Comparative Inhibitory Effects of Antigen and Antibody in the 
Staphylococcal Enterotoxin Solid-Phase Radioimmunoassay 
System

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A solid-phase radioimmunoassay employing 125I-labeled enterotoxins and polystyrene tubes coated with specific antibody has been developed for assaying the relative concentrations of antibodies to staphylococcal enterotoxins A and B. Competitive binding occurs between tube-bound antibody and free antibody for binding sites on 125I-labeled enterotoxin. The sensitivity of the system is affected by the amount of antibody on the walls of the tubes, the concentration of 125I-labeled enterotoxin added to the system, and probably by the relative binding affinities of the bound and unbound antibodies. Antibody, 0.01 to 0.07 μg/ml, inhibited the uptake of 125I-labeled enterotoxin by 20%. Both the antibody and antigen solid-phase radioimmunoassay inhibition systems can be appropriately represented by either of the following two models: 

\[ \log_2 \left( \frac{Y}{1 - Y} \right) = \alpha_0 + \alpha_1 \log X + \beta_1 \log Y, \]

where \( Y \) is bound activity, \( X \) is antigen or antibody concentration for inhibition, and \( \alpha_0, \alpha_1, \beta_1 \) are regression coefficients. Estimates from the first model were slightly more precise for the antibody system, whereas the reverse was true for the antigen system.

We have recently developed a solid-phase radioimmunoassay test for assaying staphylococcal enterotoxins A and B in purified form and in crude form (8). This test has also been used to study the antigenic relationships among enterotoxin types A, B, and C (7). Radioimmunoassay involving salt precipitation of gamma globulin has been used to determine relative antibody concentrations (3). We report here that the solid-phase radioimmunoassay test, with slight modification, may be used to determine the relative potencies of antiserum to enterotoxins A and B. Competition occurs between free antibody and tube-bound antibody for antibody-binding sites on 125I-labeled enterotoxins. Antigen and antibody inhibition in solid-phase radioimmunoassay are compared by two empirical mathematical models.

MATERIALS AND METHODS

Purified enterotoxins. Purified staphylococcal enterotoxins A and B were supplied by M. S. Bergdoll, University of Wisconsin, Madison. The purified toxins contained less than 5% impurities (1). 

Enterotoxin antiserum. Antisera to enterotoxins A and B were produced in rabbits as described previously (5). Additional antisera to enterotoxin A were obtained from R. W. Bennett, Food and Drug Administration, Washington, D. C. For use as inhibitors, the various antisera were heat inactivated 10 min at 56 C and diluted in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.2 (0.07 M NaCl, 0.07 M phosphate), to which sodium azide (0.1% final concentration) had been added as a preservative. Antibody protein was determined by quantitative precipitation (2).

Iodination of enterotoxins. Enterotoxins A and B were radiolabeled with 125I by the chloramine-T procedure (4, 6), modified as previously described (8). Labeled enterotoxin contained approximately 4 μCi of activity per μg of protein.

Preparation of antibody-coated polystyrene tubes. Polystyrene tubes (10 by 75 mm) were sensitized with antibody to enterotoxin as previously described (8). Pooled anti-A and anti-B enterotoxin sera were used and contained 5,384 and 2,975 μg of antibody protein/ml, respectively. One-milliliter amounts of sodium sulfate-precipitated antibody in PBS, containing approximately 2 to 10 μg of protein/ml, were added to polystyrene tubes with a volumetric pipette. After 2 h at room temperature, the antibody was removed, and the tubes were washed once with 2.0-ml amounts of 1% BSA in PBS. The tubes were then filled with 1% BSA and left overnight at room temperature. The BSA was removed, and the tubes were washed once with PBS and stored inverted at 4 C until used.

Counting equipment. Radioactivity was measured with a Packard Auto-Gamma Counter (model 5320). This system has a counting efficiency of approximately 62% for 125I in a 1-ml geometry and a background count rate of approximately 50 counts/min.
**Solid-phase radioimmunoassay.** Antigen inhibition tests were carried out as previously described (8), except that antigen was removed from antibody-coated tubes after incubating at 37 C for 18 h. After antigen removal, 1 ml of 1% BSA and 0.001 μg of 125I-labeled enterotoxin A or B (in 0.1 ml of 1% BSA) were added to each tube. The tubes were shaken vigorously, incubated at 37 C for 4 h, washed once with 2 ml of PBS, and counted.

For antibody inhibition, 1 ml amounts of various dilutions of antisera in 1% BSA in PBS were added to antibody-coated tubes. 125I-labeled enterotoxin, 0.001 μg or 0.0001 μg in 0.1 ml, was added to each tube. Individual tubes were shaken vigorously, incubated at 37 C for 4 h at 37 C, washed once with 2 ml of PBS, and counted for radioactivity.

**RESULTS**

Two empirical mathematical models were used to examine the antigen and antibody-inhibition systems. They are: Loge(Y/1 - Y) = a0 + α1 LogeX (model I) and LogeY = β0 + β1 LogeX (model II), where Y is the fraction of activity bound in presence of antigen or antibody, X is the antigen or antibody concentration, and α1, β0, and β1 are regression coefficients. Model I is a well known relation used in radioimmunoassay work that requires that the logit of Y (Loge(Y/1 - Y)) be related to LogeX (10). Model II is a linearization (8) of the function Y = AXβ.

Antigen inhibitions in solid-phase radioimmunoassay were carried out using both enterotoxins A and B. Figure 1 presents representative data on the inhibition of uptake of 125I-labeled enterotoxin B (0.001 μg) on anti-B-coated tubes by various concentrations of unlabeled enterotoxin B. The data are plotted for both models I and II. Correlation coefficient data (9) for the regression lines and variance data (10) for the mean (X) of the concentrations of unlabeled enterotoxin B added to the system are presented in Table 1. Correlation coefficient values for enterotoxin B inhibition indicate that both models gave satisfactory linear fits of the data. The F-ratio values (Table 1), however, indicate that model II gave a more precise fit of the data. These F-ratios are obtained by dividing the largest estimate of variance by the smallest. Since Ys are assumed to be normally distributed (10), but not their transform (i.e., Loge [Y/1 - Y] or Loge Y), an estimate of an individual Y variance was obtained at the mean (X) to compare the precision of the models. Similar inhibition data were obtained with enterotoxin A (regression data not shown).

Representative data on antibody inhibition of solid-phase radioimmunoassay involving the anti-enterotoxin B system and 0.001 μg of 125I-labeled enterotoxin B are presented in Fig. 2. As in the previous figure, data are plotted for both models I and II. The correlation coefficient and variance data (Table 1) indicate that both models gave adequate fit of the data, but model I gave a more precise fit. This is just the opposite of the data for enterotoxin (antigen) inhibition of the system (enterotoxins A and B, Table 1). Anti-enterotoxin B at a 1:100,000 dilution in 1 ml gave approximately 25% inhibition of uptake of 0.001 μg of 125I-labeled enterotoxin B. Neither pooled normal rabbit serum (1:1,000 dilution) nor anti-enterotoxin A serum (1:1,000 dilution) inhibited the uptake of labeled enterotoxin B.

A similar pattern of inhibition for the enterotoxin is seen in Fig. 3 when data are plotted for both models I and II.

**TABLE 1. Statistical comparison of models I and II for radioimmunoassay involving antigen and antibody as inhibitors**

| Inhibition system | Model | Correlation coefficient | Mean of log of antigen and antibody concn (X) | Variance of Y at X | F ratio |
|-------------------|-------|-------------------------|-----------------------------------------------|-------------------|--------|
| Enterotoxin B     | I     | -0.913                  | -5.451b                                       | 0.00170           |        |
|                   | II    | -0.909                  | -5.451b                                       | 0.00044           | 3.86f  |
| Enterotoxin A     | I     | -0.982                  | -5.451a                                       | 0.00119           |        |
|                   | II    | -0.988                  | -5.451a                                       | 0.00029           | 4.76f  |
| Anti-enterotoxin B| I     | -0.998                  | -10.297c                                      | 0.00034           |        |
|                   | II    | -0.995                  | -10.297c                                      | 0.000318          | 9.35f  |
| Anti-enterotoxin A| I     | -0.980                  | -9.393c                                       | 0.00105           |        |
|                   | II    | -0.960                  | -9.393c                                       | 0.00022           | 4.97f  |

*Loge of unlabeled enterotoxin concentration (micrograms per milliliter) for inhibition.

*Loge of decimal of anti-enterotoxin dilution for inhibition.

fSignificant at α = 0.01.
toxin A system by anti-A is seen by representative data in Fig. 3. As in the enterotoxin B antibody-inhibition system, both models I and II gave adequate fit of the data (Fig. 3 and Table 1), with model I giving a more precise fit. Anti-enterotoxin A, at a 1:50,000 dilution in 1 ml, inhibited the binding of 0.001 μg of 125I-labeled enterotoxin A by approximately 25%. Neither normal rabbit serum nor anti-B (both at a 1:1,000 dilution in 1 ml) inhibited the binding of 125I-labeled enterotoxin A to anti-A tubes.

The sensitivity of the solid-phase radioimmunoassay antibody-inhibition system was examined, and representative data are presented in Table 2. Two concentrations of antibody, 1:200 and 1:500, were used to coat the polystyrene tubes. Also, 0.001 and 0.0001 μg of 125I-labeled enterotoxin B were compared for relative sensitivity in the system, whereas only 0.001 μg of 125I-labeled enterotoxin A was examined. As can be seen, the sensitivity of the antibody-inhibition systems was increased by using 1:500 dilutions of antiserum as opposed to 1:200 dilutions. The use of antibody dilutions greater than 1:500 to coat tubes resulted in an unstable system with results that were difficult to reproduce (data not shown). The use of 0.0001 μg of 125I-labeled enterotoxin B as opposed to 0.001 μg also enhanced the sensitivity of the system. The system was unstable if less than 0.0001-μg amounts of labeled enterotoxins were added (data not shown). At 20% inhibition, using a 1:500 dilution of antiserum for coating the tubes and 0.001 μg of labeled enterotoxin, the test can detect as little as 0.02 μg of anti-B per ml and 0.07 μg of anti-A per ml. With 0.0001 μg of 125I-labeled enterotoxin B, the

![Fig. 2. Inhibition of uptake of 0.001 μg of 125I-labeled enterotoxin B on anti-B-coated tubes by various dilutions of antiserum to enterotoxin B. Y is the fraction of activity bound in presence of antiserum to enterotoxin B; X is the concentration of antibody expressed as decimal of antiserum dilution. Data are plotted for models I (O) and II (●). The scale for model II is on the left ordinate; the scale for model I is on the right ordinate.](image)

![Fig. 3. Inhibition of uptake of 0.001 μg of 125I-labeled enterotoxin A on anti-A-coated tubes by various dilutions of antiserum to enterotoxin A. Y is the fraction of activity bound in presence of antiserum to enterotoxin A; X is the concentration of antibody expressed as decimal of antiserum dilution. Data are plotted for models I (O) and II (●). The scale for model II is on the left ordinate; the scale for model I is on the right ordinate.](image)

### Table 2. Sensitivity of solid-phase radioimmunoassay antibody inhibition system

| System     | 125I-labeled enterotoxin (μg added) | Dilution of antibody for coating tubes | Antibody for inhibition* | Inhibition dilution |
|------------|------------------------------------|---------------------------------------|--------------------------|-------------------|
|            |                                    |                                       |                          | 20%               | 50%               |
| Enterotoxin B | 0.001                              | 1:200                                 | Anti-B (374)             | 1:6,800 (0.112)*  | 1:3,300 (0.231)   |
|             | 0.001                              | 1:200                                 | Anti-B (361)             | 1:96,000 (0.031)   | 1:27,000 (0.110)  |
|             | 0.001                              | 1:500                                 | Anti-B (361)             | 1:161,200 (0.018)  | 1:39,300 (0.075)  |
|             | 0.0001                             | 1:200                                 | Anti-B (361)             | 1:342,900 (0.0086) | 1:73,100 (0.040)  |
|             | 0.0001                             | 1:500                                 | Anti-B (361)             | 1:341,600 (0.0087) | 1:81,100 (0.037)  |
| Enterotoxin A | 0.001                              | 1:200                                 | Anti-A (424)             | 1:5,800 (0.058)    | 1:1,400 (0.240)   |
|             | 0.001                              | 1:200                                 | Anti-A (W)               | 1:56,800 (0.094)   | 1:13,800 (0.350)  |
|             | 0.001                              | 1:500                                 | Anti-A (W)               | 1:81,400 (0.066)   | 1:15,500 (0.359)  |

* Parentheses indicate the rabbits from which the anti-A and anti-B enterotoxin sera were obtained.

* Parentheses represent micrograms of anti-enterotoxin per milliliter present at the given dilutions.
test can detect 0.009 μg of anti-B per ml. The antigen inhibition system detects 0.001 to 0.002 μg of toxin per ml at the 33% inhibition level (8). Solid-phase radioimmunoassay then is slightly more sensitive for antigen detection than for antibody detection.

DISCUSSION

A solid-phase radioimmunoassay test for assaying staphylococcal enterotoxins A and B in purified form and in crude form has recently been developed (8). In this test 125I-labeled enterotoxins A and B compete with unlabeled enterotoxins for antibody-binding sites on the walls of polystyrene tubes which had been coated with specific anti-enterotoxin antibodies. The data presented here demonstrate that the solid-phase radioimmunoassay procedure, with slight modification, can also be used to determine relative potencies of antiserum to enterotoxins A and B. Competition occurs between free antibody and antibody on the wall of polystyrene tubes for antibody-binding sites on 125I-labeled enterotoxin.

The antigen and antibody inhibitions in solid-phase radioimmunoassay were compared by two empirical mathematical models, model I and model II (see above). The F-ratio tests performed on the variances estimated for Y at X were used to demonstrate the relative differences between models. Estimates of the correlation coefficients were all above 0.90, and this is probably satisfactory for most immunochemical studies. Both models I and II can appropriately represent either the antigen or antibody-inhibition system. Estimates from model I, however, as determined by correlation coefficients and analysis of variances (Table 1), were slightly more precise for the antibody-inhibition system, whereas the reverse was true for the antigen-inhibition system. These mathematical models are not necessarily applicable to other modifications of the radioimmunoassay systems described here.

The sensitivity of the solid-phase system for detection of antibody is enhanced by using low concentrations of antibody for tube sensitization and by using low concentrations of radiolabeled enterotoxin. Sensitivity of the antibody-inhibition system is also probably affected by the relative binding affinities of tube-bound and free antibody. Solid-phase radioimmunoassay is more sensitive for detection of enterotoxin than for detection of antibodies to enterotoxin. This is probably due to the fundamental differences in the mechanisms of inhibition by antigen and antibody in solid-phase radioimmunoassay.

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