Suppression of the Intestinal Immune Response to Cholera Toxin by Specific Serum Antibody

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The possibility that preexisting specific serum antibody could suppress a defined mucosal immune response to a topically applied antigen was studied in rats. Hyperimmune serum antibody induced by parenteral immunization of rats with cholera toxin markedly suppressed the mucosal immune response to enterically applied cholera toxin. Such antibody was far more suppressive than antibody induced by primary parenteral immunization, apparently due to its greater avidity. Transfusion of small amounts (25 to 100 μl) of hyperimmune serum suppressed the primary mucosal antitoxin response, the development of specific memory in the mucosal immune system, and, somewhat less effectively, the secondary mucosal antitoxin response. Suppression was due largely to a direct effect of serum antibody upon the interaction of absorbed enteric antigen with lymphoid tissue in Peyer's patches and, possibly, mesenteric lymph nodes; interference with antigen absorption played little or no role in the observed suppression. These results do not explain the previously reported suppressive effect of primary parenteral immunization on the mucosal immune response to cholera toxin. However, they support the notions that repeated parenteral immunization can evoke avid serum antibody without necessarily stimulating mucosa-associated lymphoid tissue and that such antibody can markedly suppress primary and secondary phases of the local immune response to mucosally applied antigen. Thus, a mechanism is demonstrated by which repeated parenteral immunization may adversely affect efforts to initiate or sustain protective mucosal immune responses.

The mechanisms which control immune responses at mucosal surfaces have not been well defined. A better understanding of such mechanisms should assist efforts to develop more effective ways to stimulate protective mucosal immune responses.

Parenteral immunization with a soluble protein can modify the local immune response to the same antigen when it is applied subsequently at a mucosal surface; both priming and antigen-specific suppressive effects have been described previously (6, 13, 15). Mucosal priming appears to require an injection route which favors the interaction of injected antigen with immunoglobulin A (IgA) precursors in mucosa-associated lymphoid tissue (15). In contrast, mucosal suppression follows parenteral immunization by a variety of routes, including those routes which evoke little or no mucosal priming (15). Although the exact mechanism of such suppression has not been explained, these observations suggest that it is caused by products of the systemic immune response, either cellular or humoral, which reach mucosa-associated lymphoid tissue and modify its responsiveness to mucosally applied antigen. One possibility is that such suppression is due, at least in part, to specific serum antibody raised by the parenteral antigen and is analogous to the well-described suppressive effect of serum antibody on systemic immune responses.

The aims of the present study were to determine whether specific serum antibody suppressed a defined mucosal immune response, the mechanism of such suppression, and the extent to which specific antibody causes the suppression observed after parenteral immunization. The mucosal immune response to cholera toxin was studied; enteric doses of this protein cause distinct primary and secondary type responses, predominantly of the IgA class, in rat intestinal lamina propria (12). The results show that hyperimmune serum raised by parenterally injected cholera toxoid markedly suppresses both primary and secondary phases of the specific mucosal immune response, whereas serum obtained after a single injection of toxoid is much less effective. They support the notion that avid serum antibody produced by systemic lymphoid tissue can directly suppress the response of mucosa-associated lymphoid tissue to subsequent topical immunization.
MATERIALS AND METHODS

Rats. The rats used were males and females of the inbred Wistar-Lewis strain from Charles River Breeding Laboratories, Wilmington, Del. Housing was in a conventional rodent colony.

Cholera toxins and toxoids. Several preparations were used. These have been described in detail elsewhere (12). Purified cholera toxoid was from Schwarz/Mann, Orangeburg, N.Y. The crude cholera toxoid used was NIH lot 001, which was made by Wyeth Laboratories and was provided by Carl Miller, National Institute of Allergy and Infectious Diseases. Purified and crude cholera toxoids were made by R. O. Thomas, Wellcome Research Laboratories, Beckenham, England. Purified materials were diluted in 0.05 M borate-buffered saline containing 0.02% gelatin, pH 7.4; crude materials were diluted in 0.9% NaCl.

Active immunization. The technique for intraintestinal immunization through a small laparotomy is described elsewhere (14). Intraduodenal or intracolononic immunization of 125- to 150-g female rats was with two doses of purified toxoid (12.5 μg each, unless otherwise stated) or crude toxoid (40 mg plus crude toxoid (1.5 mg), followed by crude toxoid (40 mg); the injection volume was 0.5 ml, and the interval between doses was 14 days. These regimens were shown previously to cause vigorous antigenic responses in intestinal mucosa (12). The intraperitoneal (i.p.) dose of purified toxoid was 40 μg in 0.2 ml; in some instances it was emulsified with an equal volume of complete Freund adjuvant (CFA) (Difco Laboratories, Detroit, Mich.).

To obtain primary immune serum, male rats weighing 200 to 250 g were given a single dose of 40 μg of purified toxoid with or without CFA i.p. At intervals, they were exsanguinated, and their sera were separated, pooled, and stored at −40°C. Hyperimmune serum was a single pool from rats which were primed i.p. with 25 μg of toxoid plus CFA, boosted intravenously 6 weeks later with 50 μg toxoid, and bled 8 to 12 days after boosting.

Passive immunization. Passive immunization was by i.p. injection of whole serum or serum fractions (see below).

Assay, purification, and characterization of antitoxin in immune sera. The antitoxin contents of serum and serum fractions were measured by passive agglutination of formalinized sheep erythrocytes sensitized with purified cholera toxoid (7). All specimens were inactivated at 56°C for 30 min before assay. A control serum manufactured by the Swiss Serum and Vaccine Institute, Berne, Switzerland [lot EC3 (A-2/67-2)], gave a titer of 1:1,024 in this assay.

Purified antitoxin was separated from immune serum by affinity chromatography on CNBr-activated Sepharose-4B (Pharmacia Fine Chemicals, Piscataway, N.J.) coupled with purified toxoid (14). Each sample was passed twice through the affinity column. Unbound serum was washed from the column (1.3 by 4.5 cm) with 0.1 M phosphate-buffered saline, pH 7.4. Specifically bound protein was eluted with 0.1 M pro- pionic acid, and 0.5-ml samples were collected onto 0.5 g of tris(hydroxymethyl)aminomethane base (Sigma Chemical Co., St. Louis, Mo.); those samples containing protein were pooled, dialyzed sequentially at 4°C against phosphate-buffered saline and 0.15 M NaCl, and then concentrated over an XM-50 membrane (Amicon Corp., Lexington, Mass.). Protein contents were determined by absorption at 280 nm (1).

The effect of 2-mercaptoethanol on purified antitoxin was studied by determining the antitoxin titer after incubating equal volumes of antitoxin and either 0.1 M 2-mercaptoethanol or phosphate-buffered saline for 4 h at 25°C.

To precipitate immunoglobulins, purified antitoxin (0.74 mg/ml) was combined with an equal volume of rabbit anti-rat IgG specific for heavy and light chains and containing 3.2 mg of specific antibody per ml (Miles-Yeda Ltd., Rehovot, Israel). The mixture was incubated at 37°C for 60 min, and the resulting precipitate was removed by centrifugation at 14,000 × g and 4°C for 30 min.

ACC In intestinal lamina propria and thoracic duct lymph. The methods used to identify and count antitoxin-containing cells (ACC) have been described previously (14). Briefly, thoracic duct lymphocytes (TDL) were obtained by chronic thoracic duct drainage, smeared on slides, and fixed with ethanol. Pieces of intestine were frozen over liquid nitrogen, and sections (thickness, 5 μm) were cut on a cryostat and fixed in methanol. In both types of preparations,ACC were identified by a fluorescent antibody technique which involved sequential staining with purified cholera toxoid, followed by staining with an immunopurified, fluorescein-conjugated rabbit antitoxin (14). The frequency of ACC is expressed as the number of ACC collected per hour among TDL or the number per cubic millimeter in the basal region of intestinal lamina propria. To determine geometric means, tissue samples having no detectable ACC were assigned a value of 110 ACC per mm², which was the lower limit of sensitivity of this scoring method.

Challenge of intestinal segments with cholera toxin. This technique has been described in detail elsewhere (11). Briefly, two ligated segments of ileum 10 to 14 cm long were prepared in fasted, anesthetized rats. The distal segment was injected with 0.5 ml of isotonic crude cholera toxoid, and the proximal segment was injected with 0.9% NaCl. The abdomen was closed. After 18 h the rats were killed, and the fluid content of each segment was measured. Results are expressed as milliliters of secreted fluid per centimeter of intestinal length. Results were considered valid only if the saline-injected segment contained less than 0.05 ml/cm.

Statistics. Unless stated otherwise, statistical analysis was by the Student t test as applied to the geometric mean frequencies of ACC in intestinal lamina propria or among TDL (15) or to arithmetic means of milliliters of fluid secreted per centimeter of intestine. Where indicated, the Mann-Whitney test was used for analysis of non-parametrically distributed results. Probability values greater than 0.05 were defined as not significant.

RESULTS

Suppression of mucosal antitoxin response by immune serum. (i) Role of immune serum in suppression after a single
i.p. dose of toxoid. Purified toxoid given i.p. both primes for and suppresses the mucosal antitoxin response to intraduodenal toxin (14, 15). Evidence for suppression is an impaired mucosal antitoxin response after two intraduodenal doses of toxin in rats first given toxoid i.p. (15). The possibility that this suppression was due to serum factors evoked by i.p. immunization was studied by comparing the suppressive effect of toxoid given i.p. with the effect of transfused serum from immunized donors i.p. (Table 1). Fluid toxoid given i.p. 14 or 28 days before enteric priming and toxoid plus CFA given 84 days before enteric priming each caused 90 to 95% suppression of the mucosal antitoxin response; toxoid plus CFA given 14 days before enteric priming was not significantly suppressive. In contrast, 6 ml of immune serum, obtained at the same intervals after toxoid was given i.p., and given i.p. to nonimmune rats 1 day before enteric priming had little or no suppressive effect. Modest suppression was observed only with serum obtained 84 days after i.p. administration of toxoid plus CFA; this was also the only serum with a detectable antitoxin titer.

(ii) Suppressive effect of hyperimmune serum: the role of antitoxic antibody. In contrast with the above-described data, hyperimmune serum obtained from animals primed i.p. with toxoid plus CFA and boosted intravenously with fluid toxoid markedly suppressed the mucosal antitoxin response when given 1 day before enteric priming (Fig. 1A). Suppression followed transfer of as little as 25 μl of serum, was equally effective when enteric immunization was with purified toxin or crude toxoid plus crude toxin, and was overcome when the enteric priming and boosting doses of purified toxoid were increased fourfold. This serum was also suppressive when given 1 day before enteric boosting (Fig. 1B); 100 μl prevented about 80% of the booster increment in the mucosal antitoxin response.

Evidence that the suppressive effect of hyperimmune serum was due to antitoxic antibody is shown in Tables 2 and 3. The suppressive effect and antitoxin content were completely removed by passage of whole serum twice through a toxoid-Sepharose affinity column, were largely recovered by elution of the column with acid, and were removed from the acid eluate by precipitation of immunoglobulin with anti-rat IgG. Table 3 also summarizes comparative data on the separation of antitoxin from serum obtained 84 days after a single i.p. dose of toxoid plus CFA (suppressive activity shown in Table 1). Antitoxin recovery from this serum was about 25% of the recovery from serum animals immunized twice. However, the calculated 50% suppressive doses of purified antitoxin in the two sera differed by more than 60-fold (antitoxin from the hyperimmune serum was more potent). Purified antitoxin from both sera resisted degradation by 2-mercaptoethanol.

Observations on the mechanism of suppression by hyperimmune antitoxic antibody. (i) Failure of transfused antitoxin to

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**Table 1. Role of immune serum in suppression of enteric antitoxin response by a single i.p. dose of purified toxoid**

<table>
<thead>
<tr>
<th>Parenteral immunization</th>
<th>No. of days before enteric priming</th>
<th>No. of ACC per mm² in jejunal lamina propria*</th>
<th>P*</th>
<th>No. of days before bleeding</th>
<th>Antitoxin titer of transfused serum</th>
<th>No. of ACC per mm² in jejunal lamina propria</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>9,200 ± 1.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxoid (40 μg), i.p.</td>
<td>14</td>
<td>550 ± 2.01</td>
<td>&lt;0.001</td>
<td>14</td>
<td>&lt;1:4</td>
<td>14,900 ± 1.15</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>460 ± 1.52</td>
<td>&lt;0.001</td>
<td>28</td>
<td>&lt;1:4</td>
<td>7,700 ± 1.62</td>
<td>NS</td>
</tr>
<tr>
<td>Toxoid (40 μg) + CFA, i.p.</td>
<td>14</td>
<td>6,000 ± 1.27</td>
<td>NS</td>
<td>14</td>
<td>&lt;1:4</td>
<td>12,000 ± 1.21</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>84</td>
<td>900 ± 1.41</td>
<td>&lt;0.001</td>
<td>84</td>
<td>1:256</td>
<td>6,000 ± 1.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Rats were given toxoid i.p. At the indicated intervals, enteric immunization with purified toxin was begun; two 12.5-μg intraduodenal doses were given 14 days apart.

b Nonimmune rats received 6 ml of immune (or nonimmune) serum i.p. at zero time; enteric immunization was with 12.5 μg of purified toxin given intraduodenally on days 1 and 15.

c Number of ACC per cubic millimeter (geometric mean ± standard error) in jejunal lamina propria 5 days after the second intraduodenal dose of toxin. The responses in rats given nonimmune serum and those given no parenteral toxoid were not significantly different; five to eight rats were used to determine each mean.

d Compared with rats given no toxoid i.p.

e Compared with rats given nonimmune serum.

f NS, Not significant.
Alter initial events in absorption of enteric toxin. It was possible that transfused antitoxin caused suppression by reaching the intestinal lumen, combining with the immunizing dose of toxin, and thus diminishing its absorption and access to mucosal lymphoid tissue. This was studied by determining whether a suppressive dose of hyperimmune serum given i.p. interfered with the normal secretory effect of cholera toxin upon ileal mucosa; it is known that antitoxin prevents this effect only if it combines with toxin before the toxin binds to the mucosal surface (2), and mucosal binding is probably the initial event in toxin absorption. Hyperimmune serum (100 µl) was given i.p. to nonimmune rats 1 day before challenge of a ligated ileal segment with a 50% effective dose of crude cholera toxin (6 mg). The mean amount of fluid secreted into the segment after 18 h was 0.21 ± 0.05 ml/cm for rats given immune serum and 0.16 ± 0.06 ml/cm for control rats given no serum (n = 11 for each mean). These values are not significantly different from each other or from the 50% effective dose response in a previously reported dose-response curve for cholera toxin in Wistar-Lewis rats (11).

(ii) Effects of transfused antitoxin on specific events during the primary and secondary mucosal immune responses to enteric toxin. The effects of transfused antitoxin on two parts of the primary response were studied. The first was the primary antitoxin response. Rats were given 100 µl of hyperimmune serum i.p. 1 day before intraduodenal priming with 12.5 µg of purified toxin; jejunal biopsy was 19 days later. The primary mucosal antitoxin response in jejunal lamina propria was 180 ± 1.50 ACC per mm² (mean ± standard error; n = 6). In animals given no serum, it was 700 ± 1.50 ACC per mm² (n = 16). The difference is statistically significant (P < 0.001 by Mann-Whitney test). The second was the dissemination of sensitized lymphocytes capable of mediating a secondary response at distant mucosal surfaces (N. F. Pierce and F. T. Koster, unpublished data). Table 4 shows that intracolonic toxin primed for a secondary response in jejunal lamina propria when the booster was given intraduodenally. This priming effect was impaired markedly by immune serum given i.p. 1 day before priming, but was not affected by serum given 2 days after priming, indicating that the suppressive effect was on events during the first 2 days after intracolonic priming.

The effect of hyperimmune serum given 1 day before boosting on the generation of ACC among hyperimmune serum.

![Graph](image-url)

**FIG. 1.** Suppression of the mucosal antitoxin response by hyperimmune serum. Rats were immunized intraduodenally with two doses of purified toxin (12.5 µg [●] or 50 µg [▲]) or with crude toxoid plus crude toxin (○), as described in the text. Hyperimmune serum was given i.p. 24 h before enteric priming or boosting. Each point represents the mean of data from 8 to 10 rats. SE, Standard error.

**TABLE 2.** Suppression of enteric immune response to cholera toxin by purified antitoxic antibody from hyperimmune serum.

<table>
<thead>
<tr>
<th>Serum or serum fraction transfused before intraduodenal priming*</th>
<th>No. of ACC per mm² in jejunal lamina propria after intraduodenal boosting*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>9,200 ± 1.15</td>
<td></td>
</tr>
<tr>
<td>Whole serum</td>
<td>180 ± 1.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum fractions from toxoid-Sepharose affinity column</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbound fraction</td>
<td>11,300 ± 1.45</td>
<td>NSa</td>
</tr>
<tr>
<td>Acid eluate</td>
<td>680 ± 1.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acid eluate + anti-rat IgG</td>
<td>13,900 ± 1.23</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Hyperimmune serum (0.4 ml or its equivalent) was given i.p. 1 day before intraduodenal priming with purified toxin (12.5 µg). Preparation of this serum is described in the text.

a Number of ACC per cubic millimeter (geometric mean ± standard error) in jejunal lamina propria 5 days after intraduodenal challenge with purified toxoid (12.5 µg). Challenge was 14 days after enteric priming; five to seven rats were used to determine each mean.

b Compared with rats given no immune serum.

c NS, Not significant.
TABLE 3. Comparison of antitoxin contents and suppressive activities of primary immune serum and hyperimmune serum

<table>
<thead>
<tr>
<th>Specimen tested</th>
<th>Antitoxin titer&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antitoxin content (µg/ml)</th>
<th>50% Suppressive dose&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary serum&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Primary serum&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Hyperimmune serum&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Whole serum</td>
<td>1:256</td>
<td>270</td>
<td>&gt;6.0</td>
</tr>
<tr>
<td>Serum fractions from toxoid-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Sepharose affinity column</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbound fraction</td>
<td>&lt;1:4</td>
<td>&lt;1:4</td>
<td></td>
</tr>
<tr>
<td>Acid eluate</td>
<td>1:64</td>
<td>1:1,024</td>
<td></td>
</tr>
<tr>
<td>Acid eluate + 2-mercaptoethanol</td>
<td>1:64</td>
<td>1:512</td>
<td></td>
</tr>
<tr>
<td>Acid eluate + anti-IgG</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1:4</td>
<td></td>
</tr>
<tr>
<td>Purified antitoxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;1,620&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as titer in equivalent volumes of whole serum.
<sup>b</sup> Dose of serum or calculated dose of purified antitoxin given before intraduodenal priming which caused 50% suppression of log<sub>10</sub> of the geometric mean antitoxin response in jejunal lamina propria (derived from data in Fig. 1A and Table 1). The point of 50% suppression was calculated as follows: antilog[(log<sub>10</sub> A − log<sub>10</sub> B)/2], where A is the response of enterically immunized animals not given immune serum and B is the lower limit of sensitivity of the assay (110 ACC per mm<sup>3</sup>).
<sup>c</sup> Rats were primed i.p. with 40 µg of toxoid plus CFA and bled 84 days later.
<sup>d</sup> Immunization is described in the text.
<sup>e</sup> ND, Not done.
<sup>f</sup> Based on the amount of protein recovered in the acid eluate of a toxoid-Sepharose affinity column per milliliter of serum applied.

TABLE 4. Effect of transfused hyperimmune serum on dissemination of primary response to intracolonic toxin

<table>
<thead>
<tr>
<th>Immune serum&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Enteric primer&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Enteric booster&lt;sup&gt;c&lt;/sup&gt;</th>
<th>No. of ACC per mm&lt;sup&gt;3&lt;/sup&gt; jejunal lamina propria&lt;sup&gt;c&lt;/sup&gt;</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Colon</td>
<td>None</td>
<td>≤110</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>Duodenum</td>
<td>≤110</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Colon</td>
<td>Duodenum</td>
<td>4,100 ± 1.57</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>0.1 ml i.p. 24 h before priming</td>
<td>Colon</td>
<td>Duodenum</td>
<td>470 ± 1.88</td>
<td></td>
</tr>
<tr>
<td>0.1 ml i.p. 48 h after priming</td>
<td>Colon</td>
<td>Duodenum</td>
<td>5,800 ± 1.17</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

<sup>a</sup> Preparation is described in the text.
<sup>b</sup> The enteric primer was 12.5 µg of purified toxin given intracolonically; the booster dose was 12.5 µg given intraduodenally 14 days later.
<sup>c</sup> Number of ACC per cubic millimeter (geometric mean ± standard error) 5 days after boosting or 19 days after priming. Five to eight rats were used to determine each mean.
<sup>d</sup> Compared with rats given 0.1 ml of hyperimmune serum i.p. before priming.

DISCUSSION

Numerous studies have described suppression of systemic immune responses by specific serum antibody; these have been summarized in recent reviews (4, 17). The present study shows that serum antibody induced by parenteral immunization also suppresses a specific mucosal immune response. Several characteristics of this suppression are virtually identical to those described for suppression of systemic immune responses. The similarities include the following. (i) Antibody raised by primary parenteral immunization had little or no suppressive effect, whereas hyperimmune serum was highly suppressive (3). (ii) The enhanced suppressive effect of hyperimmune serum was probably due, at least in part, to increased avidity of specific antibody (18); this was suggested by the much greater suppressive effect per microgram of 2-mercaptoethanol-resistant antibody from hyperimmune serum than primary serum. (iii) Hyperimmune serum suppressed the primary antibody response, the development of specific memory, and, somewhat less effectively, the secondary antibody response (17). And (iv) suppression by hyperimmune serum appeared to involve a di-
rect interaction of antibody with specific antigen (3) since it was overcome by increasing the antigen dose.

Key events in the enteric mucosal immune response include the absorption of enteric antigen (10), its interaction with lymphoid cells in Peyer's patches (8) and, possibly, mesenteric lymph nodes (9), and the subsequent dissemination via thoracic duct lymph of sensitized, IgA-committed lymphocytes or immunoblasts to other mucosal sites (8, 9, 14). This study shows that hyperimmune serum antibody exerted its suppressive effect largely on events occurring in Peyer's patches and possibly mesenteric lymph nodes, since it prevented the normal appearance and/or dissemination among TDL of specifically primed lymphocytes during the primary response and of antitoxin-containing immunoblasts during the booster response, but did not interfere with initial events in the absorption of toxin from the gut lumen. Evidence for the latter conclusion is the failure of a suppressive dose of hyperimmune serum to diminish the secretory effect of cholera toxin on the gut mucosa; although other reports have shown that serum-derived IgG antibody can traverse gut mucosa and interfere with specific antigen absorption, this occurs only when the serum antibody titer is high (16, 19). In addition, it is possible that hyperimmune antibody also suppresses the antigen-driven division of specifically sensitized IgA immunoblasts which occurs in enteric lamina propria during the local secondary response (8); this was not examined in the present study.

Despite the similarities cited above, the circumstances under which parenterally induced serum antibody may contribute to the suppression of systemic and mucosal immune responses appear to differ. Highly avid serum antibody produced during a secondary-type systemic immune response appears to have a feedback suppressive effect on that response (4, 5). Avid antibody also suppresses the primary systemic immune response in passive immunization experiments (3), but such suppression is considered unphysiological since avid antibody is neither present at nor produced by primary parenteral immunization (3). In contrast, suppression of a mucosal immune response by parenterally induced serum antibody is not a feedback effect, at least not in the strict sense that the product of a response acts to inhibit the response from which it has arisen, since, depending upon the route of injection, parenteral immunization causes little or no mucosal immune response (15). Instead, such suppression appears to be due to the effect of preexisting, systemically derived serum antibody upon the interaction between mucosa-associated lymphoid tissue and absorbed enteric antigen, a situation reproduced by the present experiments with passively transferred hyperimmune serum. Thus, one circumstance is demonstrated in which preexisting serum antibody may physiologically suppress either the primary or secondary phases of a specific immune response.

The observed effect of parenterally induced serum antibody on the mucosal immune response may have relevance for efforts to immunize against a variety of mucosal infections. It shows one mechanism by which repeated parenteral immunization, which is usually aimed at inducing maximum serum antibody titers, may interfere with the development of mucosal immune responses after natural or planned mucosal exposure to specific or cross-reacting antigens. Such mucosal immune responses may contribute to host protection or prevent asymptomatic shedding of pathogenic agents to the environment or both. To overcome such suppression, a substantially increased local antigenic challenge would be required; the magnitude of this increase may be appreciated by calculating that the hyperimmune serum from twice-immunized donors in this study contained 1,200-fold more suppressive antibody per milliliter than was achieved by transfer of one 50% suppressive dose of the same serum to a nonimmune rat, assuming that transfused antibody was distributed in an extracellular volume equaling 20% of body weight.

Finally, this study shows that serum antibody is not substantially responsible for the suppression of the mucosal immune response which appears several weeks after primary parenteral immunization (15). Other studies in this laboratory, which will be reported elsewhere, have shown that such suppression is predominantly cell mediated, apparently by antigen-specific suppressor T cells.

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LITERATURE CITED