Human immunogenicity studies on group A streptococcal C5a peptidase (SCPA) as a potential vaccine against group A streptococcal infections

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Background & objectives: Group A streptococcal C5a peptidase (SCPA) is a major virulence surface factor. Its highly conserved nature among all tested serotypes of group A streptococci (GAS) as well as animal protection studies make SCPA a prime vaccine candidate. The present study was undertaken to explore the human immunogenicity to SCPA using an indirect enzyme-linked immunosorbent assay.

Methods: Children (n=72) who had signs and symptoms of acute pharyngitis and had GAS isolated from the throat at initial visit were included. Acute and convalescent sera were collected 4 weeks apart. ELISA was performed using recombinant SCPA peptide as antigen.

Results: The mean convalescent anti-SCPA level was twice the level of mean acute anti-SCPA and the difference was statistically significant ($P < 0.0001$). There was a rise in convalescent anti-SCPA in all children aged 2-12 yr.

Interpretation & conclusion: Our observations confirmed that SCPA was highly immunogenic in children infected with group A streptococcal pharyngitis. Further studies need to be done to characterize the immune response including antibody subclass.

Key words  C5a peptidase  -  group A streptococcus  -  humoral immune response  -  vaccine

Group A streptococcus is an important human pathogen that causes a variety of infections ranging in severity from pharyngitis and pyoderma to severe invasive diseases such as necrotizing fasciitis and toxic shock syndrome. Streptococcal non-suppurative sequelae like acute rheumatic fever and rheumatic heart disease remain major public health problems among most of the world’s population. The use of antibiotics and an improved standard of living have not significantly eliminated the high morbidity and mortality of group A streptococcal infections as seen by many outbreaks of rheumatic fever and invasive disease in the United States and Europe$^{1,2}$. Observations of increased incidence of macrolide resistance among isolates of group A streptococci$^3$, as well as reports of clinical and microbiological failures after penicillin treatment$^4$, make the development of a safe and efficacious vaccine more urgent than ever.

Among the different surface proteins that have been considered as potential candidate vaccines, the group A streptococcal C5a peptidase (SCPA) plays a major role as a virulence factor by allowing the group A streptococci to establish a mucosal site of infection$^{5,6}$. The objective
of the present study was to explore the human immunogenicity of SCPA after natural infection with group A streptococcus using a standardized enzyme-linked immunosorbent assay (ELISA)\(^7\).

**Material & Methods**

The study population consisted of children (aged 2-12 yr) residing in various states in the United States, who had signs and symptoms of acute pharyngitis and had group A streptococci isolated from the throat at the initial visit. Acute and convalescent sera were obtained 4 weeks apart. The ELISA was performed as described previously\(^7\). Recombinant SCPA peptide was used as the antigen and coated overnight on microtiter plates. Acute and convalescent sera from the same patient were always run adjacent to each other on the same microplate under identical conditions. After incubation with secondary antibody, (alkaline phosphatase-conjugated goat anti-human IgG) the substrate, \(p\)-nitrophenyl phosphate was added and the enzyme reaction was stopped. Optical density at a wavelength of 405 nm was measured with an EL340 BioTek microplate reader and analyzed to obtain antibody concentrations\(^8\). Paired Student’s ‘t’ test was used to compare the means of the anti-SCPA responses in acute and convalescent sera following group A streptococcal pharyngitis. The analyses were performed using Instat for Macintosh version 2.0 (GraphPad Software, San Diego, CA). \(P<0.005\) was considered significant.

**Results**

A wide range in anti-SCPA concentrations was seen in both acute and convalescent sera of 72 children included in the study. Overall, the mean convalescent anti-SCPA was twice the concentration of mean acute anti-SCPA and this difference was statistically significant \((P < 0.0001)\). Of the paired acute and convalescent sera that were assayed, over two-thirds of the children studied demonstrated a significant rise, \(i.e., > 15\) per cent rise in convalescent anti-SCPA concentration. When the immune response to SCPA was examined according to age, it was seen that a mean rise in convalescent anti-SCPA occurred in all of the represented age groups between 2 and 12 yr. It appeared that children below the age of 5 yr had a higher magnitude of response.

When the immune response to SCPA was plotted according to M type of the infecting strain it was found that a mean rise in anti-SCPA occurred irrespective of the M type, suggesting that all M types produced SCPA.

**Discussion**

Important features of a good vaccine candidate are as follows: significant contribution to disease pathogenesis, conservation among all the different circulating strains, absence of ability to evoke human cross-reactive antibodies, protection in animals against infective challenge with the vaccine strains, and reproducible immunogenicity in the host. SCPA has fulfilled most of the above criteria. Our study clearly showed that a strong immune response to SCPA was mounted in children following natural infection with group A streptococcal pharyngitis.

As a major streptococcal virulence factor, SCPA has been shown to contribute significantly to the organism’s ability to colonize mucosal surfaces. By enzymatically cleaving C5a, the complement-derived chemotaxin, at the His\(^67\) – Lys\(^68\) bond, the leukocyte-binding site of C5a was removed, and chemotaxis was inhibited\(^5\). The resulting lack of host phagocyte infiltration at the initial site prevents bacteria from being cleared, thus allowing streptococci to establish infection. This has been clearly shown in mouse experiments involving colonization of the oral mucosa\(^6\). The presence of humoral and mucosal antibodies in mice, induced by using recombinant SCPA as an immunogen, was associated with protection against nasopharyngeal colonization after challenge with several different streptococcal M types\(^10\). These results strongly suggest cross-protection. This feature of a potential vaccine candidate is especially important in view of recent reports confirming the dynamic changes in prevailing group A streptococcal serotypes within an isolated community\(^11\), and also the well known fact that most streptococcal isolates from developing countries are characterized as non-typeable strains\(^12\). SCPA plays an additional role in virulence by facilitating intracellular invasion of epithelial cells\(^13\). This invasive property is blocked by the presence of antibody to SCPA.

SCPA appears to be highly conserved among all tested group A streptococcal strains as demonstrated
by the presence of cross-reacting antibodies among over 20 different serotypes of group A streptococci. The scpA gene is also found to be present among several different strains of group A streptococci. A similar peptidase is also expressed by human isolates of group B10-18. C (unpublished data) and group G streptococci19. The strong ability of neutralizing antibody to prevent bacterial colonization, make SCPA an ideal vaccine candidate for combatting group A streptococcal disease. However, further work characterizing the immune response including antibody subclasses, cellular immunity, as well as functional assays need to be done. Important questions that need to be addressed include normal levels of anti-SCPA in different populations, and in populations endemic for rheumatic fever and rheumatic heart disease.

It has been previously shown that human sera containing high titre of anti-SCPA IgG could successfully inhibit and neutralize SCPA activity. More recently, it has been shown that inhibition of SCPA activity is not serotype specific. Rabbits vaccinated with SCPA were found to have high titres of specific antibody, which was able to neutralize SCPA activity associated with several different serotypes of group A streptococci. The strong immune response generated in children infected with group A streptococci in our study could potentially generate non-serotype specific neutralizing antibodies which could provide some degree of protection for the host against early establishment of infection by the bacteria.

In conclusion, the present results showed that SCPA was highly immunogenic in children recently infected with streptococcal pharyngitis and the magnitude of this immune response was independent of the infecting serotype. These data, in addition to previous knowledge of the well-defined role of SCPA in virulence, lack of known cross-reactive immunity with human tissue, and ability of neutralizing antibody to prevent bacterial colonization, make SCPA an ideal vaccine candidate for combatting group A streptococcal disease.

**References**


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