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Letter to the Editor Open Access

Two New Immunoassays to Study the Binding Capacity of Staphylococcal Protein A (SpA) or Streptococcal Protein G (SpG) to Sera from Four Mammalian Species Including Wild and Domestic Animals

Angel Justiz-Vaillant[1*], **Norma McFarlane-Anderson**[2] **Wayne Mohammed**[1] **Sehlule Vuma**[1]

[1]*Pathology and Microbiology Unit, Department of Para-Clinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago, West Indies*

[2]*Biochemistry Section, Department of Basic Medical Sciences, The University of the West Indies, Mona Campus, Jamaica, West Indies*

[*]**Corresponding author**: Angel Justiz-Vaillant, Pathology and Microbiology Unit, Department of Para-Clinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago, West Indies; Tel: +868-773-5914; E-mail: angel.vaillant@sta.uwi.edu

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Description

The aim of this study was to report on the interactions between Staphylococcal Protein A (SpA) or Streptococcal Protein G (SpG) and sera from four mammalian species: raccoon, skunk, coyote, and mule using two novel techniques. SpA has a molecular weight of 42 kDa. It binds to the Fc fragment of IgG produced by several animal species. The native protein consists of five domains. Of these, four show high structural homology of about 58 aminoacids and they have binding capacity to immunoglobulins [1,2]. Streptococcal protein G, Type III bacterial Fc receptor, is a small globular protein produced by several streptococcal species and is composed of two or three nearly identical domains, each of 55 amminoacids. SpG is well-known for binding to IgG from many species [3,4].

Study of the SpA binding capacity to sera by a novel assay: Inhibition of the agglutination of SpA-bearing Staphylococcus aureus.

Most sera were commercially available (Sigma-Aldrich Co). Briefly serial dilutions of 25 µl of the various mammalian sera were added in duplicate to 96 wells micro-titer plates containing 25 µl of SpA-bearing S. aureus cells (Sigma-Aldrich Co) and incubated for 1 hour at RT. Inhibition of agglutination was seen in positive samples (containing antibodies that react with SpA) and agglutination at the bottoms of the wells was seen in negatives samples. In this test a donkey serum was used as a positive control and a chicken serum was used as a negative control.

Study of the SpG binding capacity to sera by a novel assay: Neutralization of the SpG inhibitory effect on the gel test.

Following titrations to determine the optimal concentrations, 2 ng/µl of SpG was treated with 2 µl samples of sera from mule, coyote, skunk, raccoon, chicken and donkey and centrifuged at 13 000 rpm for 5 min. The mixture was resuspended and incubated with an equal volume of a 1% suspension of sensitized human O^+ RBC in an antiglobulin gel test card for 15 min at 37°C. The card was centrifuged, visualized and photographed. After centrifugation positive reaction was graded from 0 to 4+. A 4+ reaction was indicated by a solid band of RBCs on the top of the gel. A 3+ reaction displayed agglutinated RBC in the upper half of the gel column. A 2+ reaction was characterized by RBC agglutinates that dispersed throughout the length of the column. A1+ reaction 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 as shown in Figure 1.

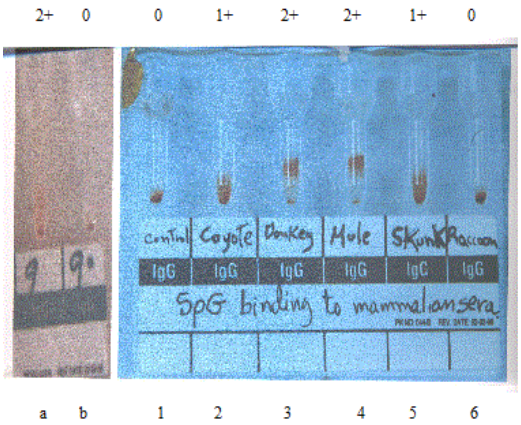

Figure 1: It shows the results of the neutralization of the SpG inhibitory effect on the gel test. a=Positive control; b=Negative control; 1=Chicken serum (another negative control) shows no binding capacity to SpG; 2=Coyote serum shows moderate binding capacity to SpG (1+); 3=Donkey serum (another positive control, 2+); 4=Mule serum shows high binding capacity to SpG (2+); 5=Skunk serum shows moderate binding capacity to SpG (1^+); 6=Raccoon serum shows no binding capacity to SpG.

ELISA

The results of the two new techniques were compared with a sensitive Enzyme-Linked Immunosorbent Assay (ELISA) to confirm the respective binding capacities of SpA and SpG with serum specimens: The 96 well polystyrene microplates (U-shaped bottom) were coated with 500 ng of SpA or SpG (Sigma-Aldrich) in coating buffer for 4 h at 37°C. The microplates were washed four times with PBS-Tween-20 and blocked with 3% non-fat milk in PBS, 25 µl/well, 1h, at Room Temperature (RT). The microplates were washed four times again. Samples were added in dilutions of 1:32. After incubation for 1h at RT the microplates were washed four times and 50 µl of peroxidase-labelled-SpLA or peroxidase-labelled-SpG diluted 1:3000 was added. The microplates were then incubated for 1h at RT, washed four times. Tetramethylbenzidine (TMB) solution (50 µl) was used.

After a further incubation of 15 min in the dark, the reaction was stopped with 3M H2SO4 and read in a microplate reader at 450 nm. The cut-off point was 0.25 for SpA-ELISA and 0.31 for SpG-ELISA.

Both novel techniques: inhibition of the agglutination of SpA-bearing *Staphylococcus aureus* and neutralization of the SpG inhibitory effect on the gel test were effective in the study of SpA or SpG binding capacity to animal sera and the results were corroborated by the ELISA (as shown in Table 1). The SpA test demonstrated a high binding capacity to sera from skunk, coyote, raccoon and mule, and the SpG test showed high binding capacity to sera from mule, moderate binding capacity to sera from coyote and skunk and very low interaction with raccoon sera as shown in Figure 1. The study of these protein-protein interactions is important since SpA or SpG can be used as a "secondary antibody" conjugated to enzymes, gold, and other molecules in immunoassays for detection of specific antibodies, immunodiagnosis of infectious diseases and in the purification of immunoglobulins in the wild animal population that lacks commercially prepared antibodies for immunotesting. The results reported here are novel in nature.

Serum samples	SpA-ELISA (Mean OD/SD)	SpG-ELISA (Mean OD/SD)
Skunk	0.67/0.008	0.49/0.006
Coyote	0.71/0.005	0.45/0.007
Raccoon	0.69/0.01	0.22/0.003
Mule	0.57/0.008	0.6/0.01
Donkey (+ control)	0.62/0.006	0.63/0.004
Chicken (- control)	0.08/0.002	0.01/0.001
Blank	0.004/0.001	0.005/0.001

Table 1: Sandwich ELISA for detection of SpA or SpG binding capacity to sera from wild and domestic animals. The mean Optical Density (OD) was calculated from duplicated samples.

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