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Potential coverage of a multivalent M protein-based group A streptococcal vaccine

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ABSTRACT

Background: The greatest burden of group A streptococcal (GAS) disease worldwide is due to acute rheumatic fever (ARF) and rheumatic heart disease (RHD). Safe, effective and affordable vaccines designed to prevent GAS infections that trigger ARF could reduce the overall global morbidity and mortality from RHD. The current study evaluated the potential coverage of a new 30-valent M protein-based vaccine using GAS isolates from school children in Bamako, Mali, a population at high risk for the development of RHD.
Methods: The bactericidal activity of rabbit antisera against the 30-valent vaccine was assessed using a collection of GAS isolates recovered during a study of the epidemiology of pharyngitis in Bamako.
Results: Single isolates representing 42 of 67 *emm*-types, accounting for 85% of the GAS infections during the study, were evaluated. All (14/14) of the vaccine *emm*-types in the collection were opsonized (bactericidal killing >50%) and 26/28 non-vaccine types were opsonized. Bactericidal activity was observed against 60% of the total *emm*-types recovered in Bamako, which accounted for 81% of all infections.
Conclusions: Multivalent vaccines comprised of N-terminal M peptides elicit bactericidal antibodies against a broad range of GAS serotypes, indicating that their efficacy may extend beyond the *emm*-types included in the vaccine.

1. Introduction

Group A streptococci (GAS) cause a broad spectrum of diseases worldwide that ranges from uncomplicated pharyngitis and skin infections to life-threatening invasive infections that include pneumonia, bacteremia, necrotizing fasciitis, streptococcal toxic shock syndrome, as well as the immune-mediated non-suppurative sequelae of acute rheumatic fever (ARF), rheumatic heart disease (RHD), and glomerulonephritis. The greatest burden of GAS disease, as defined by morbidity and mortality, is due to RHD in low- and middle-income countries. Minimum estimates suggest that RHD affects 15 million individuals resulting in ~230,000 deaths each year [1]. However, as more data become available from low-income countries [2–4], the true burden of RHD is becoming clearer and it appears that the disease burden outlined previously is considerably underestimated.

Current strategies for the prevention of ARF and RHD rely on antibiotic treatment of symptomatic pharyngitis (primary prevention) or continuous administration of antibiotics to individuals with documented ARF (secondary prevention). These prevention strategies are costly and, for the most part, are ineffective in resource-poor countries where 90% of the disease burden exists [5]. The development of a safe, effective and affordable vaccine designed to prevent the GAS infections that trigger ARF/RHD has long been considered an attractive approach. Vaccine development has faced obstacles related to the complex epidemiology of GAS infections, especially in developing countries [6], and safety concerns based on the theoretical possibility that the vaccines may elicit autoimmune responses that could mimic ARF or other sequelae of GAS infections [7].

Significant progress has been made recently in the design and clinical development of complex multivalent M protein-based vaccines [8–10]. The surface M protein is a major virulence determinant of GAS that contains opsonic epitopes [11]. M protein antibodies that are produced following natural infection [12] or vaccination [9] are bactericidal in human blood. Bactericidal

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antibodies have been associated with protection against infection with the homologous serotype of GAS [13]. A number of structure-function studies have revealed that the N-terminal peptides of M proteins contain epitopes that evoke antibodies with the greatest bactericidal activity and are least likely to cross-react with human tissue antigens [7]. This observation has been the basis for the design of complex recombinant hybrid vaccine proteins containing opsonic epitopes representing M proteins from multiple serotypes of GAS [8,10].

We have previously shown that a 26-valent vaccine containing M peptides from serotypes of GAS representing the vast majority of infections in the USA and Canada [14] was safe and immunogenic in adult volunteers [9]. In pre-clinical and clinical studies, the vaccine evoked bactericidal antibodies against all of the vaccine serotypes of GAS [9]. Recently, we constructed a 30-valent vaccine whose composition was based on more extensive surveillance data from the USA and Europe [15–17]. In animal studies, the 30-valent vaccine evoked bactericidal antibodies against all of the vaccine serotypes of GAS [10]. In addition, significant bactericidal activity was observed against a number of laboratory strains representing non-vaccine serotypes of GAS, suggesting that the potential efficacy of this vaccine may extend beyond those serotypes represented in the vaccine [10]. This observation is potentially significant because the predicted coverage of the previous 26-valent vaccine in resource-poor countries is low compared to economically developed countries [6].

In the current study, we have evaluated the bactericidal antibody activity of the 30-valent vaccine antisera raised in rabbits using a collection of pharyngeal isolates of GAS obtained from symptomatic school children in Bamako, Mali [18]. The overall goal was to assess the potential coverage of the new vaccine in a sub-Saharan African population at high risk for developing ARF and RHD and where the epidemiology and *emm*-type prevalence of GAS infections is very different from that observed in the USA, Canada or Europe [15–17].

2. Materials and methods

2.1. Immunization of rabbits with the 30-valent vaccine

Three New Zealand white rabbits were immunized with 800 μg doses of the 30-valent vaccine proteins adsorbed to alum at time 0, 4 weeks and 8 weeks, as previously reported [10]. Serum was obtained prior to the first injection and at 2 weeks following the last injection. The antibody titers against the M peptides contained in the vaccine and functional bactericidal activity of the antisera against all of the vaccine serotypes of GAS have previously been published [10]. Animal protocols were approved by the Institutional Animal Care and Use Committees of the University of Tennessee Health Science Center and the Veterans Affairs Medical Center, Memphis, TN.

2.2. Clinical isolates of GAS from school children in Bamako

The clinical isolates of GAS used in this report were obtained during a comprehensive assessment of the molecular epidemiology of symptomatic pharyngitis in school children in Bamako, Mali conducted during 2006–2009 [18]. Briefly, children ranging from 5 to 16 years of age attending any of four participating schools in Djikoroni-para and Sebenikoro urban neighborhoods in Commune 4 of Bamako were eligible. Students who presented to the school infirmary or school-associated health center complaining of sore throat were enrolled. Upon presentation, study personnel confirmed that the student was experiencing throat pain, then symptoms of pharyngitis were obtained and a brief focal examination was performed. Throat culture swabs were placed in Amies charcoal media (CultureSwab™, Becton Dickinson) and transported to the microbiology laboratory at the Center for Vaccine Development – Mali at ambient temperature within 6 h. Upon arrival in the laboratory, swabs were streaked onto 5% sheep's blood agar, a bacitracin disk (0.04 U) was placed, and the plate was incubated at 35–37 °C. GAS were beta-hemolytic, catalase-negative, bacitracin-sensitive, gram-positive cocci in pairs and chains that tested positive for group A streptococcal antigen on agglutination test. Bacterial isolates were stored at −80 °C in trypticase soy broth with 15% glycerol. All human subjects protocols were approved by the Ethics Committee of the Faculté de Médecine, Pharmacie et Odontostomatologie in Bamako, Mali, and the respective Institutional Review Boards of the University of Maryland School of Medicine, the University of Tennessee Health Science Center, and the Memphis Veterans Affairs Medical Center.

2.3. Emm-typing

DNA from the GAS isolates was extracted and the 5′portion of the *emm* gene was amplified using standard methodology developed at the Centers for Disease Control, Atlanta, GA, USA [19]. The amplified DNA product was sent to the University of Maryland Biopolymer Laboratory for sequencing. The nucleotide sequence was then compared using a standard FASTA alignment algorithm against known *emm* gene sequences on the CDC website and an *emm*-type and sub-type were assigned.

2.4. Indirect bactericidal tests

Bactericidal assays were performed as previously described [20]. Briefly, 0.05 ml of Todd-Hewitt broth containing bacteria (~50–200 CFU) was added to 0.1 ml of preimmune or immune serum and 0.35 ml of lightly heparinized non-immune human blood and the mixture was rotated for 3 h at 37 °C. Then 0·1 ml of this mixture was diluted 10- and 100-fold and 0.1 ml aliquots were added to melted sheep's blood agar. Pour plates were prepared and viable organisms (CFU) were counted after overnight incubation at 37 °C. The results were expressed as percent killing, which was calculated using the following formula: [(CFU after 3 h growth with preimmune serum − CFU after 3 h growth with immune serum)/CFU after 3 h growth with preimmune serum] × 100. For each assay, the CFU added to the test mixtures was determined and only those assays that resulted in growth of the test strain to at least five generations in the presence of preimmune serum were used to express percent killing in the presence of immune serum.

2.5. Emm sequence analyses

Primary structural homologies among *emm* sequences contained in the 30-valent vaccine and the *emm* sequences of non-vaccine types were identified using the Swiss Institute of Bioinformatics SIM alignment program [21]. The translated sequences corresponding to the N-terminal 50 amino acid residues of the mature M proteins [22] were individually aligned with the complete sequences of the 30-valent vaccine proteins using the comparison matrix PAM40, a gap open penalty of 12 and a gap extension penalty of 4. The alignments resulting in the top three scores were analyzed for their position in the 30-valent vaccine proteins and percent identity was recorded.

3. Results

During the epidemiologic study in Bamako, a total of 372 GAS were recovered from throat cultures of enrolled subjects, of which 305 were available for analysis in this report. A complete