STUDIES ON THE IMMUNE RESPONSE OF THE RHEUMATIC SUBJECT AND ITS RELATIONSHIP TO ACTIVITY OF THE RHEUMATIC PROCESS. V. ACTIVE AND PASSIVE IMMUNIZATION TO HEMOLYTIC STREPTOCOCCUS IN RELATION TO THE RHEUMATIC PROCESS [1]

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There seems to be a close relationship between infection with hemolytic streptococcus, producing skin toxin, and initiation of rheumatic activity in susceptible subjects. The failure of the rheumatic patient to develop activity of the disease process when infected with a non-erythrogenic strain of hemolytic streptococcus suggests the possibility that toxin may play an important rôle in the genesis of rheumatic lesions. To test the possibility that an increase in circulating antitoxin to streptococcus might protect the tissues of the rheumatic subject and modify the disease attack, the authors have made two studies, one on active immunization with scarlatinal (NY5) toxin, the other on passive immunization with NY5 antitoxin.

I. ACTIVE IMMUNIZATION WITH SCARLATINAL TOXIN

Its relation to streptococcus infection and rheumatic fever

Reports on the effectiveness of active immunization with streptococcus toxin vary widely. Most observers agree that the procedure prevents scarlet fever and many consider it effective in lowering the incidence of throat infections and their complications, such as rheumatic fever and nephritis. The literature has been summarized by the Pickett-Thomson Research Laboratory (1). The present study has been made to determine whether active immunization with streptococcus toxin may increase resistance to infection and whether it may modify the host's reactivity.

Character of the group under observation

The individuals under observation consisted of two classes of student nurses entering training at the Presbyterian Hospital in 1932 and in 1933. Half of each class was immunized and the other half was observed as a control group. The subjects were in good health, had not experienced recent streptococcus infection, had neither history nor signs of rheumatic disease and had in most instances lived in the vicinity of New York.

Method of immunization

Fifty-two members of one class and 61 members of the other were immunized with NY5 streptococcus toxin. The material was purified and concentrated by Dr. Michael Heidelberger. Injections were given twice a week, beginning with 500 S.T.D. (skin test doses) and reaching a maximum of 80,000 S.T.D., after a period of two or three weeks. Each individual received between 300,000 and 400,000 S.T.D.

Results

The results of immunization in comparison with the control group may be considered from three standpoints: (*a*) alteration of skin reactivity; (*b*) influence on the incidence of respiratory infection; (*c*) effect on the development of rheumatic fever and nephritis.

(*a*) *Alteration of skin reactivity.* The change in skin reactivity after immunization with NY5 toxin is seen in Table I. Before immunization, the majority of individuals were Dick positive. With one exception, all were Dick negative following immunization and remained so over a period of one year. About 80 per cent became skin negative to 20 S.T.D. following immuniza-

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TABLE I

Skin reactions to streptococcus toxin before and after immunization

	Group A—1932				Group B—1933			
	Skin reactive to			Number tested	Skin reactive to			Number tested
	1 S.T.D.	5 S.T.D.	20 S.T.D.		1 S.T.D.	5 S.T.D.	20 S.T.D.	
Before immunisation....	29	44	50	52	30	37	44	61
One month after immunisation....	2	3	19	52	0	0	8	61
Four months after immunisation....	1	8	21	50				
One year after immunisation....	1	8	21	50				

tion. Skin reactivity decreased markedly in all but three individuals.

(*b*) *Influence on the incidence of respiratory infection.* A clinical record was kept on each individual and every respiratory infection was reported to the physician in charge. Cultures of the throat were made on these occasions. During the period of observation the immunized group of approximately 100 individuals contracted 24 respiratory infections associated with hemolytic streptococcus.[2] Nearly all of these individuals had negative skin tests to 20 S.T.D. In the control group there were nineteen similar infections and one case of scarlet fever.

(*c*) *Influence on the development of sequelae.* Both the control and immunized groups included only selected individuals, as all known rheumatic subjects had been removed. Nevertheless, two individuals in the immunized group developed rheumatic carditis and one severe nephritis following cervical adenitis. Likewise in the control group there was one instance of acute rheumatism and one of acute nephritis among the nineteen throat infections.

In brief, the findings indicate that although skin reactivity to streptococcus toxin was diminished by active immunization,[3] there was no evidence that it increased resistance to streptococcus infection or prevented the development of rheumatic disease.

II. PASSIVE IMMUNIZATION WITH ANTISTREPTOCOCCUS SERUM

Two findings have been almost constantly associated with activation of the rheumatic process: first, that the effective (see Paper IV of this series (2)) organisms produce strong soluble toxins, and second, that the host develops an antibody response (antistreptolysin) at the time of onset of rheumatic activity. Whether toxin is itself important in initiating rheumatic disease or whether toxin production in vitro is merely an index of a highly active strain is unknown. It seemed possible that the introduction of streptococcus antitoxin during respiratory infection and during the symptom-free phase before the onset of an expected rheumatic attack, might throw light on the rôle played by toxin in the initiation of rheumatic activity.

Group of patients studied

Ten patients were selected for this study, including seven males and three females. Their ages were between fourteen and thirty. All were highly susceptible rheumatic subjects with varying degrees of cardiac damage who had been under observation for a period of two to six years. Six of the group, while under the authors' care, had previously contracted hemolytic streptococcus infections which had been followed by severe attacks of rheumatic fever. None had experienced streptococcus disease, so far as could be determined, for at least one year. Two throat cultures had been taken on each individual every month. In each individual the rheumatic process appeared to be quiescent at the beginning of the study. All gave negative skin tests to horse serum and were in excellent condition at the onset of pharyngitis. Each individual reported the symptoms of pharyngeal infection at the onset of the respiratory infection. Throat cultures at that time showed hemolytic streptococcus in predominance. Each subject was admitted to the wards

[2] These organisms were all beta strains of hemolytic streptococcus. Some produced toxins which were neutralized by NY5 antitoxin.

[3] Skin reactivity to streptococcus nucleoprotein was not influenced.

of the Presbyterian Hospital with the characteristic clinical picture of acute pharyngitis.

Procedure

The patients were placed in bed in the hospital and the local infections were treated in the customary manner, with the exception that no salicylates were given because of the possibility of masking rheumatic symptoms. Antistreptococcus serum was given to some of the patients during the period of pharyngitis; to others at the time the pharyngitis was subsiding and to the remainder during the quiescent interval, after infection but before rheumatic manifestations were due to appear. The material used was NY5 antiserum, lot number 223B, supplied especially for this purpose by the New York State Department of Health. This antiserum was chosen because of its broad valence and because it had previously been shown (2) to neutralize, in about 70 per cent of instances, the toxins formed by organisms effective in initiating rheumatic activity in New York City. The serum was fresh, contained [4] 800 units of antitoxin per cc. and was kept at 4° C. during this study. At the beginning of immunization small quantities were diluted with saline and given intravenously. The doses were increased in the customary manner from .01 cc. to .1 cc. to 1 cc. to make sure that the patient could tolerate 10 cc. without symptoms. Only one patient experienced any distress, and in this individual (Patient G.S.) administration of serum was discontinued. The others received antitoxin each day for five days, the total dosage varying between 40,000 and 100,000 units. The usual precautions against anaphylaxis were observed with great care. Nevertheless, two individuals of the group, although skin tests were negative and although they had not received horse serum in the past, developed severe serum disease a few days after their last dose of antitoxin.

The organisms associated with the throat infections were studied shortly after isolation. Their capacity to produce soluble toxin was tested on at least two adult silver fox rabbits in the manner used throughout these studies (2). The filtrates which caused severe reactions in dilutions 1 : 200 or greater were considered strong toxin producers (+++ or ++++); those which produced 1 to 2 cm. reaction in dilution 1 : 50, weak toxin producers (+); those which caused no reaction in dilution 1 : 10 were considered negative. Negative strains were checked by making a second and in some cases a third group of filtrates. Neutralization tests were carried out in the usual manner by incubation with an appropriate amount of NY5 antitoxin at 37° C.

Blood serum was obtained under sterile conditions from each individual before the administration of any antitoxin. Samples were similarly collected shortly after the last dose and subsequently at weekly intervals. All of the specimens were stored at 4° C. and the antistreptolysin titers were determined at the completion of the experiment.

Following the administration of serum each patient was kept in bed. Electrocardiographic tracings, white blood counts and blood sedimentation rate determinations were made twice a week. Those individuals who appeared to escape recrudescences after three weeks in bed were observed in the Out-patient Department where the laboratory studies were repeated. Tables II and III, illustrative of the records kept on each subject, and their histories, are presented in brief.

CASE HISTORIES

C. G., Number 238300. The patient was a boy of fifteen who had been under observation for two years. He had experienced a typical attack of rheumatic fever at six years of age and developed mitral stenosis. In 1932 and 1933 he was in excellent health and symptom-free until the onset of pharyngitis on March 9th. He was admitted to the Presbyterian Hospital on the first day of his infection and given 90,000 units of antiserum. Clinical observations and laboratory findings are presented in Table II. This table is divided into three phases—acute pharyngitis, symptom-free interval, attack of acute rheumatism. The findings are similar to those in the other patients who developed recrudescences. During mild serum sickness the blood sedimentation rate fell to a strikingly low level.[5] This rose on the tenth day after pharyngitis, and marked leukocytosis appeared. The antistreptolysin titer rose rapidly, and on the fourteenth day he developed severe rheumatic carditis beginning with abrupt development of pyrexia. Rheumatic activity persisted for at least three months (see Table II). The polyarthritis was more intense than he had ever experienced.

[4] It also contained 4,000 units of antistreptolysin per cc.

[5] The blood sedimentation rate has been observed to be low during periods of serum sickness with edema.

TABLE II

Data on Patient C. G., Number 238,300

Disease stage	Date	Blood			Throat flora	Clinical observations	Remarks *
		W.B.C.	Sedimentation rate	Antistreptolysin titer			
	1933		*mm.*	*units per cc.*			
Phase 1 Acute hemolytic streptococcus infection	March 9	15,000			Hemolytic streptococci predominant	First day of acute pharyngitis. Temperature 101°	Ekg. T2 is diphasic
	March 10	15,400			Hemolytic streptococci predominant	Acute pharyngitis. Temperature 101°	NY5 serum number 223B 10,000 units
	March 11		16	33		Subsiding pharyngitis	NY5 serum number 223B 20,000 units
Phase 2 Symptom-free interval	March 12					Good condition. Apparently quiescent	NY5 serum number 223B 20,000 units
	March 13		8				NY5 serum number 223B 20,000 units
	March 14						NY5 serum number 223B 20,000 units
	March 15						
	March 16			33			
	March 17		4			Serum sickness. Mild urticaria	Ekg. normal. Blood sedimentation rate perhaps depressed by serum sickness
	March 18					Nausea and vomiting. Sore throat and general glandular enlargement	
	March 29	16,900					
	March 21				Hemolytic streptococci disappearing		
	March 22						
	March 23	18,550	34			Good health. Apparently quiescent	
	March 24	21,550					
	March 25	22,350		111			
Phase 3	March 27	19,200		200		Acute rheumatism. Temperature 103°. Carditis and polyarthritis	Ekg. T2 diphasic
	March 28	21,800	66				Aspirin started
Moderately severe rheumatic attack	March 29					Continued activity of the rheumatic process	
	March 30	13,900	124				Ekg. normal
	March 31						
	April 4	9,420	115	333			Aspirin stopped
	April 7						Ekg. normal
	April 28	9,800	64			Polyarthritis	Aspirin started
	May 11	9,350	86			Persistent activity—symptoms suppressed by salicylates	Hemoglobin loss 30 per cent. R.B.C. loss 1,000,000. Ekg. normal. Discharged for convalescent care
	May 15			250			Sent to Reed Farm and had recrudescence one week later
Good health	November 9 *1934*			125		Marked improvement. Symptom free	
	January 13			83		Good health, symptom free	

* Ekg. = electrocardiogram.

M. A., Number 63035. The patient was a girl of twenty who had been under observation for eight years. During this period she had three rheumatic attacks without polyarthritis. All of these followed throat infections with hemolytic streptococcus. She had developed advanced heart disease but had been free of symptoms for more than one year when she contracted influenza in January 1933. Recovery was satisfactory. The rheumatic process remained quiescent. On February 28th she contracted hemolytic streptococcus pharyngitis and was readmitted to the Presbyterian Hospital where she was given 80,000 units of antiserum. The observations during the infection, the symptom-free period and the rheumatic attack are summarized in Table III. The findings are similar to those seen in Table II. Following acute pharyngitis, there occurred a symptom-free interval and then developed the most severe rheumatic attack that this patient has experienced. Polyarthritis was intense.

RESULTS

The findings in this study of ten patients passively immunized with NY5 antistreptococcus serum are presented together in Table IV. Six individuals developed rheumatic recrudescences and four appeared to escape. Four of the six recrudescences were severe attacks that necessitated bed care for a period of months. In three of these four, the antiserum neutralized in vitro the toxic filtrate of the hemolytic streptococcus associated with the preceding throat infection. Seven of the ten strains were toxin producers, and six of these seven infections were followed by rheumatic recrudescences. In each of the six patients who had recrudescences there was a rise

TABLE III

Data on Patient M. A., Number 63035

Disease stage	Date	Blood: W.B.C.	Blood: Sedimentation rate	Blood: Antistreptolysin titer	Throat flora	Clinical observations	Remarks
	1933		*mm.*	*units per cc.*			
Influenza	January 7	7,800		16	Normal	Prostrated, temperature 104°	Severe influenza
	January 8			14		Improving	
	January 30	6,080	12	20	Normal	Symptom-free	No sequelae from influenza
Phase 1 Acute hemolytic streptococcus infection	February 28	14,700	45	25	Hemolytic streptococci predominant	Onset of acute pharyngitis. Temperature 101°	NY5 antiserum 20,000 units
	March 2				Hemolytic streptococci predominant	Acute pharyngitis. Temperature 102°	NY5 antiserum 20,000 units
	March 3				Hemolytic streptococci predominant	Acute pharyngitis. Temperature 102°	NY5 antiserum 20,000 units
	March 4	13,700				Subsiding pharyngitis. Temperature 99°	NY5 antiserum 20,000 units
Phase 2 Symptom-free interval	March 5					Symptom-free	Ekg.—incomplete bundle branch block, present for five years
	March 6	14,700	49			Symptom-free	
	March 7					Symptom-free	
	March 8	11,600	60			Symptom-free	
	March 9			111		Symptom-free	
Phase 3 Severe rheumatic attack	March 10				Few hemolytic streptococci	Malaise, pain in right shoulder and left forearm	Mild rheumatic symptoms
	March 11					Pains in arms and legs. Temperature 100°	
	March 12					Mild glandular enlargement. Temperature 100°	Mild serum sickness
	March 13	12,800	36			Cervical adenitis. Temperature 101°	
	March 14				Few hemolytic streptococci	Severe muscle pains. Temperature 102°	
	March 15	14,600	94		Few hemolytic streptococci	Severe carditis and polyarthritis. Temperature 103°	Intense rheumatic attack
	March 16				Few hemolytic streptococci	Pyrexia. 101° to 102°	Ekg.—tachycardia
	March 21	10,280	90	125	Few hemolytic streptococci	Pyrexia. 101° to 102°	Salicylate therapy
	March 23	9,780	91	125	Few hemolytic streptococci	Urticaria	
	March 28	12,080	65		Few hemolytic streptococci	Symptoms subsiding	
	March 30	8,920	65		Few hemolytic streptococci	Symptoms subsiding	
	April 5		60			Symptoms subsiding	
	April 8		94	125		Tachycardia	
	April 17	11,100	65				Salicylates discontinued
	April 24	8,250	32				
	April 28						
	May 11					Slowly subsiding activity	Discharged from Presbyterian Hospital
Heart failure	July 25	8,260	12	83		Cardiac insufficiency	Readmitted to Presbyterian Hospital
Good health	October 26			71	Normal	Symptom-free	Living in Brooklyn
	1934 January 22			50	Normal	Symptom-free	Working as salesgirl

TABLE IV

The effect of passive immunization with streptococcus antitoxin on rheumatic subjects recovering from streptococcus pharyngitis

Patient: Name	Patient: Age	Organism: Strain of hemolytic streptococcus	Organism: Sugar fermentation type	Organism: Toxin production	Organism: Toxin neutralization with NY5 antiserum	Antiserum: Total number of units	Antiserum: Time of administration	Antistreptolysin titer: During infection	Antistreptolysin titer: During quiescent interval		Antistreptolysin titer: During period of attack		Clinical result
	years							*units per cc.*	*units per cc.*	*units per cc.*	*units per cc.*	*units per cc.*	
M.A.	16	S 65**	Pyogenes	++++	Complete	80,000	During pharyngitis	25		111	125	125	Severe attack 1 week after serum
C.G.	15	S 69	Pyogenes	+++	None	90,000	During pharyngitis	33	33	111	200	250	Severe attack 10 days after serum
F.R.	16	S 67	Subacidus	+++	Complete	100,000	At end of pharyngitis	83		143	250	250	Severe attack 17 days after serum
J.W.	15	S 90	Infrequens	+	Complete	40,000	1 week after pharyngitis	167	167	200	200	250	Severe attack 2 days after serum
J.Z.	30	S 61	Pyogenes	+++	Incomplete	100,000	During pharyngitis	71	83	100	125	100	Mild attack 16 days after serum
M.O.*	21	S 62	Equi	+++	None	80,000	During pharyngitis	25	56		111	167	Mild attack 12 days after serum
W.P.*	21	S 77	Pyogenes	+++	Complete	80,000	At end of pharyngitis	100	111	143	143		Attack apparently escaped
J.R.	15	S 76	Hemolytic II	None		40,000	During pharyngitis	100	56	63	71	63	Attack apparently escaped
P.H.	17	S100	Pyogenes	None		40,000	1 week after pharyngitis	63	62	71	100		Attack apparently escaped
G.S.	14	S 89	Pyogenes	None		1,000	At end of pharyngitis	33	33	33	33		Attack apparently escaped

* Developed severe serum sickness the day following the last dose of antitoxin.

** S designates strains from patients treated with serum.

in antistreptolysin titer at the onset of the attack. Of the four individuals who appeared to escape recrudescences, three were infected by organisms that produced no detectable soluble toxin. The antistreptolysin titer remained low in these cases. The results presented in Table IV are in accord with the findings already described (2) for patients who received no antiserum; that is, recrudescences followed infections with agents that produced soluble toxin, and occurred with a response of the antibody mechanism.

DISCUSSION

It is seen that active immunization with streptococcus toxin does not afford protection against respiratory infection with hemolytic streptococcus. It is also evident that the introduction of antitoxin just after streptococcus infection is ineffective. This result is strikingly different from the effects of passive immunization following tetanus infection. The observations suggest that if streptococcus toxin plays a rôle in the genesis of rheumatic lesions, the relationship is probably not simply one of direct damage to mesodermal tissues.

One patient, W. P. Number 257703, is of especial interest. While under observation he contracted hemolytic streptococcus pharyngitis in 1931. The organism was a strong toxin producer, and the patient developed a sharp rise in antistreptolysin titer coincident with the onset of a severe rheumatic attack. In the present study, he was also infected by a strain which produced strong toxin. However, in this instance he developed only a slight rise in antistreptolysin titer and escaped all evidence of rheumatic activity. Whether the occurrence of severe serum disease in this individual modified the antibody response is unknown. His failure to develop a recrudescence suggests that if streptococcus toxin is a factor in the production of rheumatic activity, its effectiveness is dependent upon the immune response of the host.

SUMMARY

Active immunization with streptococcus toxin neither prevents streptococcus infection nor inhibits the development of the rheumatic process.

The introduction of protective antibodies just prior to the expected attack does not decrease and may possibly increase the intensity of the rheumatic recrudescence.

The development of rheumatic activity appears to depend not only upon infection with a toxin producing strain of hemolytic streptococcus but also upon the host's immune response to this infection.

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