAL

The patients used consisted of those who responded to a request to return to the clinic for this study. The number is not large and represents only a small proportion of the total number of scarlet fever patients treated in the New Haven Hospital since scarlatinal antitoxin was first employed in January, 1924. Most of the cases were children from 2 to 15 years of age. In all, 50 former patients returned, 34 of whom had had serum treatment at least five months previously.

EXPERIMENTAL

Two methods have been used to measure the degree of antitoxic immunity to scarlet fever possessed by the individuals studied. The

first method consisted in determining the skin reactivity of the subjects to intracutaneous injections of standard amounts of scarlet fever toxin, the method commonly known as the Dick test. The second method consisted in determining the amount of scarlet fever antitoxin in the subject's blood by using his serum for blanching tests (Schultz-Charlton, 1918) and for toxin neutralization tests. The results obtained by the two methods have then been compared in order to determine how closely they agreed in providing a basis for estimating the degree of immunity possessed by each subject.

I. Skin tests of immunity

Methods. The skin tests of immunity were performed according to the accepted method of intracutaneous injection on the flexor surface of the forearm, the readings being made 24 hours after the injection (Dick and Dick, 1924).

The toxin employed in the tests was prepared in this laboratory from a strain of *Streptococcus scarlatinae* supplied by Dr. Dochez. Cultures were made in phosphate broth, pH 7.6, to which 2 per cent of defibrinated rabbit's blood had been added. After incubating for four days at 37°C. the broth was filtered through a Berkefeld "V" filter. The toxin was standardized according to the method described by Dick and Dick (1925). When diluted 1:2000 with normal saline, 0.1 cc. of the toxin-filtrate gave a skin reaction in a series of susceptible individuals approximately equal to the skin reaction produced by the standard skin test dose of toxin (D II) supplied by the United States Hygienic Laboratory in Washington, D. C. This amount was therefore considered to be one skin test dose, the present standard unit of toxin.

In order to determine the relative degree of immunity possessed and to control false reactions three skin tests were done on all cases: (1) 0.1 cc. of a 1:500 dilution of toxin equivalent to 4 skin test doses, (2) 0.1 cc. of a 1:2000 dilution, approximately equal to 1 skin test dose, and (3) 0.1 cc. of a 1:500 dilution, heated 10 hours at 100°C.

The skin reactions were interpreted as follows:

+ = positive: definite redness with some induration and perhaps tenderness, the area of erythema being over 10 mm. in diameter.

± = slightly positive: definite erythema approximately 10 mm. in diameter, with little or no induration.

± = faintly or doubtfully positive: faint erythema 10 mm. or more in diameter.

- = negative: no erythema or an erythema less than 10 mm. in diameter.

Results. The summarized results of the skin tests are shown in table 1, from which it will be seen that the immunity in the cases treated with antitoxin is conspicuously less than it is in those who received no antitoxin. In the treated group only 54 per cent failed

TABLE 1

Skin tests in former scarlet fever patients 5 months to 2 years after onset of scarlet fever

Amount of toxin	Skin reaction	Number of cases		Percentage	
		Treated with antitoxin	Not treated with antitoxin	Treated with antitoxin	Not treated with antitoxin
1 S.T.D.....	+	9	1	26	6
	±	7	0	20	0
	±	0	0	0	0
	-	18	15	54	94
4 S.T.D.....	+	18	2	53	13
	±	7	0	21	0
	±	1	1	3	6
	-	8	13	23	81
Heat control, 4 S.T.D.....	+	1	0	3	0
	±	2	0	6	0
	-	31	16	91	100

S.T.D. = skin test dose.

to react to one skin test dose, in the untreated group 94 per cent. Of the remaining 46 per cent in the treated group 26 per cent gave strongly positive reactions, while in the untreated group only 6 per cent gave strongly positive reactions.

The advantage of employing an additional test of four skin test doses of toxin is obvious, when one considers that one skin test dose gives information over only a relatively small range of immunity. By the use of this larger dose it is even more clear that the susceptibility of the treated cases is greater and their immunity less than is the case with the untreated patients. With four skin test doses the

percentage of strongly positive reactors increases from 26 per cent to 53 per cent, the percentage of negative reactors diminishes from 54 per cent to 23 per cent. In striking contrast with this, it will be seen that the percentages of positive and negative reactors in the untreated group show an insignificant change.

In Table 1 there are included among the cases treated with scarlatinal antitoxin two patients with septic complications who received the serum after the rash had faded and the stage of specific toxemia had passed. Omitting these two cases, one of whom gave a slight combined reaction and the other a positive skin test, the results are not markedly altered. The correction for these two cases has been

TABLE 2
Condensed results of skin tests

Amount of toxin	Skin reaction	Number of cases		Percentage	
		Treated with antitoxin	Not treated with antitoxin	Treated with antitoxin	Not treated with antitoxin
1 S.T.D.	Positive	14	1	44	6
	Negative	18	15	56	94
4 S.T.D.	Positive	23	2	72	13
	Negative	9	14	28	87
Heat control.	Positive	2	0	6	0
	Negative	30	16	94	100

made in table 2, in which the positive (+ and ±) reactions have been combined and the doubtful (±) reactions have been classified as negative.

Leaving out of account the two cases mentioned as having been treated late in the disease (eleventh and fourteenth days), the interval elapsing between the onset of symptoms and the time of antitoxin treatment varied from less than 24 hours to 8 days, the average time being less than 72 hours. In 13 of the 34 cases, the specific toxin of scarlet fever was actually shown to be present in the blood stream before antitoxin was administered, in four none was demonstrable, while in the remainder the test was not made. The cases varied greatly in severity and in the presence and nature of septic processes

at the time of treatment. No correlation between these variables and the degree of subsequent immunity could be established.

The contrast between the two series of cases is striking, not only with respect to the reactions to four skin test doses of toxin, but also with respect to the reactions to the standard Dick test of 1 skin test dose. In fact, the percentage of positive reactors (+ and \pm) to one skin test dose of toxin among the cases treated with serum, is slightly higher (46 per cent) than that (42 per cent) for a group of eighty student nurses, the majority of whom had never had scarlet fever, tested recently in this hospital with the same toxin. While these results undoubtedly indicate that scarlet fever patients treated with antitoxin develop on the average a less effective active immunity than untreated patients, it nevertheless seems improbable that any considerable number of those showing a + or \pm skin reaction would, under circumstances and conditions of exposure, develop a second attack of scarlet fever.

The difference in the two series of cases is too great, however, to leave reasonable doubt as to the greater degree of immunity to scarlet fever toxin developed by the untreated cases, 87 per cent of whom were negative even to 4 skin test doses, as compared with 28 per cent of those who had been treated with antitoxin.

II. Serum antitoxin tests of immunity

In order to test the validity of the results of the Dick tests as expressions of immunity to scarlet fever, the minimal blanching dose (M.B.D.) (Blake and Trask, 1925), or the highest dilution of the patient's serum which gave a positive Schultz-Charlton rash extinction test, was determined for seventeen of the cases in the above series. In addition, neutralization experiments were carried out on a smaller number of serums.

Methods. Approximately 10 cubic centimeters of blood were drawn at the time of the skin tests and the separated serum placed in a refrigerator in a "No-Air" stoppered bottle. A culture was made for sterility.

1. For the blanching tests, 0.5 cc. of the undiluted serum or of a 1:10, 1:100, or 1:1000 dilution in normal saline was injected intradermally into the fresh, uniform scarlatinal rash of a patient with

mild or moderate symptoms. Repeated readings were made from 12 to 36 hours after injection, the results in most instances being checked by two and sometimes by three observers. Complete blanching of the rash at the site of the injection is represented by a double plus (+ +). The highest dilution showing any blanching is the minimal blanching dose.

Since 0.5 cc. was injected into the skin, undiluted serum which gave a positive blanching test contained at least 2 M.B.D. per cubic centimeter. Similarly, a positive end-point in a dilution of 1:10 indicates 20 M.B.D. per cubic centimeter.

2. The method for the neutralization tests was similar to that of Henry and Lewis (1925), except that the ratio of serum to toxin was varied in multiples of ten and the amount of toxin was not constant.

Three neutralization tests were performed with each serum as follows: (1) 0.5 cc. toxin, 1:2000 (5 S.T.D.), + 0.5 cc. serum, undiluted; complete neutralization indicates at least 0.1 unit of antitoxin per cubic centimeter of serum. (2) 0.5 cc. toxin 1:200 (50 S.T.D.) + 0.5 cc. serum, undiluted; complete neutralization indicates at least 1 unit of antitoxin per cubic centimeter of serum. (3) 0.5 cc. toxin, 1:200 (50 S.T.D.) + 0.5 cc. of serum, 1:10; complete neutralization indicates at least 10 units³ of antitoxin per cubic centimeter of serum.

Dilutions were made with normal saline. The mixtures were incubated for 45 minutes in a water bath at 37°C.

One-tenth of one cubic centimeter (0.1 cc.) of each of the three mixtures of serum and toxin and of a toxin control (1:4000) was injected intradermally on the anterior aspect of the arm in test subjects susceptible to scarlet fever toxin. It was necessary to employ as a subject an individual who reacted to one-half of one S.T.D. of toxin or less, in order to bring out the reaction to a very small excess of toxin, since the amount of toxin in the first mixture injected was one-half of one S.T.D. (0.05 cc. of a 1:2000 dilution of toxin).

Readings were made 24 hours after injection, the size and intensity of any erythema which developed and the presence or absence of local swelling or tenderness being noted. The apparent dissociation of the toxin-antitoxin combination after 24 hours and the late spread

³ One unit is that amount of antitoxin which neutralizes 100 S.T.D. of toxin.

of the zones of erythema, to which Henry and Lewis (1925) called attention, were observed in the majority of the neutralization tests. Experience may show that it is possible to interpolate other propor-

TABLE 3
Serum antitoxin tests

Cases		Serum antitoxin tests							Skin tests			
Number	Name	Serum treatment	Minimal blanching doses per cubic centimeter of serum				Neutralization units of antitoxin per cubic centimeter of serum			S.T.D.		Heat control
			2	20	200	2000	0.1	1	10	4	1	
1	McAvoy	S	-	-	-	-	0	0	0	++	+	-
2	Dunham		+	±	-	-	C	0	0	++++	+++	-
3	Franklin	S		±	-	-				+	±	-
4	Canby			+	-	-				++	-	-
5	Clark	S		±	?	-				+	+	-
6	Neuman	S	+	+	±	-	C	0	0	+	-	-
7	Schoenrock	S		+	±	-	C	C	C	+	+	±
8	Delieto	S		+	±	-				±	-	-
9	Hayden	S		++	+	-	C	C	P	±	-	-
10	Williams	S		++	+	-	C	C	C	++	+	-
11	English		++	+	±	?				±	-	-
12	Kaplan	S		+	+	?				+	-	-
13	Novak	S		+	+	?				-	-	-
14	Tulp	S		+	+	?				±	-	-
15	Marsagas	S		+	+	±	C	C	P	±	-	-
16	Quinn	S	++	+	±	±	C	C	P	-	-	-
17	Nugent	S		++	+	+				++	+	-

S = treated with antitoxin. 0 = no neutralization. P = partial neutralization. C = complete neutralization.

Skin reaction to toxin:

++++ = erythema 41 to 50 mm. in diameter.

+++ = erythema 31 to 40 mm. in diameter.

++ = erythema 21 to 30 mm. in diameter.

+ = erythema 11 to 20 mm. in diameter.

± = erythema 10 mm. in diameter.

± = faintest erythema 10 to 15 mm. or more in diameter.

- = no erythema or erythema less than 10 mm. in diameter.

tions of toxin and serum antitoxin so as to obtain closer end-points of neutralization.

Results. The results of the tests are shown in table 3, together with the results of the skin test for immunity with 4 and 1 skin test

doses of toxin in the same individuals. Fourteen of the persons studied had been treated with antitoxin, three had not. It will be seen that all but one of the group contained demonstrable antitoxin in their serum as determined by the blanching test. Of these, four contained at least 20 M.B.D. of antitoxin per cubic centimeter of serum, nine contained at least 200 M.B.D. and three contained at least 2000. In the eight cases in which neutralization tests were done, seven showed the presence of antitoxin, at least 0.1 unit per cubic centimeter of serum in two cases, at least one unit in three cases, and at least ten units³ in two cases. These results support the suggestion previously made that it seems improbable that any considerable number of those who show a positive skin reaction would, under ordinary circumstances and conditions of exposure, develop a second attack of scarlet fever.

III. Comparison of skin and serum antitoxin tests

Within the limitations of the experiment, there is apparent a fairly close correlation in most of the cases between the skin reactions to toxin and the antitoxin of the serum, whether the latter be determined by means of the M.B.D. or by the end point of neutralization. The lack of agreement in Number 7 may possibly be explained on the basis of a slightly positive heat control or pseudo-reaction. Whether or not another control skin test would prove Number 10 to be allergic to a non-specific substance in the broth-filtrate has not been determined. Number 17 is another instance of disagreement between the results of the skin test and the antitoxin content of the serum. A re-test with a neutralized control instead of a heat control was refused. With these three exceptions, in no instance did the serum of an individual giving a positive skin reaction to one S.T.D. contain as much as 200 M.B.D. of antitoxin per cubic centimeter, while those giving a negative skin test to one S.T.D. all except Number 4 showed 200 M.B.D. or more of antitoxin per cubic centimeter.

It will be seen that the correlation between the skin reactions and the antitoxin content of the serum, as determined by either method, is not as close as that between the results of the blanching and of the neutralization experiments, which agree fairly well.

SUMMARY

A comparison has been attempted between the degree of late immunity to scarlet fever developed by (a) former patients who were treated with scarlet fever antitoxin during the toxemic stage of the disease and (b) those who did not receive antitoxin.

Fifty cases are presented, 34 of whom had received antitoxin and 16 no antitoxin. The skin reactivity to toxin, after an interval of from five months to two years after the onset of the disease, was determined in terms of the response to four S.T.D., one S.T.D., and a heat control of four S.T.D. Further experiments correlate the skin tests for immunity with the antitoxin content of the serum, as expressed in minimal blanching doses and in the end-points of neutralization of toxin.

DISCUSSION

Immunization to scarlet fever, in response to the stimulus of circulating toxin undoubtedly takes place, in most instances, very rapidly. Whether or not the advent of scarlet fever antitoxin therapy will so alter the natural course of immunization to the disease as to increase the incidence of second attacks of scarlet fever in individuals treated with serum, experience will show. Probably only a small percentage of those individuals in the series, in whom the Dick test remains positive, would, under ordinary conditions of exposure, contract scarlet fever. An analogous condition arises from the partial, active immunization of individuals with injections of toxin. In several instances (Dick and Dick, 1925) these individuals have subsequently contracted the disease in mild form.

Perhaps, in some instances, of relapse following inadequate antitoxin therapy is the result of the early temporary neutralization of the toxin and consequent removal of the natural stimulus to antitoxin formation.

CONCLUSIONS

1. The degree of late immunity to scarlet fever developed by patients treated with adequate therapeutic doses of scarlet fever antitoxin during the toxemic stage of the disease appears to be less than that developed by patients who did not receive antitoxin.

2. A correlation exists between the results of the skin tests of immunity to scarlet fever and the antitoxin content of the serum, whether the amount of serum antitoxin be determined by blanching or by neutralization experiments.

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