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

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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

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