Immunome Research
ISSN: 1745-7580

Vaillant et al., Immunome Res 2018, 14:2
DOI: 10.4172/1745-7580.1000157

Research Article Open Access

Inhibition of the Reactivity of Coombs Sera with IgG-Sensitized Human Erythrocytes by Streptococcal Protein-G (SpG)

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Received date: March 02, 2018; **Accepted date:** April 13, 2018; **Published date:** April 16, 2018

Abstract

Streptococcal protein-G (SpG), type III bacterial Fc receptor, is a small globular protein produced by several *Streptococcal* species and it is composed of two or three nearly identical domains, each of 55 amino acids. Streptococcal protein G has been shown to have high binding affinity to sera from various mammalian species including rabbit, human, pig, goat, sheep, cow and many other animal species. Of concern are patients with invasive infections by *Streptococcus spp*, where large amount of secreted SpG could interfere with the outcome of the gel technique by getting false negative tests. It has been shown and reported that the bacterial protein SpA was already found to inhibit the Coomb's test. We hypothesize that SpG as well as many other immunoglobulin-binding bacterial proteins with binding affinity to human IgG could cause false negative results in patients with bacteraemia. With the intention of proving this hypothesis we conducted two sets of experiments, which proved that SpG has the potential of inhibiting the gel test for the detection of sensitized erythrocytes *in vitro*. We concluded that is important to exercise caution, when evaluating a result of gel technique in patients with septicemia caused by SpG-producer *Streptococci*. The experiments used in this research were novel modifications of existent techniques and they proved reliable in demonstrating our hypothesis.

Keywords: Streptococcal protein-G (SpG); Staphylococcal protein-A; Gel test; Anti-red blood cells; Antibodies; Pre-transfusion testing

Introduction

Streptococcal protein G (SpG), type III bacterial Fc receptor, is a small globular protein produced by several *Streptococcal* species and it is composed of two or three nearly identical domains, each of 55 amino acids. SpG binds the Fc regions of IgG from many mammalian species [1]. Sloan and collaborators reported that the primary amino acid sequence of the IgG-binding domains (C1, C2, and C3) of SpG are not homologous to those of the corresponding areas (E, D, A, B and C) of Staphylococcal protein-A (SpA) [2].

A human immunoglobulin G (IgG) prerequisite for satisfactory binding affinity to SpG is the possession of at least two domains by this bacterial Fc-receptor [3]. Streptococcal protein G has been shown to have a high binding affinity to sera from various mammalian species including rabbit, human, pig, goat, sheep, and cow by using competitive RIA [4]. More recently using direct ELISA, some new interactions of SpG with free-ranging nondomestic hoofstock (order Artiodactyla) such as bison, muntjac, oryx, bontebok, elk, addax, antelope, impala, kudu/nyala, sheep, and white-tailed deer have been reported [5]. The binding of SpG to IgG from cervids, giraffes, and peccaries was successfully tested by direct enzyme-linked immunosorbent assay (ELISA) [6].

Coombs et al. [7] described Indirect Antiglobulin Testing IAT for the detection of IgG autoantibodies against human red blood cells. The gel test, developed by Lapierre in 1984 and commercialized later [8], was designed to standardize antiglobulin testing while improving sensitivity and specificity of the method. Anti–human serum immunoglobulin G (IgG) mixed with Sephadex G100 (gel phase) in a microtube traps red cell–IgG agglutination complexes during migration through the gel in a centrifugation step. Agglutination complexes are visibly detectable at various levels in the microtube as an inverse function of antibody coated on red cells. Unsensitized red cells cause a cell pellet at the base of the microtube. Nowadays the gel technique is the most sensitive test for the detection of anti-RBC antibodies and comparing it with the standard Coomb's test present easy testing and better reproducibility [8,9]. This test is essential for pre-transfusion testing.

Of concern are patients with invasive infections by *Streptococcus spp*, where a significant amount of secreted SpG could interfere with the outcome of the gel technique by getting false negative tests. It has been shown and reported that the bacterial receptor SpA was already found to inhibit the Coomb's analysis [10]. We hypothesize that many other bacterial proteins with binding affinity to human IgG could cause false negative results in patients with bacteremia, where bacteria are releasing a massive amount of these immunoglobulin-binding proteins. Two sets of experiments were carried out with the intention of proving in an "*in vitro*" experiment that SpG (as the same as its counterpart SpA) might cause inhibition of the reactivity of the Coombs sera with IgG-sensitized human erythrocytes.

Materials and Methods

Blood Transfusion Service of Jamaica (West Indies) donated blood samples (1 ml of each) and tested negative for common pathogens including HIV-1/2, HBV, HCV, and HTLV-1.

We designed an experiment (SpG effect on the gel test) to prove our hypothesis as follows: SpG (Sigma-Aldrich Co, St. Louis, Missouri) in a concentration of 25 ng/µl added to the antiglobulin cards (Micro Typing System, Inc; Diamed Diagnostika GmbH, Switzerland) that contained 1% suspension of sensitized human O^+ RBC (Micro Typing System, Inc; Diamed Diagnostika GmbH, Switzerland) and then, the cards once incubated at 37°C for 1 h were centrifuged at low speed for 10 min. D-centrifuge 12 S II was used, which was specially developed by ID-System with a capacity of 12 ID-cards, monitored by a microprocessor and with a speed of 1,030 rpm. In ID-card duplicated microtubes have analyzed the samples. SpG effect on the gel technique was tested only in the second microtube of each pair. This experiment was repeated three times and showed similar results.

Study of the neutralization of the SpG inhibitory effect on the gel test was performed as follows: Following titrations to determine the optimal concentrations, 2 ng/ µl of SpG was treated with 2 µl samples of sera from donkey and mule (non-commercially available), as well as, sera from coyote, skunk, raccoon, and chicken (Sigma-Aldrich Co, St. Louis, Missouri) and centrifuged at 13 000 rpm for 5 min. Then, the mixture was resuspended and incubated with an equal volume of a 1% suspension of sensitized human O^+ RBC (Micro Typing System, Inc; Diamed Diagnostika GmbH, Switzerland) in an antiglobulin gel test card (ID-cards, also acquired from the previous chemical company) for 15 min at 37°C. Centrifuged, visualized and photographed were the cards. From 0 to 4+ was graded a positive reaction. According to manufacturer's instructions, a solid band of RBCs on the top of the gel indicated the 4+ response. The A 3+ reaction displayed agglutinated RBC in the upper half of the gel column. RBC characterized the 2+ response that dispersed throughout the length of the column. The A1+ response was indicated by RBC agglutination mainly in the lower half of the gel column with some non-agglutinated RBCs pellet at the bottom. Negative reactions had RBC pellets on the bottom of the microtube with no agglutination within the matrix of the gel column. This experiment was repeated three times and showed similar results.

Results and Discussion

The picture below reflects the results (Figure 1). Samples 107, 109, 36, 87, 104, 39, 44, 108, 105, 21, 2+3 and 3 tested positive, proving our hypothesis of the inhibitory SpG effect on the gel test in the detection of anti-RBC antibodies. Negative samples included 20n, 2, 56, 6c, 9n and 7 (not treated with SpG).

The result of the gel test should be an important part of blood testing for auto-antibodies in transplant screening tests and blood transfusion services [11-14].

Sera from non-human mammalian species neutralized the inhibitory effect of SpG (Figure 2). Whereas mule donkey, skunk, and coyote sera nullified to an extent the inhibitory effect of SpG on the detection of anti-RBC antibodies in human sera, raccoon serum (diluted 1:64) did not interfere with the reaction, because it has a low binding affinity to SpG.

The neutralization of the inhibitory effect of SpG on the antiglobulin gel test following pre-incubation of SpG with different non-human mammalian sera was also in keeping with the fact that SpG is capable of binding IgG molecules from a variety of mammalian species and testifies the SpG capability to inhibit the reactivity of Coombs sera with IgG-sensitized human erythrocytes.

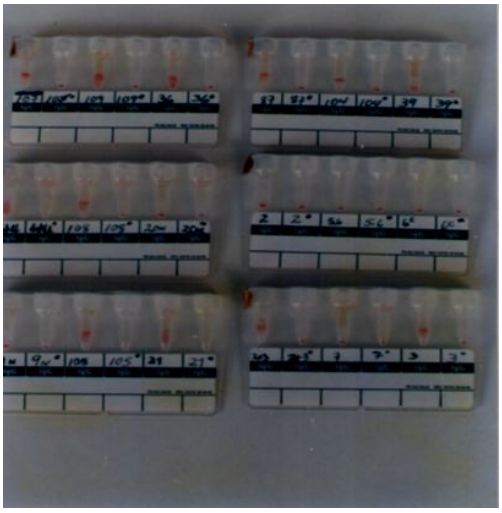

Figure 1: The inhibition of the detection of anti-human RBC antibodies by streptococcal protein G in the antiglobulin gel test; Serum samples were tested before and after treatment with protein G (samples treated with the protein are marked with an asterisk); SpG inhibited the antiglobulin gel test in the sample mention above.

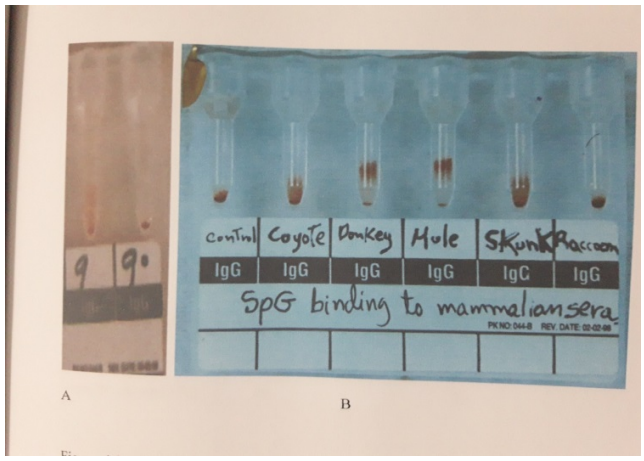

Figure 2: The neutralization of the inhibitory effect of SpG on the antiglobulin gel test following pre-incubation of SpG with different non-human Mammalia sera; In (A) is shown the inhibitory effect of SpG on the antiglobulin gel test (also shown in Figure 1); In (B) is shown the neutralization of the SpG inhibitory effect on the antiglobulin gel test by sera of different Mammalia species; The neutralization is directly related to the SpG binding capacity of IgG molecules present in the non-human mammalian sera; Thus, sera of coyote, skunk, donkey, and mule reacted with SpG and neutralized its inhibitory effect, while diluted raccoon sera did not inhibit the SpG effect on the antiglobulin test; This test nullifies the negative SpG effect on the gel test for the detection of sensitized RBC.

The potential diagnostic applications of SpA or SpG gel tests are: in pre-transfusion testing, hemolytic transfusion reaction, autoimmune or drug-induced hemolytic anemias [15-17]. SpG has been

commercially available as a reagent in antiglobulin tests [18]. Two additional micro-column affinity tests were reported [19]. Besides, two analyzes for red cell antibody screening, the anti-globulin gel test and the micro-column affinity test using SpG as an antiglobulin were documented, and they were highly sensitive and specific [20].

Many bacteria secrete IgG-binding proteins including Staphylococcal protein-A and Peptostreptococcal-protein L [21], which binds to immunoglobulins and participate in humoral immunity and inflammatory reactions. Anyone of these proteins may cause false positive reactivity in a gel test for the detection of anti-red blood cell antibodies.

Other applications of SpG includes its use in immunoblot analysis for the detection of monoclonal antibodies [22]. Hu et al. later reported an experiment for the optimization of immunoblot analysis using conjugated SpG-conjugates that avoided the false positive reactions, which often involved the use of labeled secondary antibodies and increased the detection of autoantibodies [23]. In the purification of IgG and antibody fragments from different mammalian species immobilized SpG has been used [24]. Perosa et al. reported that SpG binding to F(ab')2 is restricted, as indicated by the lack of reactivity of F(ab')2 fragments from human IgG with SpG-sepharose columns [25].

Conclusion

We concluded that is important to exercise caution when evaluating a result of gel technique in patients with septicemia caused by SpG-producer *Streptococci*. The experiments used in this research were new modifications of the existent, and they proved reliable in demonstrating our hypothesis.

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