SUPPORTING INFORMATION

Group-specific detection of 2-deoxystreptamine aminoglycosides in honey based on antibodies against ribostamycin

Inna A. Galvidis[1], Konstantin M. Burkin[1,2], Sergei A. Eremin[2], Maksim A. Burkin[1*]

[1] Department of Immunology, I.I. Mechnikov Research Institute for Vaccines and Sera, Maly Kazenny per., 5a, Moscow, 105064 Russia;
[2] Faculty of Chemistry, M.V. Lomonosov MSU, Leninsky Gory, 1, 119991 Moscow, Russia;

*Corresponding author: Tel/Fax: +7 495 9172753.
E-mail: burma68@yandex.ru (M. Burkin)

1. Examination of immunogen conjugates

The absence of specific UV-Vis spectra of AGs did not allow us to estimate changes in spectrophotometric characteristics of prepared conjugates. For this, the formation of conjugates was confirmed immunochemically in indirect ELISA. BSA-based conjugates prepared with different hapten excess over the carrier were coated on Costar 96-well plates from 3 μg/mL solutions in 0.1 mL of 0.05M carbonate-bicarbonate buffer (pH 9.6). After overnight incubation at 4°C and washing plates, a serial dilutions of antibody against neomycin (NM) were added and incubated for 1h at 25°C. The plates were washed with PBS-T three times and bound antibodies were detected using secondary goat anti-rabbit IgG conjugated to horseradish peroxidase for 1h at 37°C. After washing, 0.1 mL of substrate solution containing TMB was added and 30 minutes later, the enzymatic reaction was stopped by adding 0.1 mL of 0.5 M sulfuric acid. The absorbance was read at 450 nm.

Antibody against NM conjugated to human transferrin were previously obtained in our laboratory [1] and used here to reveal the nearest structurally related RS-determinant in prepared conjugates. As it can be seen from Fig S1, anti-NM were able of dose-dependent binding to BSA-RS conjugates. The intensity of the interaction reflected the hapten load and was proportional to the excess hapten over BSA taken for the synthesis of the conjugates.

The additional examination was conducted in competitive ELISA format where the tested conjugates served as competitors in anti-NM – Gel-RS(pi) interaction (Fig S2). The procedure of assay did not differ from that described above. Immunoreagents ratio was optimized for assay of NM [1]. The conjugates were taken at the range of concentration 1-100 μg/mL. The data obtained

are in good agreement with the analysis of binding. BSA-C6-RS×250(pi) and BSA-RS×100(pi) also showed the greatest activity as competitors, which means a higher and similar hapten load.

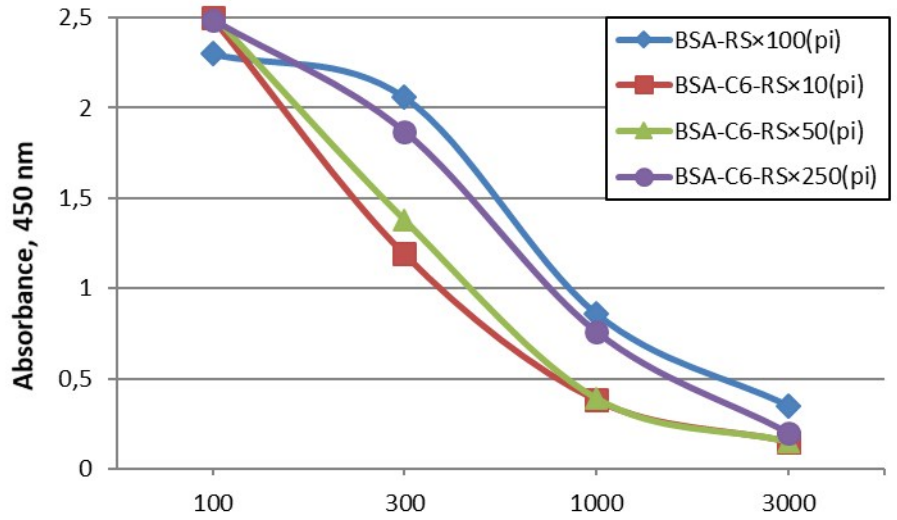


Figure S1. ELISA titration of anti-NM antibody on coated BSA-RS conjugates.
Each point is presented the average values of replicate measurements.

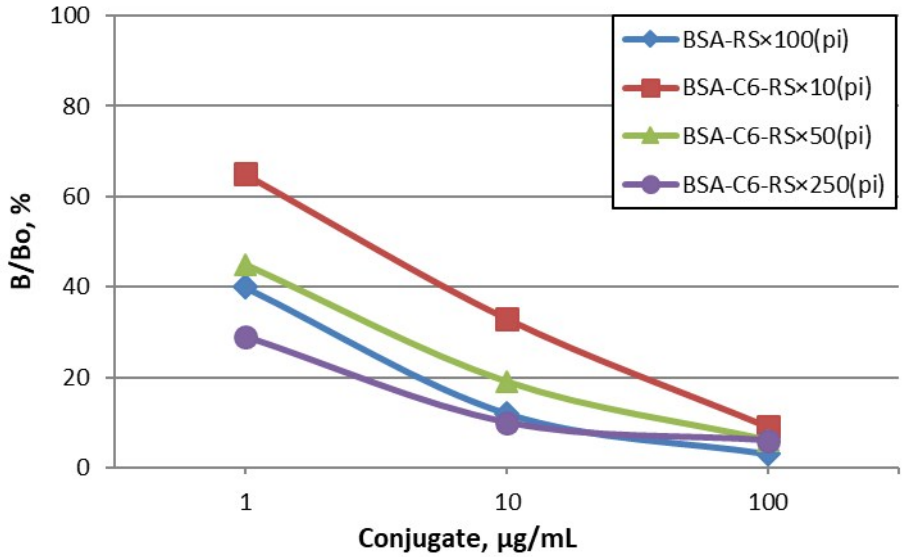
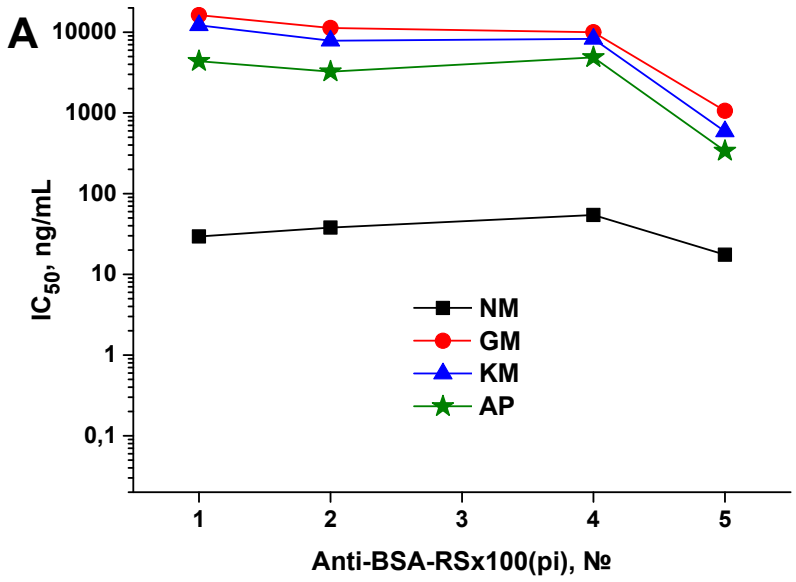


Figure S2. Inhibitory activity of BSA-RS conjugates with different hapten load on anti-NM antibody binding to Gel-RS(pi).
The average values (n=2) of relative binding (B/Bo) were obtained from replicate wells.

2. Examination of immune response specificity

To choose which immunogen design provides the better generic anti-AG response, the specificities of obtained antisera were examined in competitive ELISA with a panel of analytes. The dynamics of immune maturing during immunization course from the animals exposing the better response is

shown in Fig S3. As can be seen from the diagram BSA-RSx100(pi) initiated response mostly against NM, while AGs from the other families recognized much worse (≤5%)(FigS3A). The improvement in sensitivity observed after the 5th booster, however, was not a trend with continued immunization (Data not shown).



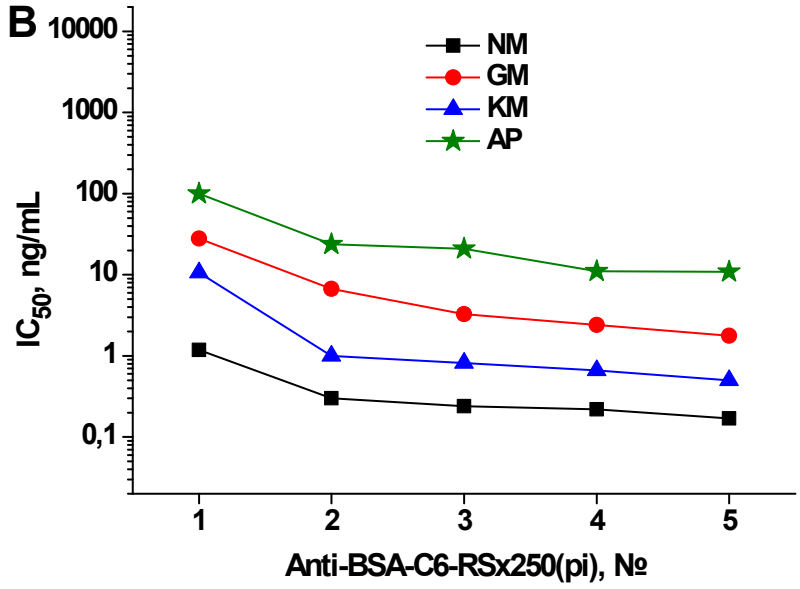


Figure S3. Dynamics changes in sensitivity of AGs determination in ELISA using anti-BSA-RSx100(pi) (A) and BSA-C6-RSx250(pi) (B) sera during the course of immunization. The IC_{50} values were calculated from the standard curves obtained for each analyte taken in triplicates. Gel-RS(pi) was used as a coating antigen.

Higher sensitivity of AGs determination (IC_{50}= 0.2 – 10 ng/mL) was achieved with C6-spacer containing immunogen, BSA-C6-RSx250(pi) (Fig S3B). A moderate improvement in sensitivity was

observed during the course of immunization demonstrating the best parameters for anti-BSA-C6-RSx250(pi) #5.

References

1. M.A. Burkin, I.A. Galvidis. Development and application of indirect competitive enzyme immunoassay for detection of neomycin in milk. Appl. Biochem. Microbiol. 2011, 47(3), 321-326.