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Egg-derived antibody for prevention and treatment of *E. coli* induced diarrhea

Mahdi Saeed
Michigan State University, USA

Antimicrobials are still the major tools for treating infectious diseases. However, the emergence of multidrug resistant bacterial pathogens and the observation that antibiotic treatment might actually increase the production of some bacterial-derived toxins, limit the usefulness of this approach, Enterotoxigenic *Escherichia coli* (ETEC) strains that produce heat stable enterotoxin are the leading cause of traveler's diarrhea and a major cause of diarrheal disease in developing countries, especially among children. They are transmitted by contaminated food and water and cause profuse watery diarrhea which results in rapid dehydration and death in human neonates. The objective of this project is to produce antibodies against STa that can serve as a therapeutic preparation against diarrheal disease caused by ETEC strains. In order to produce large amounts of high avidity antibodies, a novel approach was pursued which consists in purification of ETEC-STa, conjugation with bovine serum albumin and immunization of egg-laying hens to produce STa-specific egg yolk-derived antibodies. Chickens were selected due to the well-known capacity of birds to generate strong immune responses to protein antigens exceeding those generated in rabbits and other mammalian species, pooled egg-yolk samples from 24 different birds immunized with STa were produced. These samples were transferred and characterized in terms of levels of antibodies and antibody avidity by the Immunology Research Unit (IRU) at the University of Maryland. ELISAs to measure STa antibody titers and avidity were set up and validated using the following reagents: Lyophilized E. coli STa, lyophilized STa hyper-immune rabbit serum, STa-containing egg yolk extract from an immunized bird (positive control) and purified IgY. ELISA checker board titrations assessing different experimental conditions and reagents were performed which allowed establishing the adequate experimental conditions for measurements of STa-specific rabbit IgG and chicken IgY antibodies with good sensitivity and reproducibility. The STa antigen purified at MSU was potent and produced very high absorbance values with low background. The rabbit antiserum prepared at MSU was also highly reactive and yielded elevated antibody titers, above 1×100 EU/ml. The optimal conditions for the measurement of chicken IgY STa antibodies included: Coating with STa at 1 pg/ml in PBS buffer overnight at 4 oC, washing plates after each incubation 6 times with PBS Tween 0.05%, soaking plates for 2 min in between washes; blocking plates with PBS 0.102 Tween, incubating samples for that 37 oC, using as corlugate: HRP-conjugated AffiniPure Rabbit anti-chicken IgY, Fc Fragment Specific diluted 1/5000. A standard curve with known concentration of IgY (a commercial reagent) was used to interpolate absorbance values and to report antibody titers in mass per volume (concentrations). To measure avidity, an ELISA was performed as described above adding a 10 min overlay with 6M urea after incubation with the experimental samples with the purpose of dissociating low affinity antibodies. An avidity index was calculated for each sample as the residual titer measured in the presence vs. absence of urea. The STa IgY titers in the 24 egg yolk samples ranged between 470 and 6182 EU/ml or 390 and 5399 ng/ml (expressed in antibody concentrations). The range of avidity indices (AI) were between 54 and 100% with a mean AI of 77.2. The avidity of STa IgY antibodies was higher than the avidity of the rabbit antisera despite the higher antibody levels produced in rabbits.

Biography

Mahdi Saeed is the Professor of Epidemiology and Biostatistics in Michigan State University. He has completed his DVM degree in 1973 from University of Baghdad and PhD from Washington State University.

saeeda@msu.edu

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