PRELIMINARY COMMUNICATION

Safety and Immunogenicity of a Recombinant Multivalent Group A Streptococcal Vaccine in Healthy Adults
Phase 1 Trial

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Context Group A streptococcal infections and their sequelae represent a global health problem. Recent advances have allowed previous obstacles associated with group A streptococcal vaccine development to be overcome.

Objective To preliminarily evaluate the safety and immunogenicity of ascending doses of a recombinant fusion peptide group A streptococcal vaccine containing N-terminal M protein fragments from serotypes 1, 3, 5, 6, 19, and 24 in healthy volunteers.

Design, Setting, and Participants An open-label, uncontrolled, dose-ascending phase 1 vaccine trial of 28 healthy adult volunteers aged 18 to 50 years recruited from the metropolitan area of Baltimore, Md, between October 5, 1999, and February 26, 2003, using newspaper advertisements and posted fliers, and evaluated in the outpatient facility of the Center for Vaccine Development.

Interventions Each volunteer received 3 spaced intramuscular injections of 50 µg (n=8), 100 µg (n=10), or 200 µg (n=10) of hexavalent group A streptococcal vaccine formulated with aluminum hydroxide into the deltoid muscle of alternating arms.

Main Outcome Measures Assessments of clinical safety, including elicitation of antibodies that cross-react with host tissues, and immunogenicity as measured by enzyme-linked immunosorbent assay (ELISA) and assays of opsonophagocytic- and bactericidal-antibody responses.

Results One year of intensive follow-up revealed the vaccine to be well tolerated. There was no evidence of tissue cross-reactive antibodies or immunological complications. At the highest (200 µg) dose, vaccination elicited significant increases in geometric mean antibody levels to all 6 component M antigens by ELISA (all $P<.01$) and to 5 of 6 M types in the opsonophagocytosis assay (all $P<.05$). In addition, postvaccination increases in serum bactericidal activity of at least 30% were observed in 31 (55%) of 56 assays.

Conclusion These results provide the first evidence in humans that a hybrid fusion protein is a feasible strategy for evoking type-specific opsonic antibodies against multiple serotypes of group A streptococcus without eliciting antibodies that cross-react with host tissues, which represents a critical step in the development of a vaccine.

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GROUP A STREPTOCOCCAL INFECTION and its protean complications continue to cause morbidity and mortality throughout the world. The World Health Organization has estimated that 12 million people have rheumatic heart disease, of whom 400 000 die each year.[1] In addition, serious invasive infections, such as bacteremia, streptococcal toxic shock syndrome, and necrotizing fasciitis, plus the more common noninvasive infections, such as pharyngitis and impetigo, produce a significant burden of disease throughout the world. Vaccine prevention of even a fraction of these cases could have a major impact on the health of children and young adults, and potentially reduce the large economic impact of these infections.[2]

Efforts to develop a vaccine to prevent group A streptococcal infections have been ongoing for more than 70 years. The surface-expressed M protein is a major protective antigen of group A streptococcus and a frequent

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target for vaccine development. Numerous experimental M protein-based vaccines ranging from crude cell walls to highly purified M proteins were evaluated between the 1930s and 1970s in trials involving thousands of healthy participants.[3] Nonetheless, a potential limitation of this approach was the observation of serologic cross-reactivity between certain M protein epitopes and human tissues, including the heart, joint, and brain.[4,5] Thus, a trial in the 1960s involving a partially purified M3 protein vaccine generated controversy when the authors later reported an apparent increase in the attack rate of rheumatic fever among vaccinated children compared with historical control children.[6,7] Whether these cases were causally associated with vaccination remains uncertain. Nonetheless, clinical testing of group A streptococcal vaccines ceased for nearly 30 years.

Vaccine development efforts were revitalized in the 1980s with the discovery that type-specific, amino-terminal regions of the M protein elicited strong bactericidal immune responses and could be separated from the potentially harmful cross-reactive epitopes.[8-12] These observations provided the rationale for creating a multivalent group A streptococcal vaccine. As proof-of-principle, a recombinant fusion protein was constructed containing the amino-terminal fragments from 6 serotypes of group A streptococcus of epidemiologic importance: M24, M5, M6, M19, M1, and M3. All types are pharyngitis-producing strains that have been associated with either outbreaks or recurrences of rheumatic fever.[13,14] These 6 M types combined account for approximately 30% of cases of simple pharyngitis and 56% of rheumatic fever episodes in the United States.[15] Herein, we report a preliminary study of the safety and immunogenicity of ascending doses of the hexavalent group A streptococcal vaccine in healthy volunteers.

METHODS

Vaccine Construction

The hexavalent gene was constructed using polymerase chain reaction and oligonucleotide primers to amplify specific 5′ sequences of the chromosomal *emm* genes containing 35 to 80 codons from each component M type. The primers were synthesized to include unique restriction enzyme sites to direct the orientation and facilitate ligation of the purified polymerase chain reaction products to one another in tandem. The hexavalent gene was ligated into an expression vector containing a polyhistidine tag at the 3′ end to facilitate purification of the recombinant protein from extracts of *Escherichia coli*. Expression, purification, and formulation of the vaccine with aluminum hydroxide were performed as described.[16] The vaccine was formulated at 50 µg/0.5 mL, 100 µg/0.5 mL, and 200 µg/0.5 mL in single-dose vials each containing 750 µg of low-viscosity aluminum hydroxide adjuvant (Rehydragel LV, Reheis, NJ). Preclinical studies in laboratory animals showed that the vaccine was immunogenic, did not evoke human tissue cross-reactive antibodies, and elicited opsonic antibodies against all 6 vaccine serotypes (1, 3, 5, 6, 19, and 24) of group A streptococci.[16]

Study Design and Participants

Twenty-eight healthy adults aged 18 to 50 years were recruited from the metropolitan area of Baltimore, Md, between October 5, 1999, and February 26, 2003, using newspaper advertisements and posted fliers. Eligible participants were required to have a normal medical history, physical examination, clinical laboratory panel, electrocardiogram, echocardiogram, serum troponin T, C-reactive protein, and C3 complement, and were excluded if any of the following criteria were met: (1) personal or family history of cardiac or nonsuppurative streptococcal disease (rheumatic fever or glomerulonephritis) or collagen-vascular disease; (2) previous receipt of phentermine, fenfluramine, or dexfenfluramine because of the possible association with cardiac valvular abnormalities[17]; (3) the presence of cross-reactive serum antibodies against human heart, brain, renal, or joint tissues, using methods described below; (4) evidence of recent group A streptococcal infection (antistreptolysin O or anti-DNase B levels exceeding 240 units or positive throat culture); or (5) antibody titer exceeding 200 against 4 or more M protein fragments contained in the vaccine, measured by enzyme-linked immunosorbent assay (ELISA). The study was approved by the institutional review board of the University of Maryland, Baltimore. Written informed consent was obtained from each participant. Volunteers were paid approximately US $1100 for completing the study.

Vaccination

Groups of volunteers received 3 spaced intramuscular injections of either 50-µg (n=8), 100-µg (n=10), or 200-µg (n=10) doses of vaccine into the deltoid muscle of alternating arms. Escalation to a higher dose level required approval by an independent data and safety monitoring committee that reviewed all data from recipients of the lower dose group collected between day 0 until 14 days after the third inoculation. A day 0, 28, and 56 dosing schedule was chosen initially. When 1 of 8 recipients of a 50-µg dose experienced moderate induration and pruritus at the injection site following the third injection, the sponsor and independent data and safety monitoring committee recommended that the dosing schedule for subsequent groups be changed to 0, 28, and 112 days.

Clinical Assessment

For 7 days following each injection, participants recorded in diary form the occurrence of the following signs and symptoms: anorexia, malaise, joint pain or swelling, pain at the site of injection, urinary changes suggesting hematuria (blood-tinged or cola-colored urine), cutaneous abnormalities (rash or subcutaneous nodules), and oral temperature. Reactions were graded for severity using a predefined scale. A nurse checked each participant's arm for local reactions 1, 3, and 7 days after each injection and a physician examined each participant for signs of rheumatic fever

1, 3, 7, 14, 28, and 56 days after each inoculation, and again 6 and 12 months after enrollment. A cardiac evaluation (auscultation by a cardiologist, electrocardiogram, and echocardiogram) was performed 14 days after each inoculation and again 6 and 12 months after enrollment. Serial laboratory assessments included serum chemistries, blood cell counts, troponin T, C-reactive protein, C3 complement, and urinalysis. Antistreptolysin O and anti-DNase B titers were measured to detect intercurrent group A streptococcal infection. In addition, volunteers were instructed to contact the study site to have a throat culture performed if they experienced a sore throat.

Serologic Responses and Detection of Cross-reactive Antibodies

Serum Antibodies by ELISA. ELISA was performed to measure type-specific antibody responses to each of the 6 individual vaccine components prevaccination (ie, day 0) and 14 days following the second and third doses. Microtiter wells (Nalge Nunc International, Denmark) were coated with individual recombinant vaccine component peptides, each presented as a tandem repeat of M1, M3, M5, M6, M19, or M24 (5 µg/mL in 0.05 M carbonate; pH, 9.6).[18] Two-fold dilutions of a 1:100 dilution of volunteer sera were made using phosphate-buffered saline with pH of 7.4, plus 0.05% saline-tween (Sigma, St Louis, Mo), added to the wells and incubated at 37°C for 2 hours. The wells were washed with 0.15% saline-tween and a 1:1500 dilution of peroxidase-conjugated goat IgG to human immunoglobulins (IgG, IgA, IgM) (ICN Biomedicals, Irvine, Calif) was added and incubated at 37°C for 2 hours. After washing, 1-step turbo 3, 3′, 5, 5′ tetramethylbenzydine ELISA substrate (Pierce, Rockford, Ill) was added and the reaction was stopped after 30 minutes by adding an equal amount of 1 M sulfuric acid. A_{450} was recorded by a microplate reader (Model 550, Biorad Laboratories, Hercules, Calif). A human serum sample known to contain naturally acquired antibodies against all 6 of the vaccine serotype-specific antigens was used as a positive control in all assays. Negative controls consisted of wells containing antigen that were incubated with all reagents except the test serum. Titers were expressed as the inverse of the last dilution of antiserum that resulted in an optical density of at least 0.15. A response was defined as a 4-fold increase in antibody titer 14 days following the third vaccination compared with the prevaccination titer.

Opsonophagocytosis Assay. Opsonophagocytic antibodies for each of the M serotypes contained in the vaccine were measured prevaccination and 14 days following the third dose by using a modification of an assay previously published.[19,20] Briefly, 0.1 mL of volunteer serum and a standard inoculum of the appropriate group A streptococcal strain grown to log phase were incubated for 15 minutes at room temperature. Lightly heparinized human blood (0.4 mL) from a nonimmune donor was then added, resulting in approximately 10 streptococcal colony-forming units (CFUs) per leukocyte in the mixture. After the entire mixture was rotated end-over-end at 37°C for 45 minutes, smears were prepared on microscope slides that were air-dried and stained with Wright stain. Each experiment included tubes containing normal or immune rabbit serum raised against the M protein of the test organism as negative and positive controls, respectively. Percentage opsonization was determined by counting the percentage of polymorphonuclear leukocytes that ingested or were associated with bacteria as observed using light microscopy.

Indirect Bactericidal Tests. Bactericidal tests were performed on sera collected prevaccination and 14 days following dose 3, essentially as described by Lancefield.[21] Streptococci were grown to early log-phase, serial dilutions were made in Todd-Hewitt broth, and 0.05-mL inoculum (range, 3-64 CFUs) was added to 0.1 mL of test serum and incubated at 37°C for 15 minutes. After adding 0.35 mL of heparinized blood from a nonimmune donor, the mixture was rotated end-over-end for 3 hours at 37°C. Aliquots (0.1 mL) of this mixture were added to melted sheep's blood agar, pour plates were prepared, and viable organisms (CFU) were counted after overnight incubation at 37°C. Normal rabbit serum was included in each assay as a negative control and rabbit antiserum raised against the native M protein of each vaccine strain was used as a positive control. The results were expressed as percentage killing, which was calculated using the following formula:

[(CFU after 3 hours' growth with prevaccination serum – CFU after 3 hours' growth with postvaccination serum obtained 14 days after the third dose)/(CFU after 3 hours' growth with prevaccination serum)] × 100.

Only assays that yielded growth of the test strain to at least 5 generations in prevaccination preparations were used to express percentage killing. Assays with at least 30% killing were enumerated to maintain consistency with previously published articles.[22-24]

Assays for Tissue Cross-reactive Antibodies. Sera were tested for the presence of tissue cross-reactive antibodies prevaccination, 14 days after each inoculation, and again 5 months and 1 year after enrollment by indirect immunofluorescence assays using frozen sections of human heart, brain, kidney, and cartilage, as previously described.[25] Immunofluorescence reactions were scored by 2 independent reviewers. All assays included positive control rabbit antisera raised against intact M proteins and known to react with the target tissues.[25-27] The positive control sera and the volunteer sera were coded and placed in random order on the tissue samples so that all observations were made in a blinded fashion.

Other Antistreptococcal Antibodies. Antistreptolysin O was measured on 6 occasions by using the bacto-reagents and kit (Difco Laboratories, Detroit, Mich) and anti-DNase B level was determined with Wampole reagents (Cranbury, NJ) in the laboratory of Stanford Shulman, MD, Children's Memorial Hospital, Chicago, Ill.

Statistical Analysis

This open-label phase 1 trial was designed to detect overt reactogenicity and measure immunogenicity in a small number of intensively monitored participants without consideration for statistical power. Prevaccination vs postvaccination geometric mean log-transformed titers or percentage opsonization, as applicable, were compared by using Wilcoxon rank sum test for each of the 6 vaccine components at each of the 3 dose levels. Relationships between serum antibody responses measured by ELISA (fold-increases) and indirect bactericidal levels (percentage kill) were examined using Spearman rank correlations. Statistical significance was assessed at the 5% level ($P<.05$), evaluating 2-sided hypotheses throughout. SAS version 8.2 (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Clinical Tolerance and Assessment for Rheumatic Sequelae

Vaccination was well-tolerated. Local erythema, swelling, or tenderness were detected in 6 (21%) of 29 participants within 7 days after the first inoculation and 12 (43%) of 28 participants each after the second and third inoculations. All local reactions were graded as mild (≤30 mm and easily tolerated) with the exception of the aforementioned participant who developed moderate (31-80 mm) erythema, swelling, and itching for 1 day after the third dose of 50 µg. There was no evidence of an increase in local reactions with higher doses. Approximately 10% of participants reported nonspecific complaints (mild anorexia or malaise) within 7 days after each injection. One participant experienced a low-grade fever (37.9 °C) during the 24 hours after receiving a second injection of 200 µg that was associated with rhinorrhea and myalgias lasting 48 hours. No participant experienced unexplained or persistent fever, arthritis, carditis, subcutaneous nodules, erythema marginatum, glomerulonephritis, or persistent abnormalities of C3 complement or C-reactive protein. Tissue cross-reactive antibodies were not detected. There were no intercurrent episodes of group A streptococcal pharyngitis.

One participant (African American male) was withdrawn from the study because his absolute neutrophil count decreased to 900 cells/µL, 28 days after receiving a single 100-µg dose of vaccine that he tolerated well. He was excluded from immunological analysis, resulting in 10 analyzable participants in the 100-µg vaccine dose group. He had a borderline abnormal serum C3 at this time (92 mg/dL; normal range, 90-180 mg/dL). This participant had intermittent neutropenia (range, 1000-2500 cells/µL) and mildly depressed to low normal serum C3 levels (range, 81-114 mg/dL) that predated his first injection and continued throughout his 12-month observation period. His clinical course was benign except for intermittent fatigue and arthralgia attributed to a night job in construction. The final diagnosis was benign ethnic neutropenia,[28] not vaccine-related.

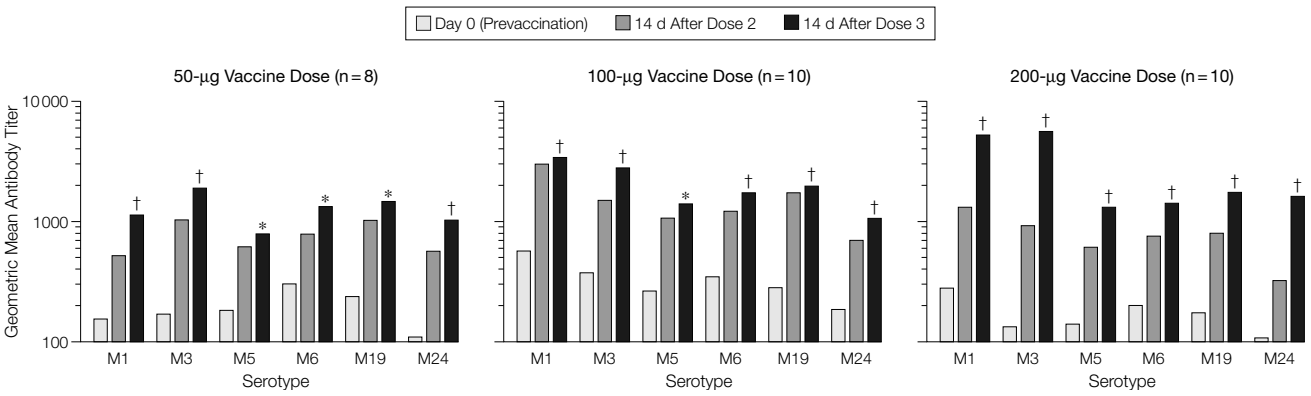
Serum Antibody by ELISA

Vaccination elicited 4-fold or more increases in 40 (83%) of 48 possible assays (ie, 6 antigens × 8 volunteers) following the 50-µg dose, in 45 (75%) of 60 possible assays (ie, 6 antigens × 10 volunteers) following the 100-µg dose, and in 57 (95%) of 60 possible assays (ie, 6 antigens × 10 volunteers) following the 200-µg dose. The geometric mean antibody titer following vaccination was significantly higher than the prevaccination titer for each antigen measured in all dose groups (FIGURE 1).

Opsonic Serum Antibody

Many participants possessed opsonic serum antibodies prevaccination (FIGURE 2). Nonetheless, 3 vaccination doses elicited increases in the geometric mean percentage opsonization.

Figure 1. Serum Antibody Responses by Enzyme-Linked Immunosorbent Assay



Geometric mean serum antibody titers by ELISA prevaccination (day 0) and 14 days following the second and third doses for each vaccine component, according to dose group. Participants received 50 µg of vaccine on days 0, 28, and 56; 100 µg of vaccine on days 0, 28, and 112; or 200 µg of vaccine on days 0, 28, and 112.
*$P<.05$.
†$P<.01$; Wilcoxon rank sum test, comparing geometric mean titers 14 days following the third inoculation to prevaccination geometric mean titers for each component M type and dose group.

Whereas responses were observed occasionally at the 2 lower dose levels, significant postvaccination increases were observed for 5 of 6 M types (all but M6) contained in the vaccine 14 days after three 200-µg doses.

Indirect Bactericidal Antibody

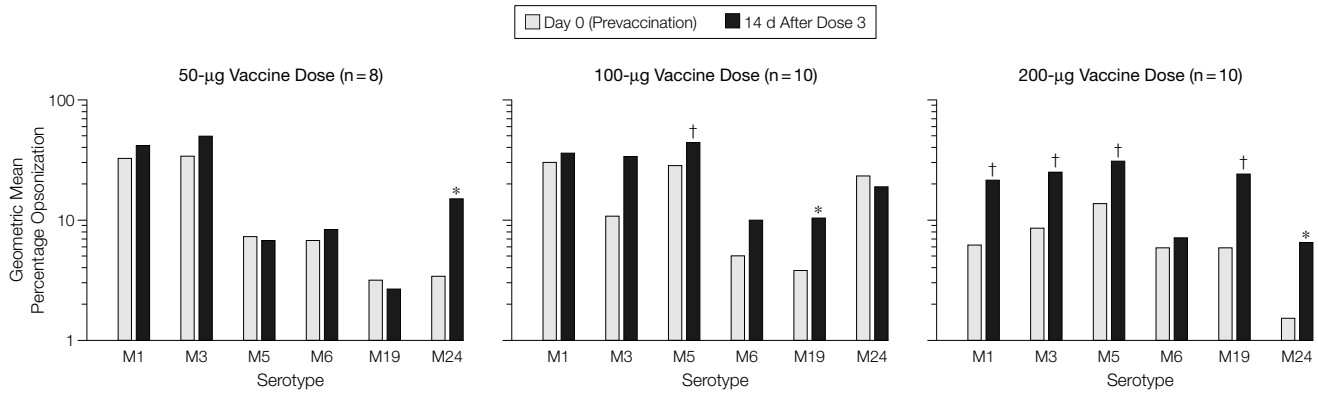
As shown in FIGURE 3, the median percentage killing attributable to the vaccine ranged from 20% to 79% in the 18 antigen-dose groups (6 antigens × 3 dosage levels). A postvaccination increase in the bactericidal response of at least 30% was observed in the majority (103 [64%] of 160) of assays for which test strain growth criteria in preimmunization samples were met, including 31 (55%) of 56 assays from participants in the 200-µg dose group; however, a dose response was not apparent.

Correlation between the fold-increases in the ELISA and percentage kill in bactericidal assays varied by M type. The strongest correlations were observed for M5 (Spearman $r=0.43$, $P=.02$) and M6 ($r=0.35$, $P=.08$).

COMMENT

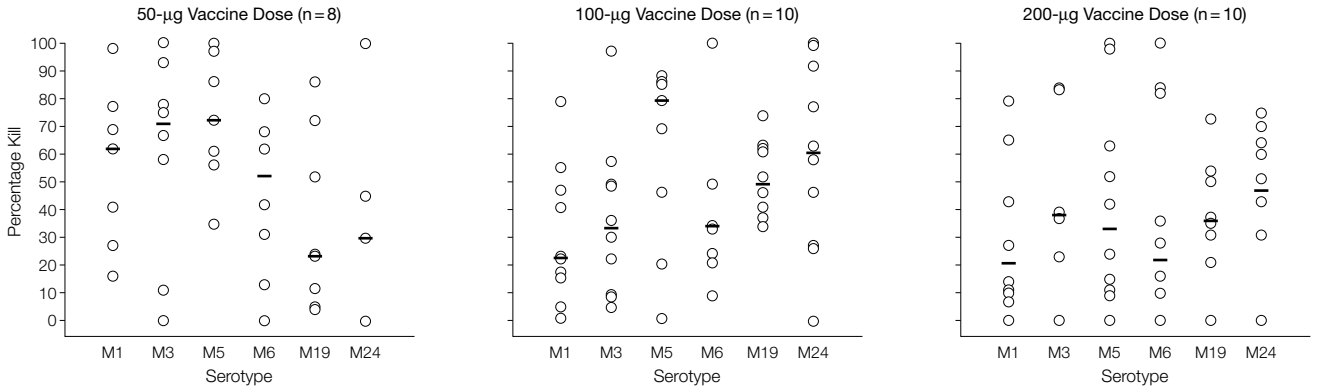
After a hiatus of 3 decades in which no group A streptococcal vaccine testing was undertaken in humans, we performed a phase 1 trial of a recombinant fusion protein group A streptococcal vaccine containing N-terminal protein fragments from 6 M types that have been associated with rheumatic fever.[13] We used a protocol in which volunteers were frequently evaluated for 12 months postvaccination by using detailed clinical and laboratory evaluations for rheumatic sequelae, glomerulonephritis, and autoimmune phenomena. Our findings, albeit in a small number of participants, suggest that in the full dose range tested, the vaccine appears safe and well tolerated and does not evoke antibodies that cross-react with human tissue. We have

Figure 2. Serum Opsonophagocytic Antibody Responses



Geometric mean serum percentage opsonization prevaccination and 14 days following the third vaccination for each vaccine component, according to dose group. Participants received 50 µg of vaccine on days 0, 28, and 56; 100 µg of vaccine on days 0, 28, and 112; or 200 µg of vaccine on days 0, 28, and 112.
*$P<.05$.
†$P<.01$; Wilcoxon rank sum test, comparing geometric mean percentage opsonization 14 days following the third inoculation to prevaccination values for each M type contained in the vaccine and dose group.

Figure 3. Serum Bactericidal Antibody Responses



Percentage reduction in surviving bacterial colonies (percentage kill) for each M type, according to dose group, using serum obtained 14 days after the third vaccination in the indirect bactericidal assay compared with prevaccination levels. Circles represent individual volunteers; lines represent median values for each M type contained in the vaccine. Individuals with the same values appear as a single circle. Participants received 50 µg of vaccine on days 0, 28, and 56; 100 µg of vaccine on days 0, 28, and 112; or 200 µg of vaccine on days 0, 28, and 112.

identified a dose (200 µg) and schedule (0, 28, and 112 days) that appears to be capable of inducing immune responses to each of the type-specific M antigens as measured by ELISA. Furthermore, significant increases in the geometric mean levels of opsonic antibodies were observed for 5 of 6 vaccine antigens and most participants also had measurable postvaccination bactericidal activity (55% of participants had a postvaccination increase in bactericidal antibody of at least 30%). Thus, these observations provide the first evidence in humans that a hybrid-fusion protein may represent a feasible strategy for evoking type-specific antibodies against group A streptococcus, and that these antibodies have functional (opsonophagocytic) activity.

Several lines of evidence suggest that the immune responses evoked by type-specific M protein epitopes will translate into clinical immunity to the group A streptococcus. Lancefield[29] demonstrated the role of type-specific immunity in preventing challenge infections in animals and later showed that humans develop long-lasting type-specific immunity following natural infection.[21] In a study by Beachey et al,[19] serum from human volunteers immunized with a type-specific peptide fragment of type 24 M protein protected mice against intraperitoneal challenge with group A streptococcus. The most direct evidence comes from clinical trials in which volunteers who were immunized either subcutaneously or intranasally with purified acid-extracted fragments of M proteins had significantly decreased rates of clinical illness following mucosal challenge with virulent group A streptococcus bearing the homologous M type.[22-24] Nonetheless, the precise immune responses that mediate clinical protection against group A streptococcal infection have not been fully elucidated. In the studies cited above, 14 of 19 participants who received a parenteral M1 vaccine were completely protected against illness following challenge with the homologous M type but only 5 of these individuals had bactericidal antibody responses to vaccination.[23] In contrast, type-specific antibodies (measured by complement fixation) recognizing the vaccine purified M protein were detected in 13 of 14 individuals who were protected against challenge. Similarly, in our study, the 200-µg dose of the hexavalent vaccine evoked 4-fold or more increases in type-specific antibodies in 95% of participants and induced significant increases in opsonic antibodies against 5 of 6 vaccine serotypes. Although increases in bactericidal antibodies occurred in most participants, they did not appear to follow a dose-response pattern and fewer participants met our arbitrary criteria for a response in this assay compared with the opsonophagocytosis assay and ELISA. Identification of the most reliable immmunological correlate of protection will require further studies of the ability of type-specific vaccines in preventing natural or experimental infections.

The multiplicity of M types presents a challenge for vaccine development. More than 80 M types have been identified by serotyping techniques and more than 120 distinct types have been elucidated by *emm* sequencing analysis. Many types can circulate in the community simultaneously[30] and both temporal and geographic variation have been observed.[31,32] It is clear that a type-specific vaccine must be multivalent; therefore, a second generation M protein-based vaccine containing 26 M types has been constructed[18] and is undergoing clinical evaluation. Serotypes included in this vaccine were selected based on epidemiological studies to identify the prevalent types responsible for the majority of infections in specific geographical regions. For example, multistate surveillance for invasive disease and pharyngitis in the United States and Canada, and additional studies from Mexico, suggested that 80% to 90% of clinical isolates were included in the 26-valent vaccine.[18,31,33] Furthermore, little sequence variation was observed in the type-specific regions of these strains.[33] In contrast, a recent study[34] suggests that the vaccine coverage would be less complete in Asia and there is little information regarding the distribution of M types in developing countries in which streptococcal infections and acute rheumatic fever are extremely common. Implementation of vaccine programs will face other complex issues, such as emergence of newly characterized *emm* types[31,35] and the possibility that nonvaccine serotypes may replace those contained in the vaccine via immune escape as has been observed with *Streptococcus pneumoniae*.[36,37]

The safety issues related to clinical development of a group A streptococcal vaccine warrant careful consideration in light of the potential risk of rheumatic fever, a disease whose pathogenesis is not well understood. Several features of the hexavalent vaccine suggest that there is a low risk of inducing rheumatic fever or other poststreptococcal sequelae. It contains no epitopes that are known to cross-react with host cardiac, joint, renal, or brain tissues[26,27,38] and it does not induce de novo stimulation of human lymphocytes, as opposed to native pep M proteins, which induce brisk blastogenic responses in lymphocytes by virtue of their superantigenic properties.[39] Experience suggests that wild-type group A streptococcus must be delivered mucosally to trigger rheumatic fever, which follows pharyngitis but has not been associated to our knowledge with invasive disease. Nonetheless, during the development of this vaccine, it will be prudent to monitor vaccine recipients carefully for clinical manifestations consistent with the nonsuppurative sequelae of group A streptococcal infection. Particular scrutiny will be necessary when trials are performed in pediatric populations who are at high risk for wild-type group A streptococcal infection to exclude the possibility that vaccination could increase the risk of autoimmune events following natural infection.

There are a few limitations of our study. We used a phase 1 trial with small sample sizes and an open-label design for safety reasons; therefore, our findings must be viewed as preliminary. Growing attention to vaccine safety has stimulated debate about the need to conduct large prelicensure trials (10 000-60 000 participants) to provide assurance that rare adverse events are not associated

with vaccination.[40] Concerns about a possible relationship between group A streptococcal vaccines and rheumatic fever are likely to trigger similar considerations. Furthermore, the absence of antibodies in vaccinated participants that cross-react with human tissues must be regarded with guarded enthusiasm because the role of these antibodies in the pathogenesis of rheumatic fever remains controversial.[41] Finally, caution should be exercised in extrapolating the immunogenicity that we observed in adults, some of whom had preexisting antibodies to the vaccine components, to immunologically naive children. Infants,[2] particularly those residing in regions of the world in which rheumatic fever is prevalent, as well as high-risk adults, such as US military recruits,[42] may be targeted for vaccination; consequently, clinical trials will be needed in both populations.

Our findings provide preliminary evidence that the hexavalent group A streptococcal vaccine is safe, well tolerated, and capable of inducing vigorous immune responses that are likely to confer protection against multiple group A streptococcal M types. Accordingly, we conclude that further development of multivalent amino-terminal M protein–based vaccines is warranted.

Author Contributions: Dr Kotloff had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Kotloff, Dale.
Acquisition of data: Kotloff, Corretti, Palmer, Campbell, Reddish, Hu, Dale.
Analysis and interpretation of data: Kotloff, Corretti, Wasserman, Dale.
Drafting of the manuscript: Kotloff, Palmer, Wasserman, Dale.
Critical revision of the manuscript for important intellectual content: Kotloff, Corretti, Campbell, Reddish, Hu, Dale.
Statistical analysis: Wasserman.
Obtained funding: Kotloff, Dale.
Administrative, technical, or material support: Palmer, Campbell, Reddish, Hu, Wasserman, Dale.
Study supervision: Kotloff, Dale.
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