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Candidates for the development of vaccines against *Streptococcus pyogenes* infections

Diana Ionescu [1.2.3], Bogdan Ionescu [1.2.3,*], Lia-Mara Ditu [1,2], Violeta-Corina Cristea [3,4], Bogdan Sacagiu [3], Maria Duta [3], Gabriela Neacsu [3], Ramona Gilca [3], Veronica Lazar [1,2]

[1] Faculty of Biology, University of Bucharest; Research Institute of the University of Bucharest, Romania
[2] Research Institute of the University of Bucharest, Romania
[3] Central Reference Laboratory Synevo, Bucharest, Romania
[4] University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

*corresponding author e-mail address: ***bogdanionescu87@yahoo.com***

ABSTRACT

Streptococcus pyogenes is an extracellular pathogen that causes a wide variety of infections from respiratory tract infections to deep tissue afflictions. Complications occur frequently and can be life threatening. *Str. pyogenes* is responsible annually for about 616 million cases of pharyngitis worldwide. The attention regarding the increased rate of streptococcal infections and complications led to the attempt of development a vaccine against *Str. pyogenes.* The conception of the first vaccine started in the 1930s. A major breakthrough was represented by Rebecca Lancefield's studies that highlighted the possibility of incorporating purified M proteins into vaccines. Today a commercial vaccine is still not available in part due to the high diversity of M protein serotypes, the molecular mimicry process and to the fact that the other vaccine candidates are still in the pre-clinical stages.

Keywords: *Streptococcus pyogenes,* vaccine, antigens

1. INTRODUCTION

Str. pyogenes (β-hemolytic on sheep blood agar with) is an extracellular pathogen that causes a wide variety of infections from respiratory tract infections (pharyngitis, pneumonia), skin to deep tissue afflictions (impetigo, cellulitis, necrotizing fasciitis). Complications can be life threatening and they include: scarlet fever, septicemia, meningitis, diseases with an autoimmune component (post-streptococcal sequela - rheumatic fever, glomerulonephritis) (Koning, 2012). *Str. pyogenes* is responsible annually for about 616 million cases of pharyngitis worldwide and 111 million cases of skin infections among children in less developed countries (Carapetis et al, 2005). All the data recorded in recent years concerning the increased rate of streptococcal infections and complications led to the need of developing a vaccine against *Str. pyogenes.* The attempts to conceive a vaccine against *Str. pyogenes* started in the 1930s. The intravenous administration of neutralized *Str. pyogenes* strains to children diagnosed with rheumatic fever resulted in a reduced number of recurrent infections. Rebecca Lancefield pointed out the importance of the M protein regarding the possibility of incorporating this purified protein in vaccines that will stimulate a protective immune response against *Str. pyogenes*. A commercial vaccine is still not available. The development of an efficient vaccine based on this protein was difficult because of the molecular mimicry process which causes antibodies against purified M protein to cross-react with other proteins belonging to the heart tissue, skeletal muscle and other organs (Ellis and Brodeur, 2003).

2. M PROTEIN BASED VACCINE

Since the discovery of the M protein by Lancefield, there have been many studies undertaken in this field. Massella developed vaccines based on M proteins in 1968 using a template represented by a partially purified M3 protein that had been extracted from serotype M3 streptococci using HCl. Vaccinated people developed anti-streptococcal antibodies. The vaccine was administered to individuals suffering from rheumatic fever. There were three episodes of rheumatic fever related to this vaccine, but there was no direct evidence that the vaccine was responsible for the development of rheumatic fever. In a more detailed study regarding this vaccine it was highlighted that 3 out of 21 individuals vaccinated developed rheumatic fever following streptococcal infections, caused by *Str. pyogenes*, which occurred during immunization (Guilherme et al., 2006).

In the 1970s, development of vaccines based on the M protein was restarted by the work done involving Fox and Wittner, who studied earlier vaccines against this protein and the immune response triggered in mice and humans. Fox and his colleagues immunized 200 healthy adults and children with M serotypes 1, 3, 6, 12, and 24. Vaccines that affected the mucous membranes as well as parenteral vaccines were tested and showed a 70% efficacy rate (Guilherme et al., 2006).

In 1979, Beachey and his team took advantage of the pepsin extraction method commonly used on M proteins and produced a highly purified PepM24 extract. PepM24 that contains the N-terminal half of the M protein was mixed with alum as an adjuvant and was used to vaccinate a small group of 12 volunteers. They developed specific antibodies against the type 24 *Str.*

pyogenes and had no delayed hypersensitivity reaction and no cross-reaction with other antigens.

Further studies conducted by Beachey and his team are viewed as the first studies conducted on people with a synthetic vaccine against the M protein (Guilherme L et al, 2006). One of the major issues of the immunization against *Str. pyogenes* infections is represented by the existence of a wide variety of M protein serotypes. M-protein is strain specific. Infection with one strain does not provide immunity from infection with another strain. For effective protection against rheumatic fever, a vaccine based on a combination of M protein serotypes isolated from rheumatogenic strains would be necessary because of the wide array of M serotypes found in the different regions of the world. In 1986, studies by Beachey and colleagues have described antibodies against a hybrid peptide containing copies of M serotypes 5 and 24. This vaccine induced the production of antibodies against both serotypes of the M protein, which suggests that effective immunization using multivalent synthetic vaccines is possible (Staali et al., 2006).

More recently, Dale explored tetravalent and octavalente hybrid recombinant protein vaccines. Four serotypes of the M protein have been included in this peptide vaccine containing the N-terminal half of M protein serotypes 24, 5, 6, and 19. Samples from immunized rabbits with polyvalent vaccines were found to offer protection against all serotypes of *Str. pyogenes* (Staali et al., 2003). Recombinant vaccines containing up to eight serotypes of M protein have been found to be effective in inducing serotype-specific opsonization responses in rabbits (Dale, 2008).

To enhance the effects of a vaccine, M protein sequences were combined with an inactivated subunit of the *E. coli* B toxin. Intranasal immunization with a recombinant protein fragment of the M5 serotype protected against intraperitoneal infection in studies undertaken on mice. The *emm-5* gene was introduced into the *Typhimurium* serotype of *Salmonella enterica* in order to create a compound which has been effectively used to orally immunize BALB /c mice

Bessen and Fischetti demonstrated that the infection and colonization processes were influenced by passive administration of specific IgA antibodies against the M protein. The IgA antibody against the salivary M6 purified protein was put in contact with the M6 streptococcal protein before intranasal introduction. This specific IgA antibody decreased mortality rates of infections caused by *Str. pyogenes* strains which presented the M6 protein. When the specific N-terminal region was introduced to the structure of the vaccine it offered protection against homologous serotypes of the M protein. The specific IgG antibody against the M protein caused the opsonization of the bacteria, whereas specific IgA antibody M protein had no effect in regard to opsonization (Ellis and Brodeur, 2003).

A greater protection against multiple serotypes of the M protein, prevention of the colonization process and stimulation of the host's immune responses was achieved by immunization with synthetic peptides corresponding to conserved regions found in the C-terminal region of the M protein. Vaccination of mice with peptides corresponding to highly conserved regions of the M proteins which conjugated with a subunit of the cholera toxin B protected the mice against colonization by *Str. pyogenes*. While the synthetic peptides introduced through the intranasal pathway did not induce the opsonization of the pathogens and did not provide protection against systemic infections, they reduced the nasopharyngeal colonization of the mucosal surface (Steer et al., 2013).

Studies conducted by Bronze and colleagues demonstrated that the immune response specific for the mucosal region was improved by topical administration of vaccines. The M protein-based vaccines used in humans did not induce cross-reactive reactions with the heart muscle and did not lead to the development of rheumatic fever or other afflictions in the vaccinated individuals. Over the years, many volunteers have received streptococcal vaccines with good results, suggesting that immunization against streptococcal infection in humans is possible. Since streptococcal complications are of an autoimmune nature and molecular mimicry may possibly play a role in their pathogenesis, safety issues are a major factor in the development and use of vaccines against *Str. pyogenes* in humans (Steer et al., 2013).

The most recent vaccination strategy aimed either for the N-terminal region specific to M proteins or the highly conserved C-terminal region of these proteins. The vaccine that uses the specific N-terminal region induces bactericidal protection and production of antibodies against specific serotypes of M protein, while vaccines using the C-terminal region offer protection against multiple serotypes and prevent colonization of the mucosal surfaces. Studies suggest that parenteral immunization or localized immunization offers an efficient protection against streptococcal infection (Steer et al., 2013).

3. VACCINE CANDIDATES NOT BASED ON M PROTEIN

3.1. C5a peptidase. C5a peptidase is another promising candidate for a vaccine. Intranasal administration of a modified form of the enzyme induces high levels of the salivary IgA antibodies and serum IgG antibodies in mice. Immunized mice quickly eradicated the streptococcal infections after the intranasal introduction of the vaccine. The aim of this vaccine was to prevent initiation of upper respiratory tract infections before the microorganisms have the opportunity to colonize the mucosal epithelium. Given that C5a peptidase antigen is stable and presents a 95-98% conserved structure in different M serotypes, a vaccine containing this protein would likely induce protection against all serotypes. This idea was confirmed in the case of mice. The immunization with a C5a peptidase isolated from the M49 serotype strains reduced the capacity of M 1, 2, and 11 serotypes to persist in the oral mucosa of the mice. The mechanism by which antibodies directed against streptococcal peptidase increase the rate of clearance is not fully understood. The most likely explanation is that the antibody neutralizes the activity of the protease, thereby affecting the microorganisms which inhabit the human tissues. This in turn could enhance local inflammatory responses and activation of

mononuclear phagocytes. Alternatively, the antibody directed against C5a peptidase could activate the complement through the classic pathway, which will increase non-specific inflammatory responses and the influx of phagocyte cells. In addition, the peptidase is not likely to induce the production of antibodies reactive against the tissue, although this hypothesis has not been tested. Evidence in support of this thinking is the study of group B streptococci that express a C5a peptidase, which is 98% identical at the level of the amino acid sequences to the C5a peptidase produced by *Str. pyogenes*. There are no reports that group B streptococcus can induce a cross-reactive immune response or lead to the development of rheumatic fever (Steer et al., 2009).

3.2. Sfb1. The *Str. pyogenes* fibronectin-binding proteins are important in bacterial adherence to host cells. Two proteins have been identified with the capacity to bind fibronectin. These proteins are named SfbI and protein F. The structural analysis showed that both proteins are identical. In addition to its role in adherence, the SfbI protein also provides support in the internalization of *Str. pyogenes* into nonphagocytic cells. As the entry site for *Str. pyogenes* is represented by the upper respiratory tract, the stimulation of an efficient mucosal response against this protein can lead to the prevention of bacterial colonization. The SfbI protein is expressed by the large majority of streptococcal strains which exhibit different M serotypes and the region involved in the adherence to fibronectin is highly conserved. Studies on this protein have shown that anti-SfbI antibodies do not induce a cross-reactive reaction with other antigens. This knowledge makes the SfbI protein a promising vaccine candidate (Steer et al., 2009).

The process of developing this protein in a vaccine against *Str. pyogenes* infections has been undertaken by Prof. Singh Chhatwal and his team. The immunization of mice through the intranasal pathway with only this protein or a compound formed between this protein and a subunit of cholera B toxin stimulated the production of specific IgG antibodies in the serum and IgA antibodies in the lung mucosa. Over 50% of the strains studied expressed the Sfb1 protein which suggests, if we taken in account its role in adherence to human epithelial cells, that the strains that possess this protein are more likely to cause upper respiratory infections. The study confirmed the previous statement regarding the lack of cross-reactive reactions with tissue proteins (Steer et al., 2009).

3.3. Streptococcal proteinase. This proteinase is an extracellular cysteine protease also referred to as exotoxin B produced by all group A streptococci. The protease is secreted as a zymogen which is cleaved to a mature enzyme under certain conditions. In the large majority of *Str. pyogenes* infections such as pharyngitis and rheumatic fever the host organism produces antibodies against this toxin. Studies regarding this protease have shown that the levels encountered in patients suffering from severe infections are lower than the ones found in mild infections. Finding a way to counter the expression of this protease may offer protection against severe infections. Immunization of mice with a neutralized form of this toxin prolonged their survival after acquiring a streptococcal infection. A passive protection was obtained after administration of antibodies against this toxin. Studies on this proteinase have led to consider the exotoxin B as a potential vaccine candidate (Guilherme et al., 2011).

4. OTHER VACCINE CANDIDATES

The surface antigens expressed during *in vivo* infection can eventually become candidates for a multivalent vaccine. Vaccines that protect a large number of individuals against streptococcal infection may consist of several streptococcal antigens and surface exotoxins to offer protection against invasion, colonization and possible complications. Long-term immunization can be obtained with the help of this vaccines but in order to obtain this result, the scientist have to overcome the challenge represented by the development of a long-lasting immunity against *Str. pyogenes* strains that adhere to the mucosal surface (Steer et al. 2013).

4.1. The lipoteichoic acid (LTA). LTA is a constituent of the cell surface of gram positive bacteria, promoting the bacterial attachment to epithelial cells. When the lipoteichoic acid (*LTA*) was administered in combination with a subunit of the cholera B toxin (a mucosal adjuvant) to mice, the levels of anti-*LTA* IgG in serum and anti-*LTA* IgA antibodies increased after administration of this vaccine (Steer et al., 2013).

4.2. FBP54 protein. Another candidate for the development of a vaccine is the FBP54 protein involved in fibronectin binding, which is common in almost all *Str. pyogenes* strains. The immunization of mice with this protein induced high levels of specific IgG antibodies in serum and of salivary IgA antibodies. Other forms of administration including subcutaneous resulted in significantly longer survival of immunized mice in the case of *Str. pyogenes* infection in comparison to unimmunized mice (Guilherme et al., 2011).

4.3. Streptococcal Hemoprotein Receptor (Shr). The streptococcal hemoprotein receptor (Shr) is a surface-localized GAS protein implicated in heme-containing proteins binding (hemoglobin, myoglobin, heme-albumin and hemoglobin-haptoglobin complex) (Dahesh et al., 2012). Shr is a promising vaccine candidate that is capable of eliciting bactericidal antibody response and conferring immunity against systemic GAS infection in both passive and active vaccination models (Huang et al., 2011).

4.4. Streptolysin S. Streptolysin S (SLS) is a cytolytic toxin produced by *Streptococcus pyogenes* strains. A synthetic peptide based on the streptolysin S toxin induced antibodies against this toxin. This peptide enhanced phagocytosis induced by specific anti-M protein antibodies. This capacity makes this peptide a candidate for multivalent vaccines (Steer et al., 2013).

4.5. Spe A and Spe C exotoxins. Modified versions of streptococcal exotoxins A (*Spe A*) and C (*Spe C*), which do not possess the superantigenic properties of their counterparts, determined protective antibody responses against streptococcal toxic shock syndrome in rabbits. Studies regarding these exotoxins have shown that they offer protection against the effects of the shock syndrome, but the probability that they can be developed in a wider vaccine option is reduced (Steer et al., 2013).

4.6. S. pyogenes cell envelope proteinase SpyCEP. *SpyCEP* is a serine protease expressed on the surface of the human pathogen *Str. pyogenes* that play an important role in the development of

invasive streptococcal infections, enabling the dissemination process by inactivation of the neutrophil chemoattractant interleukin-8 (Zingaretti et al., 2010). A vaccine based on this protein can offer protection in the case of intravenous infections and lower respiratory tract infections with *Str. pyogenes*. The fact that *SpyCEP* is expressed on the surface of the bacterial cells highlights the potential of a vaccine based on this protein, which will be able to enhance the bacterial clearance (Turner et al., 2009, Guilherme et al., 2011).

5. CONCLUSIONS

The struggle to produce a promising vaccine against *Str. pyogenes* began several decades ago. A number of possible vaccines have been developed that showed promising results. At this moment there are several vaccine models in development. Future perspectives on preventing streptococcal infections and complications take into account the development of a vaccines based on a combination of specific proteins. In order to obtain an effective vaccine, the high diversity of M protein serotypes has to be overcome by using a multivalent vaccine based on the whole protein structure or on regions represented by the C5a peptidase (inducing protection against 4 M protein serotypes), Sfb1 (which does not induce cross-reactive reaction) as well as the streptococcal proteinase. The protective immune response generated by the vaccine is amplified by the adjuvants represented by modified structures of: the lipoteichoic acid, *FBP54* protein, *streptolysin S*, *Spe A*, *Spe C* and *SpyCEP* with different implications on the evolution of the infectious process generated by *Str. pyogenes*.

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