

Staphylococcal Protein A as a Pharmacological Treatment for Autoimmune Disorders

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Abstract

Staphylococcal protein A (SpA) is a key virulence factor that enables *Staphylococcus aureus* to evade host innate and adaptive immune responses. The immunomodulatory properties of SpA have led to a hypothesis that it may have pharmacological applications as a treatment for autoimmune disease. Clinical trials are underway to test whether ultrapure SpA can be used to treat immune thrombocytopenia and rheumatoid arthritis. Here, we examine the potential of SpA as an innovative drug to manage autoimmune movement disorders.

Keywords: Staphylococcal protein A; *Staphylococcus aureus*; Tourette syndrome; PANDAS; Dopamine receptors; Autoimmunity

Introduction

Staphylococcal protein A (SpA) is a protective antigen expressed by *Staphylococcus aureus* that allows the bacterium to manipulate innate and adaptive host immune responses [1–6]. *S. aureus* is a commensal organism that forms part of the microbiome in healthy humans; however, under certain circumstances it can behave as an invasive pathogen and cause life-threatening infections. SpA, an immunoglobulin-binding protein, is expressed on the bacterial surface and is secreted freely into the extracellular environment as the bacterium grows [7]. It is expressed by all *S. aureus* strains. SpA binds to the Fc portion of human and animal immunoglobulins, a defense mechanism that provides protection from opsono-phagocytic killing. Furthermore, SpA associates with the Fab portion of VH3-type IgM B cell receptors [8], mediating their cross-linking and leading to activation and clonal expansion of B lymphocytes [9] and their subsequent apoptotic collapse (Figure 1). Recombinant SpA (purified from *Escherichia coli*) does not induce B cell clonal expansion; rather, it induces collapse of VH3 clonal B cells directly [10,11]. It is worth noting that B lymphocytes that express VH3-encoded immunoglobulins play specific roles in

various autoimmune diseases [12]; therefore, they may constitute effective pharmacological targets for the treatment of these diseases.

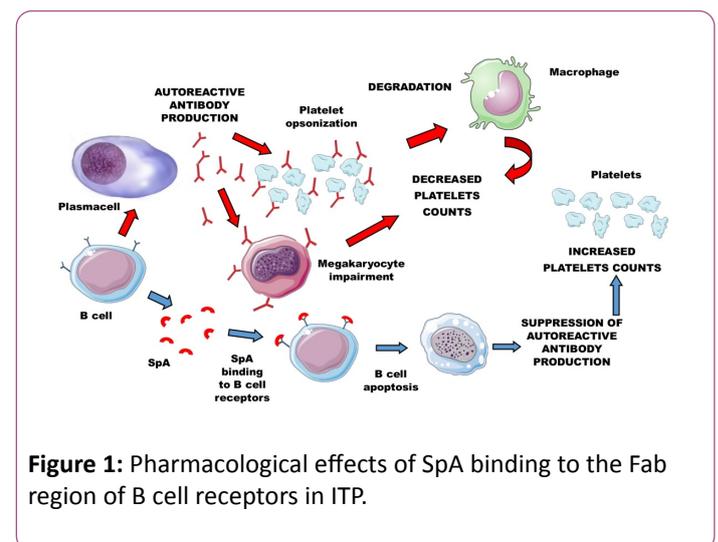


Figure 1: Pharmacological effects of SpA binding to the Fab region of B cell receptors in ITP.

B cells and plasma cells are involved in the pathogenesis of ITP. They are abnormally regulated and produce autoreactive antibodies, which bind platelets and megakaryocytes, inducing their impairment and/or phagocytic degradation in the spleen and liver. SpA binding to the Fab regions of the B-cell receptor promotes B-cells apoptosis and leads finally to inhibition of autoreactive antibody production and platelet degradation.

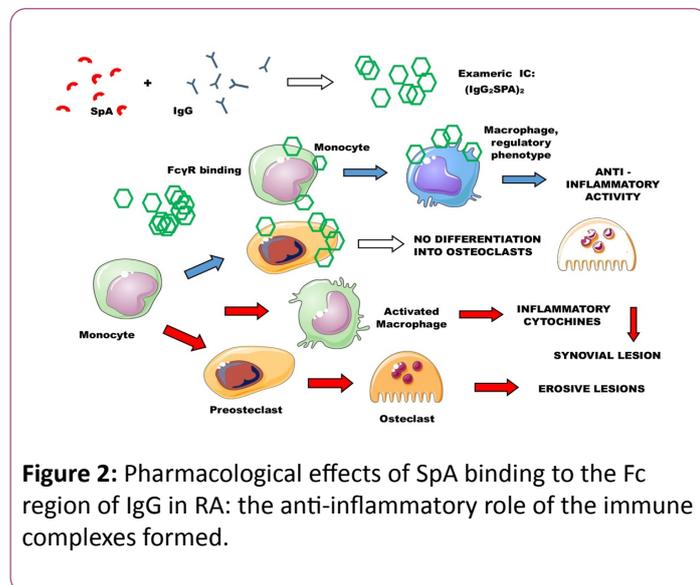
Background

SpA has high affinity for the Fc portion of IgG. IgG antibodies and IgG-containing circulating immune complexes can be selectively removed by extracorporeal exposure of a patient's plasma to protein A immobilized on a matrix [13]. In the 1990s, the US Food and Drug Administration approved a medical device containing SpA covalently linked to silica beads (PROSORBA®, Cypress Bioscience, Inc., San Diego, CA, USA) for plasma-adsorption treatment of patients with refractory rheumatoid arthritis (RA) or refractory immune thrombocytopenia (ITP).

However, the “Guidelines on the Use of Therapeutic Apheresis in Clinical Practice” [14] state the following: “Improvement in ITP may result indirectly from *in vivo* immunomodulation by the release of protein A into the patient, which can induce targeted B cell depletion”. Indeed, leakage of protein A from the matrix, probably due to the activity of serum protease, is thought to occur [13,15].

Animal Studies

SpA has been tested in animal models and has proved to be a successful treatment for several autoimmune pathologies; for example, SpA alleviates antibody-induced nephritis and renal failure associated with systemic lupus erythematosus in mice [16]. In addition, the efficacy of SpA as a therapeutic agent was evaluated in a murine model of collagen-induced arthritis (CIA) [17], which mimics RA in humans. SpA can co-opt circulating IgG molecules and form small, defined hexameric complexes that interact with monocytes, macrophages, and pre-osteoclasts. Formation of these complexes results in Fc γ receptor type I-dependent polarization of macrophages to a regulatory phenotype (Figure 2), thereby rendering them unresponsive to activators such as interferon- γ . The anti-inflammatory complexes can also directly inhibit differentiation of human pre-osteoclasts into osteoclasts “*in vitro*” (Figure 2). Moreover, administration of SpA during the early stages of disease alleviates the clinical and histologic erosive features of CIA in mice [17].



Monocytes can differentiate into either macrophages or osteoclasts depending on their response to specific biological signals. These cells are a primary source of the inflammatory environment that produce synovial and erosive lesions. SpA binds to the Fc portion of circulating IgG and generates small hexameric immunoglobulin complexes (IgG₂SpA)₂ that interact with monocytes, macrophages, and pre-osteoclasts. Formation of these complexes results in Fc γ receptor type I-dependent polarization of macrophages to an anti-inflammatory regulatory phenotype and inhibits pre-osteoclasts differentiation into osteoclasts.

Ultrapure SpA has been used to successfully treat a murine model of ITP (PRTX-100, Protalex, Inc., Florham Park, NJ, USA) [18]. ITP is an autoimmune bleeding disorder in which autoantibodies or immune complexes bind to platelet surface antigens; autoreactive T cells then target and destroy platelets and megakaryocytes in the spleen and bone marrow. Platelet counts in mice treated with PRTX-100 increase to normal levels within 1–2 weeks, and none of the mice die during the experiments [18].

Toxicology studies have also been performed in monkeys [19]. The monkey is considered to be the best predictive animal model due to its similarity to humans with respect to SpA binding to IgG, B cells, and monocyte/macrophages. Weekly intravenous doses of SpA (up to 100 μ g/kg) are well-tolerated and essentially non-toxic. The majority of treated monkeys develop antibodies against SpA. However, no evidence of a hypersensitivity response is observed.

Human Studies: RA And ITP

Two single-dose Phase I studies examined the safety, pharmacokinetic, immunogenicity, and pharmacodynamic activity of highly purified SpA in human volunteers [20]. The majority of subjects developed detectable anti-protein A antibodies after dosing, with no evidence of a hypersensitivity response. A notable pharmacodynamic effect is a transient post-dose reduction in circulating lymphocytes. SpA dosing increases transcription of multiple genes regulated by type-1 interferons in peripheral blood mononuclear cells; up-regulation of several such genes correlates with the degree of lymphopenia observed 24 h after dosing. This study demonstrates for the first time that small intravenous doses of SpA (0.3–0.45 μ g/kg) are safe and well-tolerated in humans.

Following this first toxicological study, a Phase Ib randomized, double-blind, placebo-controlled, dose-escalation study of ultrapure SpA (PRTX-100) and methotrexate was conducted in patients with active RA [21]. The most common treatment-related adverse events are nausea, muscle spasms, dizziness, flushing, fatigue, worsening of RA, and headache. However, most cases of drug-related RA flares are followed by prolonged reductions in RA activity, along with improved symptoms and a reduction in swollen joint counts. No serious adverse events are related directly to SpA (PRTX-100), and none occur in the group receiving the highest dose. As shown in the previous study [20], the majority of subjects develop detectable anti-protein A antibodies, with no evidence of a hypersensitivity response. Although this study did not determine the highest dose of PRTX-100 that could be administered to RA patients on a weekly basis with acceptable toxicity, the results suggest that, at least at the two highest doses tested, PRTX-100 has a positive effect on disease activity. These findings warrant further Phase 2/3 clinical trials to confirm the positive results and to verify if the reduction of RA activity is temporary or permanent.

The promising results obtained in the mouse model of ITP [18], and the promising preclinical data indicating that the drug has the potential to treat ITP by reducing immune-mediated destruction of platelets, support further investigations to

evaluate the safety and efficacy of SpA in ITP patients. Data from initial cohorts in two dose escalation trials (PRTX-100 at a dose of 1 µg/kg or 3 µg/kg) [22] demonstrate an acceptable safety profile, and support continued enrolment of higher-dose cohorts. In one of the trials, a platelet response is observed in one of six patients treated with the lowest dose. Clinical trials examining higher-dose cohorts are underway [23], and updated data from patients treated in Phase 1/2 and European Phase 1b studies will be released in the future.

Autoimmune Movement Disorders

Our proposal to extend experimentation of SpA to the pharmacological treatment of autoimmune movement disorders arose from a clinical case involving a girl with Tourette syndrome [24]. This case presented with characteristics that are similar to those of cases of PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Group A Streptococcal Infections), a clinical condition in which tics and obsessive compulsive disorders follow acute *Streptococcus pyogenes* infections [25,26]. The child presented with high titers of anti-streptolysin O (ASO) and anti-strepto DNase (DNase-B) antibodies and showed a positive reaction to four autoimmunity tests (out of a panel of five) that detect the presence of autoantibodies against brain antigens (Moleculera Labs, Oklahoma City, OK, USA). The assays measure the titers of antibodies against dopamine D1 and D2 receptors [27], lysoganglioside-GM1, and beta-tubulin, in addition to antibodies that activate calcium/calmodulin-dependent protein kinase type II (CaM kinase II) by binding to receptors on neural cell lines.

Microbiological monitoring indicated that the child was an intermittent nasopharyngeal carrier of *S. aureus*, and that a significant improvement in motor tics occurred during the *S. aureus* colonization phase. The nostril is the main ecological niche in which *S. aureus* resides, although the genetic and environmental determinants of carrier status are not fully understood. At any moment in time, about 20% of the general population carries *S. aureus*, while ~30% are transient carriers and ~50% are non-carriers [28,29]. A complex immunological equilibrium exists between host defense mechanisms and the differential expression and roles of *S. aureus* virulence determinants during colonization and disease [30].

This clinical case was of much interest to us because of the observed “see-saw effect” between the host immune response and tic expression. A significant improvement in motor tics occurs during the *S. aureus* colonization phase (nasopharyngeal-, oropharyngeal-, and gut-positive bacterial cultures). Furthermore, the colonization phase is associated with downregulated production of antibodies against *Streptococcus pyogenes* (the etiological agent of PANDAS) and, most importantly, of autoantibody production against D1 and D2 dopamine receptors. Dopamine is a crucial neurotransmitter required for motor control; autoimmune reactions against its neuronal receptors may alter central dopamine pathways and lead to movement and neuropsychiatric disorders, especially in childhood. After decolonization, clinical conditions revert to the poor scores previously observed with a parallel increase of antistreptococcal antibody production. This result was

consistent with data from animal models showing that a pro-inflammatory Th17 cell-associated immune response is required for *S. aureus* nasal decolonization [31]. Ultimately, the colonization phase triggers an immunomodulatory response, whereas the clearing process triggers a pro-inflammatory response. A sequential “uncoupling” of the anti-inflammatory and pro-inflammatory phenomena occurs. These results confirm data from other authors indicating that the pro-inflammatory and anti-inflammatory properties of *S. aureus* are uncoupled and can be expressed separately [32].

Several components of the *S. aureus* cell wall exert anti-inflammatory effects by mediating IL-10 production in macrophages and by downregulating pro-inflammatory cytokine responses, thereby circumventing Th1/Th17 adaptive immune responses during infection [33]. However, the *S. aureus* virulence determinants expressed during colonization and infection are different [34]. SpA is a virulence factor released extracellularly at an early stage to promote both colonization and immune evasion [3]. The beneficial downregulation of antibody production observed during the *S. aureus* colonization phase suggests, albeit indirectly, possible involvement of SpA in the process.

Conclusion

The safety, tolerability, and pharmacokinetics of SpA in animal models, and of ultrapure SpA (PRTX-100) in human studies, together with encouraging preclinical data, suggest that this protein could soon be utilized as an effective treatment for selective autoimmune disorders such as RA and ITP. Clinical trials are ongoing. The improvement of motor tics accompanying reduced production of autoantibodies against D1 and D2 dopamine receptors supports our proposal to include SpA in new clinical trials aimed at identifying innovative pharmacological strategies for the treatment and management of autoimmune neuropsychiatric and movement disorders.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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