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Antigenic Cross-Reactivity of Staphylococcal Enterotoxins

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The antigenic cross-reactivity of staphylococcal enterotoxins types A, B, and C was assessed using anti-A and anti-B antitoxins in the solid-phase radioimmunoassay test. Heterologous reactions were observed. At the 33% inhibition level, B was 18,000 and 5,400 times more effective as an inhibitor in its homologous system than were the heterologous enterotoxins A and C, respectively. Similarly, in the A system, A enterotoxin was 55,000 and 25,000 times more effective than were B and C toxins, respectively, in inhibiting A-anti-A reactions.

Antigenic distinctiveness is the basis for identification of staphylococcal enterotoxins. To date, there are four well defined types: A, B, C, and D (1). Recently, another serological type has been identified (2).

With the continuing discovery of new serological types, it is of interest to examine quantitatively the serological cross-reactivity between the enterotoxins. This possibly could lead to a serological procedure for enterotoxin assay based on common antigenic specificity.

Cross-reactions have been observed in immunodiffusion reactions between heterologous enterotoxins and antisera that have been incubated for extended periods (1). These observations do not, however, allow for the quantitation of these cross-reactions. Gruber and Wright (3) observed heterologous reactions of enterotoxins and anti-enterotoxins in an ammonium sulfate coprecipitation radioimmunoassay procedure. Although quantitation of cross-reactivity of enterotoxins was possible, no data on competitive binding were presented.

Recently, we have developed a solid-phase radioimmunoassay procedure for enterotoxin assay, involving competitive binding, which is both sensitive and quantitative (5). Solid-phase radioimmunoassay is applied here to evaluate quantitatively the cross-reactivity of purified staphylococcal enterotoxins A, B, and C.

MATERIALS AND METHODS

Purified enterotoxins. Enterotoxins types A and B are the same as those used in a previous study (5). Purified enterotoxin C was supplied by M. S. Bergdoll, the University of Wisconsin, Madison. The purified toxins contained less than 5% impurities (1). The same toxins were used both as inhibitors and for radiolabeling.

Solid-phase radioimmunoassay. Details of the solid-phase radioimmunoassay procedure, including source and treatment of antisera, radioiodination of enterotoxin, preparation of antibody-coated polystyrene tubes, and counting equipment have been described in detail (5).

Briefly, 1-ml amounts of purified unlabeled enterotoxins in 1.0% bovine serum albumin were added to antibody-sensitized polystyrene tubes (10 by 75 mm). The tubes were incubated at 37 C for 24 hr, after which 0.001 μg of ^{125}I-labeled enterotoxin in a 0.1-ml volume was added. The tubes were shaken 10 times; incubated at 37 C for 4 hr; washed once with 2 ml of phosphate-buffered saline, *p*H 7.2, and counted for radioactivity to determine the extent of inhibition of binding of labeled enterotoxin by unlabeled toxins.

Calculations. Thirty-three per cent inhibition titers were determined as described elsewhere (5).

RESULTS

Table 1 contains data on the inhibition of uptake of ^{125}I-enterotoxin B by anti-B-coated tubes. The inhibitors are unlabeled staphylococcal enterotoxins A (SEA), B (SEB), and C (SEC), and Brain Heart Infusion broth (BHI) (4). As can be seen, the homologous toxin SEB was very effective in inhibiting uptake of ^{125}I-labeled SEB, requiring only 0.0019 μg/ml for 33% inhibition. Interestingly, SEA and SEC, at relatively high concentrations, also inhibited uptake of ^{125}I-labeled SEB by anti-SEB. An amount (50 μg), for example, gave 50 and 36.6% inhibitions, respectively. At the 33% inhibition level, SEB was 18,000 times more effective than SEA and 5,400 times more effective than SEC. BHI was virtually noninhibitory. This suggests that the inhibitions by heterologous toxins are not due to nonspecific factors. (This point is further explored in Table 3.)

Table 2 contains data on the inhibition of uptake of ^{125}I-SEA by anti-SEA-coated tubes. The

TABLE 1. *Inhibition of binding of ^{125}I-SEB by anti-SEB-coated tubes*[a]

Inhibitor	Inhibitor concn. (μg/ml)	Per cent inhibition	
		Duplicates	Avg
SEB	0.1	94.7, 94.9	94.8
	0.01	79.8, 79.9	79.8
	0.005	65.9, 67.0	66.4
	0.0025	47.0, 44.8	45.9
	0.001	15.0, 12.8	13.9
SEA	50	48.9, 51.1	50.0
	10	5.7, 10.0	7.8
	1	3.4, 3.0	3.2
SEC	50	38.9, 34.3	36.6
	10	33.4, 32.4	32.9
	1	3.0, −1.9	0.5
BHI	Undiluted	13.2, 3.2	8.2
	1:10	1.1, −1.0	0.0

[a] Abbreviations: staphylococcal enterotoxins A (SEA), B (SEB), and C (SEC); and Brain Heart Infusion broth (BHI).

TABLE 2. *Inhibition of binding of ^{125}I-SEA by anti-SEA-coated tubes*[a]

Inhibitor	Inhibitor concn. (μg/ml)	Per cent inhibition	
		Duplicates	Avg
SEA	0.1	87.8, 92.0	89.9
	0.01	79.0, 79.0	79.0
	0.005	65.5, 66.1	65.8
	0.0025	56.0, 54.5	55.2
	0.001	37.0, 36.8	36.9
SEB	50	37.0, 35.1	36.0
	10	16.2, 7.7	11.9
	1	1.0, −3.9	−1.4
SEC	50	51.5, 56.2	53.8
	10	20.9, 25.2	23.0
	1	4.8, 13.2	9.0
BHI	Undiluted	2.2, 0.9	1.5
	1:10	3.0, −6.0	−1.5

[a] Abbreviations: staphylococcal enterotoxins A (SEA), B (SEB), and C (SEC); and Brain Heart Infusion broth (BHI).

inhibitors are the same as those in Table 1. Again the homologous unlabeled toxin, SEA here, was the most effective inhibitor, with 0.0008 μg/ml resulting in 33% inhibition of uptake of ^{125}I-SEA. Again the heterologous toxins showed definite inhibition—36.05% at 50 μg/ml for SEB and 53.85% for SEC at the same concentration. At the 33% level of inhibition, SEA was 55,000 times more effective than SEB and 25,000 times more effective than SEC. Again BHI was without effect. As noted previously (5), no uptake of heterologous ^{125}I-labeled enterotoxins was observed in noncompetitive binding of anti-A- and anti-B-coated tubes.

Despite the lack of significant inhibition by BHI of the SEB and SEA radioimmunoassay systems, the possibility still exists that the inhibitions observed with high concentrations of heterologous enterotoxins may have been due to non-antigen-related factors associated with the structure of the enterotoxins. For this reason, enterotoxins SEB, SEA, and SEC were examined as inhibitors in a nonenterotoxin solid-phase radioimmunoassay system. Table 3 contains data on the inhibition of uptake of ^{125}I-labeled bovine pancreatic ribonuclease by anti-ribonuclease-coated tubes. The inhibitors consisted of unlabeled ribonuclease, SEB, SEA, and SEC. Ribonuclease was an effective inhibitor at extremely low concentrations. SEB, SEA, and SEC, each at a concentration of 50 μg/ml, were noninhibitory. It is evident, then, that the heterologous enterotoxin inhibitions presented in Tables 1 and 2 are specific.

TABLE 3. *Inhibition of binding of ^{125}I-ribonuclease by anti-ribonuclease-coated tubes*[a]

Inhibitor	Inhibitor concn. (μg/ml)	Per cent inhibition	
		Duplicates	Avg
Ribonuclease	0.1	91.5, 92.1	91.8
	0.01	66.0, 61.7	63.8
	0.005	48.4, 48.5	48.4
	0.0025	29.8, 30.0	29.9
	0.001	10.2, 11.5	10.8
SEB	50	5.3, 1.1	3.2
SEA	50	−1.9, 1.9	0.0
SEC	50	0.2, −7.6	−3.7

[a] Abbreviations: staphylococcal enterotoxins A (SEA), B (SEB), and C (SEC).

DISCUSSION

In a previous study involving enterotoxin solid-phase radioimmunoassay (5), crude culture extracts of heterologous enterotoxins inhibited (up to 25.2% in one case) the SEB system. This suggested that enterotoxins were antigenically related. BHI, the culture medium for the production of these toxins, also inhibited the SEB system similarly. It was not possible, therefore, to unequivocally attribute the inhibitions with crude heterologous toxins to serological cross-reactivity with anti-SEB.

The present study clarifies the nature of the antigenic relationships of the enterotoxins. Purified enterotoxins at sufficient concentration do cross-react with antibodies to heterologous en-

terotoxins. The specificity of these reactions was established by using purified enterotoxins as inhibitors, by using a nonenterotoxin inhibitor (BHI), and by using a nonenterotoxin solid-phase radioimmunoassay system (ribonuclease-anti-ribonuclease). As an aside, it was noted that the nonspecific effects of BHI were diminished with a 24-hr incubation with antibody-coated tubes as compared with a 4-hr incubation (5).

Gruber and Wright (3), using an ammonium sulfate coprecipitation antibody technique, demonstrated that some enterotoxins react extensively with heterologous antisera. Their assay system differed from that presented here in that it did not involve competitive binding of enterotoxin and antienterotoxin. The solid-phase radioimmunoassay technique presented here is a competitive binding technique; unlabeled enterotoxin competes with labeled enterotoxin for antibody binding sites.

There are several possible mechanisms for the heterologous reactions. (i) The staphylococcal enterotoxins could share minor antigenic determinants. This appears unlikely since inhibition in some cases approached 50%. If this truly reflected the percentage of determinants that the toxins had in common, the relatively high concentrations of heterologous toxins should not have been needed to obtain this degree of inhibition.

(ii) The cross-reactions could reflect minor contaminations of purified enterotoxins by heterologous enterotoxins. One pattern of inhibition reported here, however, seems to rule against this. SEC at 10 and 50 μg/ml had about the same inhibiting ability in the SEB system (Table 1). If this inhibition was due to minor contamination of SEC by SEB, a fivefold increase in the concentration of SEC should have resulted in a more dramatic increase in inhibition. Repeated inhibitions of the SEB system by SEC yielded similar patterns.

(iii) The inhibitions by heterologous toxins could be attributed to different but structurally related determinants. This would explain the necessity for relatively high concentrations of heterologous toxins for effective inhibition.

From the practical and applied point of view, the heterologous reactions present no problems in diagnostic studies because of the extremely high homologous-to-heterologous inhibition ratios. The data presented here may have some relevancy as to the origin of the various enterotoxins. For example, is there a central enterotoxin serotype from which all others are derived by some genetic mechanism? Antigenic relationships provide phenotypic information on this subject. Further studies should be done with different preparations of enterotoxins and antitoxins to provide answers to questions such as this.

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