Protective Study with a Group A Streptococcal M Protein Vaccine

INFECTIVITY CHALLENGE OF HUMAN VOLUNTEERS

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ABSTRACT Healthy adult male volunteers were immunized with purified M protein from Group A streptococci, Type 1. The vaccine was administered subcutaneously as an aluminum hydroxide-precipitated antigen in three monthly doses. Control subjects received a placebo of the aluminum hydroxide adjuvant. To test the efficacy of the immunization, vaccinees and controls were challenged with a virulent strain of Type 1 streptococci applied to the pharynx. The immunization and challenge of the vaccinated and control subjects (19 men in each group) were carried out as a double blind experiment. All subjects were carefully screened by physical and laboratory examinations before and after the immunization and infectivity schedules. 30–50 days after the last injection, the vaccinees and control subjects were infected with the streptococci. Careful surveillance was maintained to evaluate the extent of acquired streptococcal infection. Throat cultures, leukocytes counts, temperatures, and physical signs and symptoms were monitored daily. All subjects received 1.2 million U of penicillin intramuscularly no later than 6 days after inoculation with the culture. Illness was judged by the appearance of exudative pharyngitis and cervical adenopathy accompanied by a positive throat culture. By these criteria, 9 of the 19 placebo controls, and 1 of 19 vaccinees were ill. No residual illness or clinical complications was observed after the penicillin treatment. It is concluded that the alum-precipitated M protein vaccine afforded protection against an upper respiratory Type 1 streptococcal infection.

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INTRODUCTION

Acquired immunity after a Group A streptococcal infection is type-specific, and is believed to result primarily from the induction of bactericidal (opsonic) antibodies. These antibodies are directed against the M antigens, the cell wall proteins responsible for the specificity of the 50 or more serotypes within Group A (1). Methods for the purification of M proteins and the induction of active immunity have been studied in this laboratory for the past 7 yr (2–4). Purified M protein vaccines were shown in experimental animals to be immunogenic in the absence of local or systemic reactions. These experiments led to cautious human clinical experimentation with M protein vaccines (5, 6). To date, over 150 adults and 90 infants and children have been immunized with M protein vaccines of the more common sterotypes, 1, 3, 6, and 12. Generally, three doses of 50 μg of M protein precipitated with alum sufficed to induce primary bactericidal antibodies in about 75% of young children. By selecting subjects without delayed cutaneous hypersensitivity to 1-μg intracutaneous doses of the soluble M protein, local and systemic reactions to the immunogenic vaccine have been largely avoided.

Long-term field trials with populations of children or young adults at risk in communities where streptococcal upper respiratory disease is prevalent will be the ultimate test of the efficacy of M protein vaccines. In the meantime, the protective capacity of a Type 1 M protein vaccine has been assessed by immunizing a limited number of adult volunteers and challenging them with

live homologous streptococci. The details and outcome of these infectivity experiments are presented.[1]

METHODS

M protein vaccine and reagents. Purified M protein was prepared from cell walls of Group A streptococcus, strain CDC SS-496, Type 1. This organism was passed through mice three times to improve M protein production and virulence. Procedures for the purification and preparation of alum-precipitated M-1 protein (APM-1)[2] and soluble M-1 protein have been described in detail (3, 4). One dose of APM-1 consisted of 90 μg of purified M protein combined with 2.5 mg of aluminum hydroxide in 0.5 ml of Ringer's lactate buffer (RLB) (Abbott Scientic Products Div., Abbott Laboratories, South Pasadena, Calif.). Placebo vaccine consisted of 0.5 ml of RLB containing 2.4 mg of aluminum hydroxide/dose. Subjects received the vaccine or placebo in three doses at monthly intervals. The skin test antigen contained 10 μg of soluble purified M protein in 1.0 ml of RLB; 0.1 ml of this protein solution was used to determine cutaneous delayed hypersensitivity to Type 1 M protein. All injectable materials were within acceptable limits when assayed in laboratory animals for safety, toxicity, and pyrogenicity according to protocols of the United States Public Health Service, Division of Biologics Standards (7). Screening of subjects for penicillin allergy was carried out with 0.05 ml doses of penicilloyl polylysine, 6×10^{-5} M (Cilligen, Sigma Chemical Co., St. Louis, Mo.).

Selection of subjects. Healthy male volunteers between the ages of 21 and 35 yr were screened by physical and laboratory evaluations, including electrocardiograms. Subjects with histories of heart or kidney disease or known allergies were rejected. Intradermal skin tests on the volar surface of the forearm were carried out with soluble M-1 protein (1.0 μg in 0.1 ml) and we rejected those subjects with a delayed cutaneous reaction greater than 0.8 cm erythema and induration. Subjects with an immediate wheal and flare reaction to an intradermal dose of penicilloyl polylysine were also rejected, as were subjects whose sera contained Type 1 bactericidal antibodies before immunization. All volunteers were informed of the details and possible hazards of this clinical experiment.

Antibody assay. Serum from each subject was collected just before the vaccine or placebo inoculations and 30 days after the last dosage. Type-specific antibody was assayed by a microcomplement fixation assay (8) and the indirect bactericidal test (9). In the indirect bactericidal assay, the number of colonies growing on a blood agar plate after incubation with human leucocytes and test serum were counted. The level of bactericidal antibodies was compared to normal rabbit serum. In scoring results, + equals a 0.5 log or greater diminution in the last two bacterial dilutions, ± equals a 0.2–0.5 log diminution in the last two bacterial dilutions, and 0 equals no significant difference between colony counts after incubation in the test serum when compared to growth in normal rabbit serum. The percentage of complement fixed was determined in an assay system containing the test serum diluted 1:100 and 0.1 μg of Type 1 M protein in 0.5 ml of buffer. Previous studies have shown a general correlation between the presence of complement-fixing M protein-specific antibodies and bactericidal activity (8).

Culture and infectivity procedures. 18-h cultures of Group A Type 1 streptococci grown in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) were washed in sterile 0.01 M phosphate-buffered saline (PBS) and resuspended in PBS to approximately 4×10^8 colony-forming U/ml, standardized by spectrophotometric measurement and subsequent colony counts on sheep blood agar plates. All subjects were infected between 30 and 50 days after the third dose of vaccine or placebo. A sterile cotton-tipped applicator was dipped once into this culture and the suspension was painted liberally over the pharyngeal and tonsillar areas. Throat cultures were obtained with cotton swabs wiped around the tonsillar ring and crisscrossed over the pharynx. The swabs were rolled at one edge of a sheep blood agar plate over a swath about 1 × 3 cm, and microorganisms in that area were streaked over the plate with a loop. During the course of immunization, throat cultures were obtained monthly from all subjects and no intercurrent Group A streptococci were observed.

Groups of 12–14 men, divided about equally between vaccinated and control subjects, were infected simultaneously. The first two groups of men were quartered individually under strict quarantine. Subsequently, groups of 10–12 subjects were maintained in an isolated 12-bed ward with about 4 ft of space between beds. The outcome of the experiment was independent of those two arrangements of quarters. Throat cultures were taken immediately before infection was initiated and daily thereafter. Temperatures were measured every 12 h and peripheral blood for leucocyte counts was obtained daily. Neither the subjects nor the personnel carrying out the laboratory and clinical evaluations were informed of the status of the volunteers as control subjects or vaccinees until the completion of the experiment.

After inoculation with the microorganisms, the men were observed for 6 days, during which time or immediately thereafter each man received an intramuscular injection of 1.2 million U of benzathine penicillin G and procaine penicillin G (Bicillin C-R, Wyeth Laboratories, Inc., Philadelphia, Pa.). All men whose throat cultures were positive during the 6-day observation period received a second dosage of penicillin 5 days after the first injection. Those who exhibited more severe signs and symptoms of infection (malaise with fever, chills, and physical prostration) received the penicillin therapy 18–24 h after the onset of symptoms. In these cases, the infection was treated 3–4 days after inoculation with the streptococci. The scoring system for the evaluation of illness is outlined in Table I.

RESULTS

Among the 19 vaccinees, postimmunization sera of 17 exhibited a significant (15% greater) increase in the type-specific complement-fixing antibody titer when compared to the preimmunization sera, and 5 of these immune sera contained opsonic antibody as well. None of the sera from the subjects who received placebo showed significant changes in the Type 1 antibody levels. These data are shown in Tables IIA and B.

Before challenging the vaccinees and placebo control subjects, it was necessary to ensure that the Type 1

[1] This clinical trial was carried out with inmate volunteers at the Florida State Correctional Institution, Raiford, Fla.

[2] *Abbreviations used in this paper:* APM-1, alum-precipitated M-1 protein; PBS, phosphate-buffered solution; RLB, Ringer's lactate buffer.

TABLE I
Evaluation of Infection

Parameter	Evaluation		Scored
Fever	Degrees over base line for 24 h or longer	<1.0°C	0
		1.0°C–1.5°C	1
		>1.5°C	2
Leukocyte count	Increase over base line for 24 h or longer	<100%	—
		>100%	+
Throat culture	Colonies on a blood agar plate from pharyngeal swab; maximum in 24-h period	0	0
		1–10	1
		10–100	2
		100–500	3
		>500	4
Clinical upper respiratory signs*	Erythema: mild redness to extensive hyperemia with edema Exudative pharyngitis: occasional plaques to extensive bilateral exudation Cervical adenopathy: swollen, tender nodes		0–4
Symptoms*	Sore throat, headache, malaise, chills, nausea		0–4

* Maximum in 24 h.

streptococcus strain was capable of inducing an upper respiratory infection. Accordingly, six men whose sera contained no Type 1 bactericidal antibodies were selected. These men were inoculated in the pharynx with a swab from a washed culture of streptococci (see Methods) containing 4×10^6 colony-forming U of streptococci/ml (Group I, Table IIC).[3] The men were observed for 6 days to evaluate the extent of infection according to the parameters outlined in Table I. Five of the six men acquired positive throat cultures which persisted until the penicillin treatment on the 6th day after infection. Three of these five men showed obvious signs and symptoms of streptococcal infection as evidenced by pharyngeal erythema with exudate, enlarged, tender cervical lymph nodes, fever, leucocytosis, and moderate malaise.

When we had thus established that the Type 1 streptococci were capable of inducing a clinical infection, the vaccinees and placebo control subjects were challenged in the same manner as the six untreated subjects of group I. 12–14 men at a time, equally distributed among groups II and III, were infected 30–50 day after receiving the last injection of M protein or placebo. Table IIA summarizes the result of the immunization and infectivity challenge of the 19 vaccinees. The degree of illness, defined by clinical signs and symptoms of an upper respiratory infection, was graded "none," "questionable," or "obvious." By the criteria of exudative pharyngitis and cervical adenopathy (i.e., enlarged, tender nodes) concomitant with the presence in the pharynx of Type 1 streptococci (confirmed by serotyping), 1 of the 19 vaccines had an obvious illness. Four others had mild signs and minimal symptoms and were classified as questionably ill. The remaining 14 men showed no significant signs or symptoms of infection and therefore were considered not ill. In contrast to the vaccinees, the rate of acquisition of infection was considerably higher among the subjects who had received placebo injections. In group III (Table 2B) nine men were obviously ill and five others had mild signs and symptoms, although two of these latter five subjects may not have acquired an infection owing to the absence of culturable Group A streptococci from throat swabs. It should be noted that positive throat cultures were obtained from all subjects who were classified as obviously ill. However, three other vaccinees, 8, 14, and 15, harbored considerable numbers of streptococci in the upper respiratory tract without clinical illness during the 6-day observation period.

Except for those subjects whose infection was accompanied by obvious malaise with fever and prostration, some of the symptoms of the subjects were not always reliable criteria of illness. Minor complaints of "headache" and "sore throat" were nearly as frequent among

[3] The virulence of this Type 1 streptococcal strain had been previously established with four volunteers (medical students) about 10 mo before the present immunization trials began. These four subjects developed upper respiratory infections similar in severity to subjects 3, 4, and 6 described in Table 2C. Because of the prolonged storage of the streptococcal cultures at −70°C and several transfers thereafter in laboratory media, it was deemed necessary to reconfirm the virulence of the strain as described above.

TABLE IIA

Response to Challenge in APM-1-vaccinated Subjects (Group II)

Subjects: APM-1 vaccinees	Antibody assay*				Response to infection											
	% Complement fixed		Bactericidal test					Upper respiratory signs			Symptoms					
	Pre.	Post.	Pre.	Post.	Throat culture	Fever	Leukocyte count	Erythema	Exudate	Nodes	Sore throat	Headache	Malaise	Chills	Nausea	Estimated illness
7	5	89	−	−	0	0	−	0	0	0	0	0	0	0	0	None
8	14	89	−	−	3	0	−	0	0	0	0	0	0	0	0	None
9	8	85	−	±	4	0	−	2	1	1	2	0	2	0	0	Obvious
10	0	70	−	+	0	0	−	0	0	0	0	0	0	0	0	None
11	0	64	−	−	4	0	−	1	0	1	0	0	0	0	0	Questionable
12	0	10	−	−	2	0	−	2	0	0	0	0	0	0	0	Questionable
13	2	60	−	±	0	0	−	0	0	0	0	0	0	0	0	None
14	0	92	−	±	4	1	−	1	0	0	0	1	0	0	0	None
15	0	100	±	+	3	0	−	1	0	0	0	0	0	0	0	None
16	26	92	−	−	4	0	−	1	0	1	2	0	0	0	0	Questionable
17	0	87	−	+	0	1	−	0	0	0	1	0	0	0	0	None
18	0	95	−	±	0	0	−	0	0	1	0	1	1	0	0	None
19	3	5	−	±	0	0	−	0	0	0	1	0	0	0	0	None
20	3	80	±	±	0	0	−	0	0	0	1	1	0	0	0	None
21	0	34	−	+	0	0	−	0	0	0	1	1	0	0	0	None
22	0	93	±	+	0	0	−	0	0	0	0	0	0	0	0	None
23	8	95	±	−	4	0	+	1	0	1	0	0	0	0	0	Questionable
24	0	27	−	±	0	0	−	0	0	0	0	0	0	0	3	None
25	0	15	−	−	0	0	−	0	0	0	0	1	0	0	0	None

* Pre., serum 1 day before first vaccine dose; Post., serum 30 days after third vaccine dose.

vaccinees as control subjects, infected or not. In this respect, most of subjects were heavy smokers and the ambient daytime temperature ranged between 25°C and 35°C, seasonal for the area of Florida where this study was carried out.

Fig. 1 shows the daily clinical course of four subjects, illustrating a number of phenomena typified in these trials. A positive throat culture did not appear in two subjects until 24 h after the organism was introduced. Vaccinee 23, whose illness was questionable, had a mild

TABLE IIB

Response to Challenge in Placebo Control Subjects (Group III)

Subjects: placebo controls	Antibody assay*				Response to infection											
	% Complement fixed		Bactericidal test					Upper respiratory signs			Symptoms					
	Pre.	Post.	Pre.	Post.	Throat culture	Fever	Leukocyte count	Erythema	Exudate	Nodes	Sore throat	Headache	Malaise	Chills	Nausea	Estimated illness
26	0	0	−	−	0	0	+	1	0	0	0	0	0	0	0	None
27	80	85	−	−	4	0	+	1	2	2	2	0	0	0	0	Obvious
28	10	10	−	−	0	0	−	0	0	1	0	0	0	0	0	None
29	0	0	−	−	3	0	+	1	0	0	0	0	0	0	0	None
30	30	40	−	±	4	0	+	2	2	2	2	0	2	0	0	Obvious
31	8	0	±	±	4	0	−	2	2	1	1	0	1	0	0	Obvious
32	0	0	−	−	4	0	−	2	1	2	1	1	1	0	1	Obvious
33	2	5	−	−	2	2	−	3	1	1	1	0	2	0	2	Obvious
34	0	0	−	−	3	0	−	1	0	1	1	0	0	0	0	Questionable
35	97	95	±	±	4	2	−	2	2	2	2	2	1	0	0	Obvious
36	0	0	−	−	0	0	−	1	0	1	0	0	1	0	0	Questionable
37	21	21	±	±	0	0	−	0	0	0	1	1	0	0	0	None
38	0	0	±	±	4	0	−	3	2	2	2	1	2	1	0	Obvious
39	8	3	−	±	0	0	−	1	0	0	0	0	0	0	0	None
40	0	4	−	−	4	0	−	1	0	1	0	1	0	0	0	Questionable
41	0	0	−	−	0	0	−	1	0	1	1	1	0	0	0	Questionable
42	14	11	±	−	4	2	+	2	3	2	3	0	2	0	2	Obvious
43	7	5	±	±	2	2	+	2	2	2	2	2	4	2	3	Obvious
44	0	0	−	−	2	0	−	2	0	0	1	0	0	0	0	Questionable

* Pre., serum 1 day before first placebo dose; post., serum 30 days after third placebo dose.

TABLE IIC

Response to Challenge in Untreated Control Subjects (Group I)

Subjects: controls, no placebo	Antibody assay*		Response to infection											
						Upper respiratory signs			Symptoms					
	% complement fixed	Bactericidal test	Throat culture	Fever	Leukocyte count	Erythema	Exudate	Nodes	Sore throat	Headache	Malaise	Chills	Nausea	Estimated illness
1	0	–	0	0	–	0	0	0	0	1	0	0	0	None
2	0	–	3	1	+	2	2	3	1	1	2	0	1	Obvious
3	12	–	4	0	–	0	0	0	1	0	0	0	0	None
4	8	–	2	0	–	1	0	1	0	1	0	0	0	Questionable
5	0	–	2	2	+	2	2	3	0	0	2	0	0	Obvious
6	0	–	4	2	+	2	1	1	2	1	2	0	0	Obvious

* Serum sample obtained 2 days before challenge.

erythematous pharynx and a slightly tender cervical lymph node, but no symptoms in spite of an elevated leukocyte count. Vaccinee 18 rapidly eliminated all streptococci from his pharynx and exhibited no significant clinical signs or symptoms. In control subjects 30 and 35, clinical manifestations appeared within 48 h of introduction of the streptococci. The infections were obvious enough to make a definite diagnosis, and penicillin was administered on the 4th day in order not to prolong the discomfort of the subjects. In the three cases with positive throat cultures in Fig. 1, all were negative 24 h after treatment, although in some of the other subjects, a few Group A Type 1 streptococci persisted for 24–48 h after antibiotic intervention. As previously mentioned, a second penicillin injection was administered to all infected subjects to ensure the complete elimination of the streptococci.

Six vaccinees whose preliminary skin test resulted in a delayed cutaneous reaction of 0.3–0.6 cm exhibited a moderate swelling and tenderness at the sites of APM-1 injections, lasting 24–48 h. Four of these men had a transient fever of 1–2°C for 12–24 h after the first two APM-1 injections. Complete laboratory and physical examinations of all subjects were carried out 3 wk after the penicillin treatment, and no residual illness resulted from this trial.

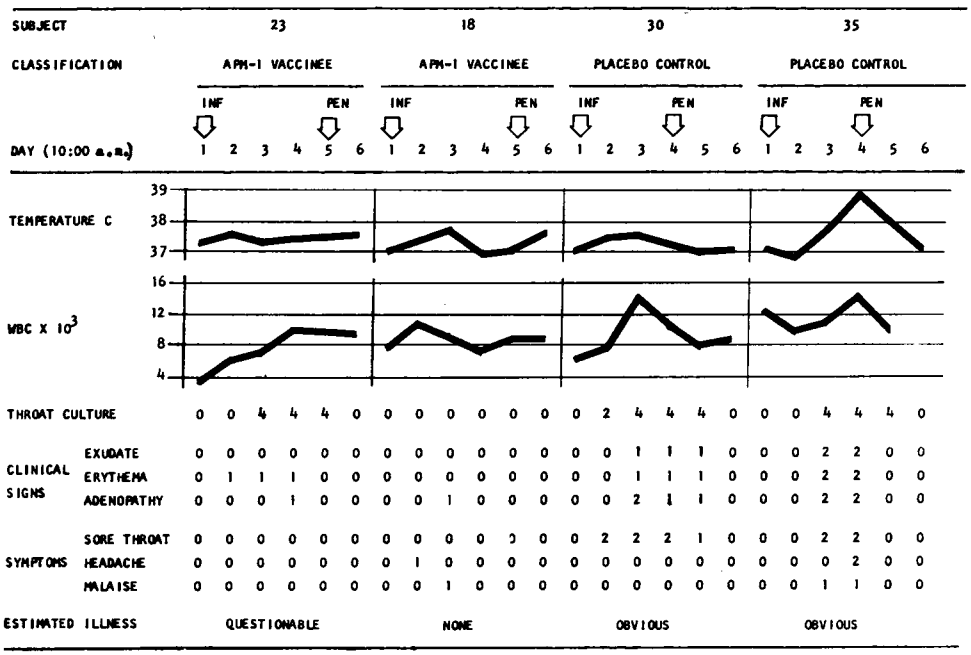

FIGURE 1 Clinical course of four subjects at the time of streptococcal challenge. Arrows indicate days on which subjects were infected with the culture and treated with penicillin. See Table II for explanation of the clinical evaluation. WBC, white blood cell count.

The results obtained from the three groups of men were analysed statistically by a system of hypergeometric distribution (10). In evaluating the criteria of obvious illness, we included the requisites of exudative pharyngitis and/or tonsillitis, cervical adenopathy, and a positive throat culture. The volunteer without all of the above symptoms or signs was considered "not ill" for analytical calculations. In this dichotomous classification of the 44 subjects, 1 of 19 vaccinees was clinically ill, and 12 of the 25 controls were clinically ill. The probability (P) that the data could be the result of a chance distribution is less than 4 in 1000. If we consider only the 19 subjects who received APM-1 and the comparable 19 placebo controls, 1 of the vaccinees and 9 of the controls acquired a clinical infection; a statistical analysis of these results with 38 subjects yields a $P < 0.01$.

DISCUSSION

Our previous data on the use of a microcomplement-fixing assay for type-specific antibody showed that with serotypes 3, 6, and 12, high titers of complement-fixing antibodies were generally indicative of bactericidal activity (8). It was also observed that these complement-fixing reactions were type-specific. We have no explanation as to why the Type 1 antibodies from the present vaccinees, although exhibiting relatively high titers in the microcomplement fixation reaction, were only bactericidal in about a third of these subjects. Approximately half of the vaccinees appeared to have no previous exposure to Type 1 streptococci, inasmuch as their antibody titers were not elevated until after the second or third dose of vaccine. There was no correlation between the appearance of rapid secondary response after the first dose of vaccine and the degree of illness resulting from the challenge.

The data indicate that although immunization with purified Type 1 M protein may present the acquisition of clinical illness in subjects challenged with homologous streptococci, the role of measurable bactericidal levels of antibody has not been clarified. The one subject, 9, among the vaccinees who developed an obvious clinical illness had type-specific antibodies measurable by complement fixation and the indirect bactericidal test. Another immunized subject, 25, had little, if any, type-specific antibody after immunization but nevertheless within 24 h completely cleared the streptococci from his pharynx and was free of clinical illness. Subjects 8, 14, and 15, in the immunized group, carried the streptococci in their pharynx for 5 or 6 days without becoming ill. Because subjects were treated with penicillin 4–6 days after exposure to the streptococci, it was not possible to determine whether carriage of the streptococci in the immunized subjects would be self-limiting and without accompanying clinical disease. Although an elevated leukocyte count and fever were less frequent among vaccinees than controls, these two signs were the least reliable factors per se in judging the acquisition of infection during the 6-day observation period. As demonstrated in the examples of Fig. 1, two control subjects manifested obvious clinical illness; one had an elevated leukocyte count without fever, and other exhibited a fever with only a slightly elevated leukocyte count.

The chronology of acquisition of a positive throat culture was of interest. In nearly half of the vaccinees and controls whose throat cultures were positive for Type 1 streptococci, few if any colonies could be detected from throat swabs taken 12–24 h after the organisms were inoculated into the pharynx. During this initial period, the streptococci (it was estimated that at least 10^5 were deposited) appeared to be completely cleared from the pharyngeal and tonsillar mucosa. In those subjects who proceeded to develop clinical symptoms, which in all cases appeared 36–72 h after exposure, the streptococci reappeared in the throat cultures. Although fewer immunized subjects in comparison to the controls in group III were colonized (8, 13, respectively) with Type 1 streptococci, the difference was not statistically significant. Therefore the vaccine, although preventing the manifestation of clinical illness, may be less effective in preventing streptococcal colonization.

In epidemiological studies carried out at the Streptococcal Disease Laboratory, Warren Air Force Base, Wyoming, shortly after World War II, Ramelkamp attempted to induce streptococcal infections in volunteer military and civilian personnel (11). By direct swab transfer from the pharynx of a naturally infected subject, susceptible volunteers were successfully inoculated with Type 19 streptococci. Results with one subject were described in detail and the sequence of events leading to the appearance of symptoms was similar to those described in this report. Throat cultures were negative until about 40 h after inoculation, when the subject complained of a sore throat. 24 h later, exudate and malaise with several degrees of fever were observed; only a slight elevation in the leukocyte count was noted. The infection was terminated when penicillin was administered 64 h after the streptococci had been introduced.

A number of attempts to immunize small groups of humans with M proteins have been described during the past 20 yr (see reviews by Gill [12] and Stollerman [13]). In most of these trials, local and systemic reactions to the vaccines resulted in abandonment of the projects, in spite of the fact that type-specific antibodies could be induced in some trials. It has been demonstrated that microgram amounts of Group A streptococcal cell wall-carbohydrate complexes are capable of rapidly inducing necrotic lesions in experimental animals (14). These substances in partially purified or crude M antigen vaccines

employed in early studies may have been responsible for the adverse reactions. Delayed cutaneous hypersensitivity to other antigens could also have been a deleterious factor.

In 1945, at a United States Naval Base, a protective study was carried out (15). This was only previously reported clinical trial in which the efficacy of a streptococcal vaccine was measured in terms of actual protection. Over 1,000 personnel were immunized with heat-killed or ultraviolet-inactivated Types 17 and 19 streptococci in three doses of approximately 10^9 cells/injection. The streptococcal attack rate among the immunized men, followed over a period of months, was not diminished when compared to unimmunized controls. No attempts were made to determine the antibody responses to the vaccines, and it may be surmised that the antigenic dose was not adequate to afford type-specific protection.

Massell, Michaels, Amezcua, and Siner, and Massell, Honikman, and Amezcua (16, 17) reported a streptococcal immunization study in which children, siblings of rheumatic fever patient, were immunized with as many as 18 weekly doses of partially purified M proteins. They succeeded in inducing type-specific antibodies in some of these children. However, among the vaccinees, two and possibly three cases of rheumatic fever developed in subsequent months because of streptococcal infections by heterologous serotypes. The authors stated that these cases of rheumatic fever could have developed as a result of "sensitization" to the vaccine material, allowing for greater susceptibility to the nonsuppurative complications during later exposure to Group A streptococci. It should be noted that in these experiments, Massell and colleagues employed an M antigen which, from a description of their methodology, could only be described as partially purified. Their dosages were approximately 100 times those used by Fox, Pachman, Wittner, and Dorfman (6), who induced bactericidal antibodies in children with purified M proteins.

It is well established that group A streptococci share antigens in common with mammalian heart tissues. This was first demonstrated by Kaplan and Meyeserian, and Kaplan and Svec, who observed that cell wall antigens chemically or physically associated with M protein fractions cross-reacted with human myofibers (18, 19). Several reviews summarizing research on the autoimmune hypothesis of rheumatic fever have been published (20, 21), but no cause-and-effect relationship has been established between the presence of antiheart antibodies and rheumatic fever. Data may be cited which do not support the role of M proteins in the induction of nonsuppurative complications of Group A streptococcal infections. Purified M proteins did not induce antimyofiber antibodies in immunized humans or experimental animals, nor could M proteins absorb antiheart antibodies from the sera of rheumatic fever patients (22). Zabriskie and Freimer (23) claim that the cardiac cross-reacting antigen is in the streptococcal cytoplasmic membrane, completely separable from the M protein. Thus, the safety of the vaccine employed in the present study has yet to be clinically challenged or overwhelmingly verified. The utility of streptococcal immunization awaits the outcome of further cautious clinical experimentation and, ultimately, field trials.

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REFERENCES

1. Lancefield, R. C. 1970. Current knowledge of type-specific antigens of Group A streptococci. *J. Immunol.* **89**: 307.
2. Fox, E. N., and M. K. Wittner. 1965. The multiple molecular structure of the M proteins of Group A streptococci. *Proc. Natl. Acad. Sci. U. S. A.* **54**: 1118.
3. Fox, E. N., and M. K. Wittner. 1966. Antigenicity of the M proteins of Group A hemolytic streptococci. II. Antibody response in rabbits to vaccines prepared with oil emulsions and aluminum hydroxide. *J. Immunol.* **97**: 86.
4. Fox, E. N. 1968. Purification and properties of Group A streptococcal M protein. *In* Current Research on Group A Streptococcus. R. Caravano, editor. Excerpta Medica Foundation, Publishers, Amsterdam. 68.
5. Fox, E. N., M. K. Wittner, and A. Dorfman. 1966. Antigenicity of the M proteins of Group A hemolytic streptococci. III. Antibody responses and cutaneous hypersensitivity in humans. *J. Exp. Med.* **124**: 1135.
6. Fox, E. N., L. M. Pachman, M. K. Wittner, and A. Dorfman. 1969. Primary immunization of infants and children with Group A streptococcal M protein. *J. Infect. Dis.* **120**: 598.
7. United States Public Health Service. 1965. Regulations for Biological Products, Title 42, Part 73, General Safety. 14.
8. Wittner, M. K., and E. N. Fox. 1971. Microcomplement fixation assay for type-specific Group A streptococcal antibody. *Infect. Immun.* **4**: 441.
9. Lancefield, R. C. 1957. Differentiation of Group A streptococci with a common R antigen into three serological types, with special reference to the bactericidal test. *J. Exp. Med.* **106**: 525.
10. Lehmann, E. L. 1959. Testing Statistical Hypotheses, John Wiley & Sons, New York. 143.
11. Rammelkamp, C. H., Jr. 1955–56. Epidemiology of streptococcal infections. *Harvey Lect.* 113.
12. Gill, F. A. 1960. A review of past attempts and present concepts of producing streptococcal immunity in humans. *Quart. Bull. Northwest. Univ. Med. Schl.* **34**: 326.

13. Stollerman, G. H. 1967. Prospects for a vaccine against group A streptococci: the problem of the immunology of M proteins. *Arthritis Rheum.* **10**: 245.
14. Schwab, J. H. 1965. Biological properties of streptococcal cell wall particles. I. Determinants of the chronic nodular lesion of connective tissue. *J. Bacteriol.* **90**: 1405.
15. Young, D. C., et al. 1946. Failure of type-specific *Streptococcus pyogenes* vaccine to prevent respiratory infections. *U. S. Nav. Med. Bull.* **46**: 709.
16. Massell, B. F., J. G. Michael, J. Amezcua, and M. Siner. 1968. Secondary and apparent primary antibody responses after group A streptococcal vaccination of 21 children. *Appl. Microbiol.* **16**: 509.
17. Massell, B. F., L. H. Honikman, and J. Amezcua. 1969. Rheumatic fever following streptococcal vaccination. Report of three cases. *J. Am. Med. Assoc.* **207**: 1115.
18. Kaplan, M. H., and M. Meyeserian. 1962. An immunological crossreaction between group A streptococcal cells and human heart tissue. *Lancet.* **1**: 706.
19. Kaplan, M. H., and K. H. Svec. 1964. Immunologic relation of streptococcal and tissue antigens. III. Presence in human sera of streptococcal antibody cross-reactive with heart tissue. Association with streptococcal infection, rheumatic fever and glomerulonephritis. *J. Exp. Med.* **119**: 651.
20. Zabriskie, J. B. 1967. Mimetic relationships between group A streptococci and mammalian tissues. *Adv. Immunol.* **7**: 147.
21. Kaplan, M. H. 1967. Multiple nature of the cross-reactive relationship between antigens of the group A streptococci and mammalian tissue. *In* Cross-reacting Antigens and Neoantigens. J. J. Trentin, editor. The Williams & Wilkins Co., Baltimore, Md. 48.
22. Fox, E. N., and B. J. Grossman. 1969. Antigenicity of the M proteins of group A hemolytic streptococci. V. The absence of antigenic determinants common to mammalian heart muscle. *J. Immunol.* **102**: 970.
23. Zabriskie, J. B., and E. H. Freimer. 1966. An immunological relationship between the group A streptococcus and mammalian muscle. *J. Exp. Med.* **124**: 661.