Role of M protein in pathogenicity of *Streptococcus pyogenes*

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Abstract: M proteins perform a significant role in *Streptococcus pyogenes* pathogenicity, which is considered one of the causative agents of numerous human diseases. This protein contributes to enhancing the ability to cause infections through multiple mechanisms, including adhesion to host cells and evasion of the immune system. This research highlights the role of M protein as a potential therapeutic target by exploring its associated biological mechanisms. The findings suggest that targeting this protein may contribute to the development of new vaccines and treatments against infections.

***Keywords*:** *Streptococcus pyogenes*, Virulence factors, M protein

1. Introduction to β-hemolytic *Streptococcus pyogenes*

Streptococcus pyogenes is a β-hemolytic and Gram-positive microbe with a low G+C% DNA content. known as group A streptococci (GAS) [20]. Typically spherical or ovoid, with a diameter of less than 2 µm, facultative anaerobes are nonmotile, non-spore-forming, and found in chains or pairs [27]. Usually containing a hyaluronic acid capsule. It is capable of fermenting trehalose, salicin, and lactose to produce acid without releasing gas, extracellular bacteria that breaks down arginine and non-hydrolyzing sodium hiprate or esculin, is resistant to optochin and sensitive to bacitracin, and does not grow at temperatures of 10 or 45 °C [31][27].

Streptococcus species are members of the Firmicutes phylum under the order of Lactobacillales and Streptococcaceae family, belonging to the lactic acid group of bacteria [1]. Their nutritional requirements are complex, as are fastidious organisms typically cultured on blood-supplemented agar medium. The presence of an external source of catalase

promotes streptococcal growth and enables the detection of hemolysis, both of which are critical for subsequent identification steps. *S. pyogenes* can be cultured on selective mediums that are specific to bacteria that are positive for gram stain, like agar with phenylethyl alcohol or Columbia agar with nalidixic acid and colistin. And the inability to grow in conditions with 40% bile salts or 6.5% NaCl [18].

Most of these organisms are common components of the typical human microbiome, including the mouth, pharynx, tonsil, vagina, and lower gastrointestinal (Gl) system. When other normal microbiota is reduced, the inoculum of bacteria is increased, virulence factors are raised, and/or when acquired immunity is weakened, this bacterium will cause disease [1]. Although a sore throat is a mild ailment, 0.3–3% of individuals with GAS pharyngitis may experience severe side effects, including rheumatic heart disease (RHD) and its sequela, acute rheumatic fever (ARF) [22]. Which primarily affect school-age children 5–15 years of age [28]. The first step in *S. pyogenes* disease is successful colonization of the upper respiratory mucosa or skin of a human. Various different adherences for epithelial cells have been qualified, such as M-protein, Pili, Lipoteichoic acid, and Fibronectin-binding proteins [14].

The research focuses on investigating the biological mechanisms that make M protein an important therapeutic target and suggests the potential to exploit this knowledge in the development of new vaccines and treatments against infections. The study emphasizes the importance of future research to identify the best strategies for targeting this protein in prevention and treatment.

2. Virulence factors of *Streptococcus pyogenes*

2.1. Definition and importance of virulence factors

GAS infections are multifactorial processes and complex [28]. Because of its acute and unique infections, *S. pyogenes* has been categorized as a carnivorous bacterium rather than other types that can be observed to cause inflammation that extends deep into the tissues and *S. pyogenes* also possesses a variety of additional pathogenicity elements that enable them to adheres to host tissues, penetrate the skin's tissue layers, and proliferate. They also possess the ability to evade and avoid immune

responses [2]. The virulence factors that are located on the surface of the human host are primarily responsible for GAS ability to colonize and initiate an infection such as carbohydrates (A- CHO), capsule, peptidoglycan, M protein, and Lipoteichoic acid (LTA) which are included in the bacteria's antigenic structure (Antigenic structure) [2][28]. And secreted factors enable the bacteria to disperse to the tissues deeper layers, helping to elude an organized host immune response, such as toxins and hemolysin that are excreted externally (exotoxins), as well as the enzymes streptokinase, hyaluronidase, and DNase which work to lysis DNA, and proteases from proteolytic enzymes [28].

2.2. Types of virulence factors in *Streptococcus pyogenes*

A- The capsule: Some strains of group A bacteria possess a capsule made up of the high- molecular-weight linear polymer hyaluronic acid, composed of β(1-4)-linked disaccharide repeating units of D-glucuronic acid and (1-3)-β-DN-acetylglucosamine, that serves as the bacteria's first line of defense. The function of a hyaluronic acid capsule aids the organisms resisting complement-dependent killing by phagocytic cells. It also influences group A streptococci's ability to connect to epithelial cells by modifying the interaction of M protein with other surface molecules and acting as a ligand for binding to the CD44 receptor on epithelial cell surfaces [13].

B. Lipoteichoic acid (LTA)

The cell wall of GAS is composed of a thick layer of peptidoglycan and integrated Lipoteichoic acids (LTAs) [13]. It consists of 1,3-phosphodiester-linked glycerophosphate with a small lipid moiety (a diacylglycerol), which facilitates the bonding of the teichoic acid domains to the membrane of bacteria [34]. LTA is the first step to adhesion, resulting in low affinity and reversible binding to the bond, according to one definition [25]. LTAs are hypothesized to be necessary for group A streptococci's initial attachment to pharyngeal epithelial cells, other cell types, and host proteins such as fibronectin. In addition to LTAs, various other group A streptococcal adhesions have been discovered, including fibronectin-binding proteins (e.g., protein F1 [SfbI], protein F2 [SbfII], FPB54, and PFBP) [13]. LTA rapidly displays the lipid moiety. The amount of LTA bound to the GAS surface affects the development of biofilms since

hydrophobicity is a requirement for biofilm formation [34]. Additionally, a considerable hydrophobin that increases the hydrophobicity of various Gram-positive bacteria is also present [6]. LTA is a protein with two distinct locations: It can combine with GAS surface proteins like M and M-like proteins to form complexes. It is covalently connected to the membrane. As they attach themselves to surface proteins, rather than membranes, LTA easily reveals its lipid moiety, which makes it a significant factor influencing the hydrophobicity of the GAS surface. Additionally, LTA stimulates the mitogenesis of lymphocytes [34].

C- Fibronectin-binding Protein

A fibronectin-binding protein, is one of the crucial proteins in *S. pyogenes* that helps the bacterium adhere to respiratory epithelial cells. At least 11 fibronectin-binding proteins have been found in *S. pyogenes*. Because it attaches to epithelial cells, this protein is essential to *S. pyogenes'* pathogenicity because it enables the bacterium to securely stick to host cells and not escape. Furthermore, it has been shown that some fibronectin-binding proteins have antiphagocytic properties, which prevent the bacteria from being opsonized by complement [26][29].

D- M- protein

Rebecca Lancefield identified the M-protein, a unique and important antigenic component present in *S. pyogenes* cell walls, approximately ninety years ago [15]. The *emm* gene encodes it [13]. According on its 5′ end sequence of the *emm* gene that codes for the M protein *S. pyogenes* is categorized. There are already more than 200 known *emm* genotypes with more than 1000 subtypes, along with a few unknown *emm*- type sequences [12]. These M types are associated with pharyngitis, have a mucoid colony shape, are often OF negative, and trigger a strong type-specific immune response [13].

E. Invasions (secreated) of Virulance Factor

1.Hemolysin (streptolysin O (SLO) and streptolysin S (SLS))

This particular protein breaks down blood in red blood cells along with platelets, neutrophils, and lyse. It also creates pores that lead to cytolysis. These toxins can produce complete hemolysis of type β hemolysis in a variety of eukaryotic organisms, with differing effects [2][3].

The first kind, streptolysin O, is oxygen-sensitive and mostly responsible for the β-hemolysis seen in the stabbing regions of surface-inoculated SBA plates or surrounding subsurface colonies of group A streptococci in whole plates because of its oxygen lability [13]. SLS is a short, oxygen-stable, non-immunogenic peptide that requires a carrier molecule in order to operate, in contrast to SLO. It builds up on the membranes of platelets, leukocytes, and erythrocytes and, by a process that is yet unknown, induces cytolysis. It was proposed that SLS interacts with the main erythrocyte anion exchange protein band, resulting in osmotic shift, Cl-influx, and erythrocyte lysis [28]. These two toxins have been shown to have immunomodulatory properties in addition to their cytolytic activities. SLS causes pain by activating sensory neurons. Neuropeptides are released as a result, and these prevent the infection site's neutrophil recruitment and the pathogen's subsequent death [22].

2.Streptococcal pyrogenic exotoxin (Spe)B

Is a broad-spectrum cysteine protease that the bacteria produce as a 40 kDa inactive zymogen that matures to 28 kDa via autocatalysis. It has been shown in patients and animals that all sequenced strains release it, however the expression patterns of different *emm* types differ [19]. SpeB can cleave a broad range of host and bacterial proteins and has a vast number of substrates. To diminish antibody-mediated regulatory phagocytosis, SpeB, for instance, can break down immunoglobulins IgA, IgM, IgD, IgG, and IgE into tiny fragments. Additionally, it can cleave E-cadherin and occludin, as well as ECM fibronectin and vitronectin, allowing *S. pyogenes* to penetrate deeper tissues and breach the epithelial barrier [19][29]. Furthermore, C3b is a strong opsonin that attracts phagocytes to infected lesions; SpeB can degrade this opsonin to prevent phagocyte migration. It has also been demonstrated that SpeB has two proinflammatory pathways. The first entails infiltrating the infected skin epithelial cells and directly cleaving and activating GSDMA, which in turn causes cellular pyroptosis. The second method includes directly cutting and activating IL-1β and IL-36γ precursors [32]. On the side of bacterium, SpeB eliminates anchoring proteins from the surface, including numerous proteins that bind to fibronectin, the M protein, and C5a peptidase. Additionally, it hydrolyzes

virulence factors that are secreted, such as EndoS, SLO, and Sags [28].

3.Superantigens (SAgs)

Superantigens, also known as SAgs, are extremely powerful immune system mitogens that cause human and some other mammalian lymphocytes to proliferate and become hyperactivated. The traditional method of activation entails the binding of peptides to major MHC (histocompatibility) class II molecules expressed by antigen-presenting cells, including dendritic cells, macrophages, and B-lymphocytes. The specialized T cells are activated when the peptide/MHC complex is identified by an antigen-specific T-cell receptor (TCR). The spectrum of cells that can be triggered by the TCR is limited by its epitope specificity. By binding non-specifically to MHC and TCR molecules, superantigens can cause cell activation. Thus far, *S. pyogenes* has been found to harbor around 13 different toxins: SpeA, SpeB, SpeC, SpeF, SpeG, SpeH, SpeI, SpeJ, SpeK, SpeL, SpeM, and the streptococcal mitogenic exotoxin Z, also known as mitotoxin Z (SmeZ) [34]. Systemic toxicosis may occur due to the presence of one or more chromosomal superantigens, such as SMEZ, as GAS isolates lacking any phage encoded superantigens have been discovered in individuals experiencing toxic shock. The beginning of STSS has been connected to a deficiency of humoral immunity that neutralizes SAg, and specific host HLA haplotypes are known to be especially vulnerable to SAg activation. This suggests that the hosts immunogenetics may have a greater influence on the course of invasive GAS disease than the superantigen repertoire of the GAS isolate [24]. Pyrogenic exotoxins A (SpeA) and C (SpeC) caused by streptococcal bacteria: Numerous strains of *S. pyogenes* secretes these pyrogenic exotoxins, which cause the scarlet fever rash and a number of symptoms of streptococcal toxic shock syndrome, also referred to as toxic shock like syndrome (TSLS) [10].

. The SpeC is intimately linked to other streptococcal infections including streptococcal traumatic syndrome shock (STSS). The SpeC is intimately linked to other streptococcal infections including streptococcal traumatic syndrome shock (STSS). This gene encodes a mature protein consisting of 208 amino acids with a molecular weight of 24,354. The mature SpeC amino acid sequences and the type A exogenous toxin amino acid

sequences and are identical [30]. There are five SpeC allele variants known (SpeC1– SpeC5). The most prevalent alleles in *S. pyogenes* strains are SpeC1 and SpeC2 [38].

4.Hyaluronidase

Hyaluronidase production by *S. pyogenes* facilitates the organisms spread through connective tissue; therefore, it has been identified as one of the spreading factors of microbial origin because it can attack the hyaluronic acid found in the cement substance of host tissues [13].

5.Biofilm Formation

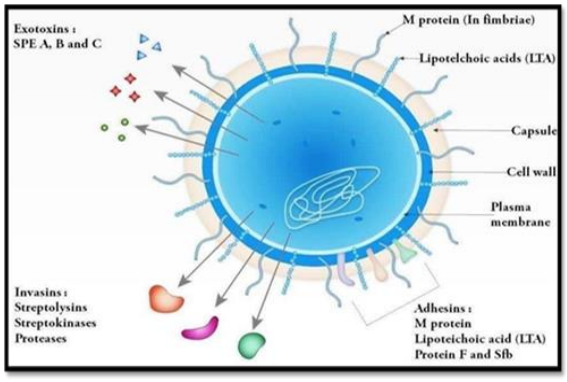
A sessile colony of bacteria that is attached to a substrate, an interface, or one another by means of a self-produced extrapolymeric matrix is known as a bacterial biofilm [37][36]. Biofilm development can be described in five stages. To put it briefly, Stage 1 consists of planktonic cells that are transiently attached to a surface. In this stage, many cells can still move independently, and only a few extrapolymeric components are limked with the connected cells. During the second stage, cells start producing more extracellular polymers, which results in a more stable attachment. During 3 and 4 stages, the biofilm architecture is established and mature. Phase 5 deals with individual or cluster of cell dispersion from the biofilm structure. The cycle of biofilm development can be repeated by these cells, and they are free to spread and recolonize. Mature *S. pyogenes* biofilms are composed of DNA, proteins, and a substance termed glycocalyx that contains polysaccharides [37].

Figure 1: surface of the *Streptococcus pyogenes* [13].

3.Structure and function of M protein

3.1. Primary structure of M protein

It is a fibrous protein that protrudes from the bacterial cell's surface, is attached, and spreads through the peptidoglycan layer [2][13], in electron microscopy, it manifests as "tuft like structures" [34]. It is made up of the proline/glycine-rich C-terminal region, a hydrophobic area that acts as a membrane anchor and the point

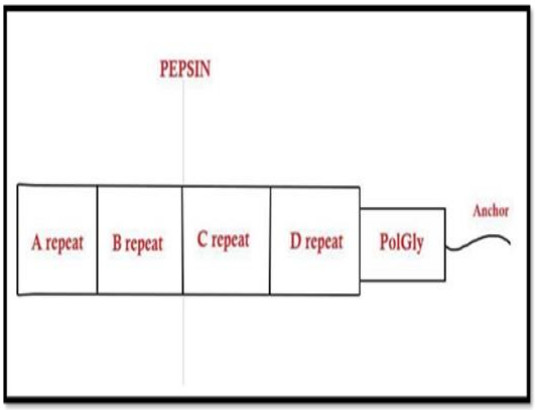


at which the M protein binds to the

bacterial cell wall [2]. M proteins are further categorized according to their reactivity with certain antibodies that target the C- terminal repeats. M proteins are divided into two classes: class I proteins react with these antibodies, and class II proteins do not. Class II strains are designated as SOF+/OF+ because they contain serum opacity factor (SOF), while class I strains are designated as SOF-/OF- because they do not [16]. Protein M is also made up of a highly variable region called the N-terminal that offers antibody protection in this area, which explains the cause of its pathogenicity [17]. M protein comparable to that of Staphylococcal A protein in structure that ranges in size from 41 to 80 KD This protein has a great deal of polymorphism [32].

3.2.M protein's secondary and tertiary structures

Despite the variety of antigens, all M proteins have a dimeric, helical, coiled structure that leads to fibers (approximately 50–60 nm in length) emerging from the bacterial cell wall [2]. Furthermore, (as seen in the emm4 protein) emm proteins bind to IgA via a short stretch of residues close to the N-terminus. There are a variety of methods to extract this protein, including using nonionic detergents and pepsin digestion (at pH 5.8, which yields a product called PepM). The extracted M protein exhibits significant antigenic activity in both cases [16]. (figure 2).



Figure(2): The M-protein's basic structure. The pepsin cleavage point is indicated and typically happens after the 228th amino.

4.Role of M protein in adhesion

4.1. Adhering to the host's cells

It has been demonstrated that M proteins from several serotypes—M1, M3, M5, M6, M18, and M24—all contribute to the adherence of GAS to immortalized cell lines like Detroit 562 and HEp-2. Furthermore, it has been reported that a number of host cell molecules bind to specific M proteins, including collagen types I and IV, CD46, heparin sulfate and dermatin sulfate,

beta2- microglobulin, and sialic acid. However, the interactions with these receptors vary according on the serotype. For instance, it has been observed that the M6 protein binds CD46, whereas the M18 protein does not [4]. A person can have repeated *S. pyogenes* infections with many M types due to the fact that there are over 150 distinct varieties of M protein [23]. Changes in the distribution of M serotypes across different regions over time are likely influenced by the prevailing immunity patterns in those populations, along with the introduction of new M serotypes [35].

4.2.Mechanisms of adhesion

M proteins' immune-modulatory properties are primarily responsible for their contribution to GAS virulence. By directly binding to and recruiting various host components, such as fibrinogen and plasminogen, to the streptococcal surface, they can provide resistance against both innate and adaptive immune responses. M proteins also induce programmed cell death in macrophages by inducing the NLRP3 inflammasome machinery, which in turn causes the processing and secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18, albeit in an M-type-specific manner. Several studies have demonstrated the function M proteins play in host colonization via their sticky contact with receptors on epithelial cells, such as membrane cofactor protein (MCP; also known as CD46) and cell-surface glycans, though serotype-specific differences in these interactions have been reported [4], additionally, M protein provides phagocytosis resistance by non-immunely binding to the IgG antibody's FC region [2].

5.Role of M protein in biofilm formation

5.1.Definition and significance of biofilm formation A biofilm of bacteria is characterized as a sessile population of microorganisms that are enclosed within a self-produced matrix of extracellular polymeric substances and are attached to a substrate, interface, or to each other [36][37]. Because biofilm-forming bacteria are resistant to destruction, the tonsil lymphoid tissue becomes permanently altered and remains infected. Because the biofilm in the tonsils is too large for the for macrophages to swallow, it will interfere with the tonsil lymphoid tissue's normal functioning and eventually lead to chronic or recurrent infections. It has been shown that biofilms, which are usually associated with persistent or recurrent microorganisms, provide defense against immunological reactions and antibiotic treatments [2]. Biofilms pose a major risk to human health since they are inherently resistant to host defenses and can withstand conventional antibiotics up to a thousand times better. *S. pyogenes*, a species of bacteria known to live in biofilms, may survive for a very long time over both biotic and abiotic surfaces, like

books, soft toys, cribs, and other hard surfaces [37].

5.2.M protein's role in biofilm formation

Numerous studies (both in vitro and in vivo) have shown that different GAS serotypes exhibit significant variability in their ability to form biofilms, indicating that GAS does not solely adopt a planktonic lifestyle. Although the importance of M or M-like proteins for biofilm formation is well established, there are a number of additional factors, including adhesins such as pilli, AspA, Scl1, and SpeB, that affect the development of biofilms. However, the role of the GAS capsule in this process has not been completely clarified and remains a topic of debate [4].

6.Experimental methods to study M protein

Cho and Caparon conducted a study to examine if virulence factors, like M protein, were essential in formation of biofilm; the HSC5 strain that is wild-type M14 serotype (having an intergenic plasmid integrated at it) and the HSC5 strain that has insertional damage of the *emm* gene. Biofilms were grown on a C medium that was rich in peptides while low in carbohydrates for 24, 48, 72, and 96 hours at 23 °C utilizing a microtiter plate assay [5]. At any time interval, the HSC5 strain with an *emm* gene disruption was unable to produce biofilms. Since this test was used to identify the initial cell-surface contacts essential for creating a biofilm, Cho and Caparon claim that these findings indicate that M protein regulates the early stages of the development of biofilm. In addition, development in flow chambers was examined under the same conditions utilizing the *emm* gene-disrupted HSC5 strain and the wild-type. Like wise to the microtiter assay, a disruption in the *emm* gene prevented biofilm formation under flow conditions. Based on these observations, Cho and Caparon propose that the M protein is crucial for the earliest cell- surface contacts in the generation of biofilm [5].

Courtney *et al*. went on to investigate whether the members of the family of M protein have a part in the production of *S. pyogenes* biofilms. More particularly, researchers wanted to know if these M protein family members were attaching lipoteichoic acid (LTA) in a way that increased its hydrophobicity. Lipoteichoic acid is reported to have increased the hydrophobicity of bacteria. The hydrophobicity of various serotypes of *S. pyogenes* is determined by the production of surface proteins that create complexes with LTA, exposing the ester-linked fatty acids of LTA to the surface of *S. pyogenes*. Biofilm production was investigated utilizing mutated strains from serotypes M1, M2, M4, M5, M6, M18, M24, and M49 that were created by allelic substitution of specific genes encoding the M protein(s). Mixing glucose with tryptic soy broth (TSB) increased biofilm development threefold while having no effect on planktonic growth. However, the best

growing medium (and one utilized for biofilm production) was Todd-Hewitt broth with yeast (THY), which was selected as the gold standard for future research in this subject. formation of biofilm varied across all serotypes. Biofilm creation was highest in serotypes M2 and M6, and lowest in serotype M49 [6].

Courtney *et al*. proved that M proteins are involved in biofilm development hydrophobicity and promote hexadecane adhesion. A competitive inhibiting enzyme-linked immunosorbent test (ELISA) showed that M proteins influence the quantity of protein- bound LTA. To see if M protein levels influence hydrophobicity and biofilm creation, Courtney et al. created a recombinant strain that expresses emm1, resulting in a twofold increase in Emm1 synthesis in the M1 serotype. The outcomes demonstrated that higher emm1 levels increased protein- bound LTA, biofilm formation, and hydrophobicity when compared with the wild-type and emm1-inactivated strains. Courtney et al. argued that the creation of complexes between LTA and M proteins directly led to hydrophobicity and biofilm development in the majority of *S. pyogenes* serotypes, albeit this link could not be shown in all serotypes. The scientists also hypothesized that the lack of a direct link could be related to specific serotypes that possess numerous members of the M protein family and that inactivating a single member is insufficient to affect these activities [6].

7.Disease manifestations associated with M protein

Successful attachment of surface streptococcal ligands to specific receptors on host cells results in interactions between the pathogen and the host. Early tonsillo-pharyngeal infection stages are caused by different molecules that are anchored in the cell wall of *S. pyogenes* adheres to human cells more readily when certain M proteins are present, either directly or through fibronectin [9]. A human host's upper respiratory tract or skin are colonized as part of the pathophysiology of GAS disease. GAS biofilm formation promotes persistence in the human host. Both the M protein and fibronectin-binding proteins are essential for the subsequent phagocytic uptake of GAS into respiratory epithelial cells. This mechanism of intracellular invasion allows GAS to access an intracellular habitat and is an early stage in the pathophysiology of systemic infection [3].

There are two types of infections caused by bacteria:

1-Suppurative infections: Pharyngitis, Tonsillitis, Sinusitis, Puerperal ،media otitis, Erysipelas ،Cutaneous cellulitis Necrotizing fasciitis, Meningitis, Scarlet fever, Bacteraemia [33].

2-Non-Suppurative infections: Endocarditis, Acute glomerulonephritis, Streptococcal toxic shock syndrome It usually occurs after infections

of the pharynx, tonsils, skin and rheumatic fever [31].

8.Present Status of GAS Vaccines

The development of GAS vaccines can be broadly classified into two groups: vaccinations according to M proteins and vaccines designed to target non-M protein antigens [32].

8.1.M Protein-Based Multivalent Vaccines

M protein on the GAS surface is a key factor in its ability to cause disease and also acts as a protective antigen. Antibodies against the M protein enhance phagocytosis and provide protection against infection. However, some parts of the M protein can cross-react with human tissues, raising concerns about autoimmune responses. Studies have shown that the M protein's N-terminal generates strong immune responses without reacting with human tissues, making it a focus for multivalent vaccine development. These vaccines aim to protect against multiple serotypes of the bacteria [7].

A- 26-Valent M-Protein-Based Vaccines

Peptide fragments from 26 different GAS serotypes were utilized in the 26-valent M-protein- based vaccination. It was evaluated on 26 healthy humans who showed no symptoms of autoimmune reactions or cross-reactions with human tissue after it successfully produced antibodies in rabbits. A 30-valent vaccine has since taken the place of this one [32].

B- 30-Valent M-Protein-Based Vaccine (StreptAnova)

The vaccine StreptAnova targets thirty GAS serotypes that are prevalent in Europe and North America. It is composed of four recombinant proteins. Rabbits respond well to it, developing antibodies against 72 genotypes (30 of which are included in the vaccination and 42 of which are not). It is immunogenic and well-tolerated without inducing autoimmune problems, according to phase I trials. With the ability to cover 80.3% of African isolates, theoretical worldwide coverage is 48% [32].

Conclusion

S. Pyogenes is thought to be highly pathogenic due in part to M protein, which is considered one of the key factors contributing to the virulence of *S. Pyogenes*. By interacting with the immune system and facilitating adherence to host cells, this protein enhances the bacteria`s ability to cause infections and evade the hosts immune response. Therapeutic targeting of M protein holds significant promise for developing effective strategies to combat bacterial infections caused by *S. Pyogenes*. As we gain a deeper understanding of the biological mechanisms behind this protein increases, it may contribute to improved methods for prevention and treatment.

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