Effect of Dietary Restriction on Total and Bacterium-Specific Mucosal Secretory Immunoglobulin A in Bile-Diverted Intestinal Self-Filling Blind Loops

STEVEN N. LICHTMAN,† PHILIP M. SHERMAN, AND GORDON G. FORSTNTER*

Division of Gastroenterology, Department of Paediatrics and Research Institute, The Hospital for Sick Children, and Department of Paediatrics, The University of Toronto, Toronto, Ontario M5G 1X8, Canada

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The effect of starvation on the mucosal secretory immunoglobulin A (sIgA) response to bacterial antigens was studied in bile-free rat self-filling blind loops constructed at the end of a Roux-en-Y branch of jejunum. Rats were fed a 50% restricted diet for 1 to 4 weeks after surgery. sIgA was measured in the mucosa and lumen by an enzyme-linked immunosorbent assay. Dietary restriction caused a final rise of luminal sIgA which was less than 50% of that of normally fed controls. Luminal bacteria counts were not different in the two groups. The percentage of total sIgA precipitated with intestinal bacteria was not significantly affected by dietary restriction, and there was no change in the specific binding of sIgA to several bacterial species. Nonprecipitated sIgA exhibited a low but significant specific binding to bacteria in both diet-restricted and fed rats. Diet restriction therefore reduced the total sIgA response to luminal bacteria, but the specific bacterial binding capacity per microgram of sIgA was not altered. In these short-term experiments diet-restricted animals appeared to be capable of secreting sIgA in excess of requirement, since the nonprecipitable luminal fraction contained free sIgA with binding capacity for bacteria. The ability of sIgA to react with specific antigens may therefore be of more significance as an indicator of bacterial susceptibility than the measurement of total sIgA.

Interaction between diet and host resistance has been known to exist for many years (7). Increased rates of infection and mortality have been shown in malnourished patients (7, 19). Malnutrition alters a variety of immune responses to a large group of pathogens (5, 6, 23, 27). Bacterial overgrowth of the small intestine is a frequent occurrence in malnourished infants and may contribute to chronic diarrhea. The flora is characteristically fecal, consisting of commensal organisms normally found in the colon (16). Recently we showed that bacteria within bile-diverted intestinal blind loops stimulate the secretion of mucosal secretory immunoglobulin A (sIgA) (14). The rapidity and the magnitude of the response suggested that it was anamnestic (13). Since it is possible that malnutrition may predispose the small intestine to bacterial habitation by altering local immune responses, we have studied the impact of diet restriction on the sIgA response to bacterial overgrowth in experimental blind loops.

**MATERIALS AND METHODS**

**Animals.** Specific-pathogen-free, weaned male Wistar rats (Woodlyn Laboratories, Guelph, Ontario) weighing approximately 200 g were used in all experiments. All rats were housed individually in wire-bottomed mesh cages. They were fed a ground nonpurified diet (Purina Laboratory Chow; Ralston Purina Co., St. Louis, Mo.) in wide-bottomed, heavy ceramic dishes so as to reduce spillage; the daily intake was determined by weighing. Rats were paired so that the underfed animals received only 50% of the previous day's intake of a rat fed ad libitum.

**Blind loops.** Self-filling blind loops (SFBL) were constructed at the end of a Roux-en-Y limb (RY-SFBL) as described previously (14). The Roux-en-Y limb was constructed 7 cm distal to the ligament of Treitz as a functional isoperistaltic side branch 5 cm in length. At the end of this branch, an antiperistaltic blind loop was made, as in the classical SFBL preparation. The RY-SFBL was therefore separated from the flow of chyme by a 5-cm length of self-emptying jejunum. Rats were killed by cervical dislocation under light ether anesthesia. The blind loop or a corresponding jejunal segment from control rats that had had no operation was removed, and the lumen was perfused with 10 ml of 150 mM NaCl and then opened longitudinally. The mucosa was removed by scraping with a glass slide, cooled on ice, weighed, and homogenized in ice-cold 5 mM disodium EDTA, pH 7.4 (100 ml/g of wet weight), in a Waring blender for 20 s. The homogenates were stored at −70°C until used. Luminal contents were stored at 4°C after the addition of sodium azide, aprotinin (Sigma Chemical Co., St. Louis, Mo.), and phenylmethylsulfonyl fluoride to final concentrations of 0.02%, 500 IU/ml and 0.5 mM, respectively.

**Specific bacterial subcultures.** Enteric bacteria were subcultured onto blood agar and identified by standard methods (9, 12). Anaerobes were cultured in Blackburn minimal medium (18) at 37°C. Aerobes were cultured in Penassay broth (Difco Laboratories, Detroit, Mich.) at 37°C. After 48 h the contents of the culture flasks were centrifuged at 30,000 × g for 30 min and brought to a final concentration of 1.7 × 10^11 to 5 × 10^13 CFU/ml. Bacterial sonicates were prepared from bacterial subcultures after dialysis in phosphate-buffered saline (PBS) for 24 h by sonication for 20 s. The sonicates were stored at 4°C until use.

sIgA. sIgA was measured by an enzyme-linked immunosorbent assay as previously described (14). Briefly, goat anti-rat slga (Miles Laboratories, Inc., Elkhart, Ind.) was affinity purified with an sIgA-linked affinity column (ReactiGel; Pierce Chemical Co., Rockford, Ill.). Microtiter wells
TABLE 1. Weight gain, mucosal protein, and anaerobic bacteria in rats fed normal and restricted diets

<table>
<thead>
<tr>
<th>Diet and rats</th>
<th>Wk</th>
<th>No. of rats</th>
<th>Wt gain (g/day ± SD)</th>
<th>Total mucosal protein (mg ± SD)</th>
<th>Luminal anaerobic bacteria (log_{10} CFU/ml ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>8</td>
<td>5.3 ± 0.5</td>
<td>37.0 ± 11</td>
<td>4.5 ± 1.2</td>
</tr>
<tr>
<td>RY-SFBL</td>
<td>2</td>
<td>10</td>
<td>4.9 ± 1.3</td>
<td>44.0 ± 9.0</td>
<td>8.5 ± 0.4*</td>
</tr>
<tr>
<td>RY-SFBL</td>
<td>4</td>
<td>9</td>
<td>4.7 ± 0.8</td>
<td>36.6 ± 10.8</td>
<td>9.0 ± 0.8*</td>
</tr>
<tr>
<td>Restricted diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>8</td>
<td>0.9 ± 0.6*</td>
<td>28.0 ± 6.0</td>
<td>4.8 ± 0.9</td>
</tr>
<tr>
<td>RY-SFBL</td>
<td>2</td>
<td>6</td>
<td>0.3 ± 0.8*</td>
<td>36.2 ± 14.0</td>
<td>8.6 ± 0.5*</td>
</tr>
<tr>
<td>RY-SFBL</td>
<td>4</td>
<td>5</td>
<td>0.5 ± 0.6*</td>
<td>24.6 ± 9.0</td>
<td>9.6 ± 0.4*</td>
</tr>
</tbody>
</table>

a Comparison of controls with no operation with RY-SFBL groups; P < 0.001.

b Comparison of normal diet with restricted diet groups; P < 0.001.

(Dynatech Laboratories, Inc. Alexandria, Va.) were coated with 0.5 μg of anti-sIgA in 150 ml of PBS and incubated for 18 h at 4°C. The wells were washed 3 times with 0.05% Tween 20 in PBS and washed with 200 ml of 2% bovine serum albumin for 3 h at 25°C. IgA standards and unknown samples in 1% bovine serum albumin–PBS were added to each well in 150-ml portions and incubated for 18 h at 4°C. Wells were washed three times with PBS-Tween and then incubated with 0.5 μg of anti-IgA–alkaline phosphatase conjugate in 150 ml of PBS for 3 h at 25°C. An additional three washes with PBS-Tween, alkaline phosphatase was assayed by the addition of paranitrophenyl phosphate in 10% diethanolamine buffer (pH 9.8, 1 mg/ml), and the plates were read at 410 μm in a Dynatech enzyme-linked immunosorbent assay reader with reference to a standard curve of rat sIgA purified from bile (14). Particulate and soluble sIgA from the luminal washings. Luminal washings were centrifuged at 100,000 x g for 30 min at 4°C. The supernatant was decanted, dialyzed against PBS (pH 7.4) at 4°C for 24 h, and stored at 4°C. The precipitate was suspended and washed three times with PBS by centrifugation. To obtain soluble IgA from the precipitate, 8.0 ml of 3 M potassium thiocyanate (pH 6.8) was added for 1 h at room temperature. The potassium thiocyanate supernatant was collected by centrifugation at 100,000 x g for 30 min, dialyzed against PBS for 24 h at 4°C, and stored at 4°C. In 36 assays 92.6 ± 4.2% (standard deviation) of the precipitated sIgA was solubilized.

Bacterial counts. Strict anaerobic bacteria were estimated in the luminal washings as described previously (20), and results were expressed as log_{10} CFU per milliliter of initial lumen content.

Binding of sIgA to specific bacterial species. Microdilution wells were coated with bacterial sonicates derived from cultures containing 1.7 x 10^{11} to 5 x 10^{13} CFU/ml at 4°C for 18 h as previously described (14). Plates were then washed, and wells were block with 1% bovine serum albumin for 3 h at room temperature. Subsequently a known amount of sIgA (approximately 10 μg/ml) from different luminal samples was incubated in the bacterium-coated wells at 4°C for 18 h. Affinity-purified anti-sIgA–alkaline phosphatase conjugate was then applied for 18 h at 4°C. Color development in the presence of the paranitrophenol solution for alkaline phosphatase was proportional to the amount of specific sIgA bound to the bacterium-coated well.

Absorption of sIgA by specific bacterial antigens. A 1-ml sample of the supernatant fraction from the blind loop lumen was incubated at 4°C for 18 h with 1 ml of a sonicated bacterial suspension (1.7 x 10^{11} to 5 x 10^{13} CFU/ml) or 1 ml of PBS. sIgA bound to particulate antigen was removed by centrifugation at 30,000 x g for 30 min at 4°C. Binding of supernatant sIgA to immobilized bacterial sonicates was determined as above.

Protein. Protein was determined by the method of Lowry et al. (15).

Statistical treatment. The data were analyzed by the two-tailed Student t test and the Mann-Whitney test for nonparametric data. The significance (P) was the same for both procedures, making it unlikely that the t-test results were biased by outlying values.

RESULTS

Table 1 shows the growth parameters of both the control group of rats and the rats who had an RY-SFBL to exclude bile and intestinal content. Rats with restricted diets gained significantly less weight per day than normally fed animals. Although there was an overall slightly positive weight gain in the diet-restricted rats, 5 of the 11 animals with an RY-SFBL actually lost weight. The total mucosal protein in the RY-SFBL and the corresponding segment of the control jejunum was not significantly reduced by dietary restriction, nor was the mucosal wet weight or loop weight (data not shown). Despite significant suppression of weight gain the diet-restricted rats were vigorous, consumed their entire daily allocation of chow, and had no evidence of serious illness. The anaerobic bacterial population of the RY-SFBL was significantly greater than that of the control jejunum, as previously reported (14). Dietary restriction had no effect on the concentration of anaerobic bacteria in either the control jejunum or the RY-SFBL. Mucosal and luminal sIgA results for the control jejunum and the RY-SFBL are compared in Fig. 1. In control animals that had had no operation the sIgA content of the mucosa or lumen was not affected by diet restriction and was not different at the beginning and end of the experimental period. RY-SFBL mucosal sIgA was lower than that of the control jejunum mucosa at the first postoperative week in both diet-restricted and normally fed groups but remained significantly lower only in the diet-restricted animals at 2 and 4 weeks. Mucosal sIgA in normally fed and diet-restricted rats was not significantly different at any postoperative week. RY-SFBL mucosal sIgA was not therefore significantly affected by dietary restriction. As previously reported (14), the sIgA in the lumen of the RY-SFBL from normally fed rats rose markedly at 1 week when compared with that in the control jejunum and maintained the same high level at 2 and 4 weeks. A much smaller rise occurred in the diet-restricted
rats with RY-SFBL and did not become significantly greater than the jejunal values in rats that had had no operation until 2 and 4 weeks postoperation. When normally fed and diet-restricted RY-SFBL results were compared at each postoperative week, slgA was significantly lower in the diet-restricted animals, not exceeding 50% of the level found in the normally fed group. Thus, a restricted diet and retarded weight gain were associated with significantly lower levels of luminal slgA.

To obtain an indication of the amount of luminal SlgA bound by particulate bacterial antigens, the luminal contents of the RY-SFBL were centrifuged at 4°C to separate a precipitate from a particulate-free supernatant. Both total luminal slgA and total slgA in the particulate-free supernatant were significantly reduced in diet-restricted animals (Table 2). The mean precipitate slgA was also lower, although not significantly, with the result that precipitate slgA as a percentage of the total slgA was the same in both groups.

Since bacterial populations were the same in the two groups, one explanation for these results might be that the avidity of slgA for specific antigen was reduced by dietary restriction. The ability of slgA from the lumen of the RY-SFBL to bind to specific bacterial antigens was therefore tested (Table 3). In addition to a mixed culture of aerobes from human feces, Bacteroides spp. and Escherichia coli isolates from the blind loop were examined because these organisms are the most abundant anaerobes and aerobes in the SFBL and play a prominent role in the pathophysiological events (14, 18). SlgA released from the 100,000 × g precipitate by KSCN was bound by the different bacteria to a greater extent than the slgA from the 100,000 × g supernatant. Dietary restriction did not affect the binding capacity of the precipitated slgA. Also, the binding of the supernatant slgA from the two groups of rats to the bacterial antigens was not significantly different, suggesting that even though the diet-restricted animals produced less slgA, there still remained an excess of unbound slgA after complexing with available antigen.

To determine whether the binding values obtained with supernatant fractions were an accurate reflection of uncomplexed supernatant slgA, the supernatant was absorbed with bacterial antigen for 18 h at 4°C before the amount of slgA capable of binding to antigen in the microtiter wells was measured. Preincubation of nonprecipitable supernatant luminal slgA with a given bacterial sonicate led to significantly reduced binding of slgA by the same immobilized bacterial antigen (Table 4). The small slgA-binding capacity of the luminal supernatants was therefore truly due to residual binding capacity for specific bacterial antigens. This reserve was equally demonstrable in luminal supernatants from normally fed and diet-restricted animals. There is no evidence, therefore, that the degree of diet restriction achieved in these experiments critically reduced reserve binding capacity in the intestinal secretions.

**DISCUSSION**

Local secretion of slgA molecules with specific affinities for intraluminal bacterial antigens plays an important role in

<table>
<thead>
<tr>
<th>Antigen source</th>
<th>Diet</th>
<th>100,000 × g precipitate</th>
<th>100,000 × g supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> (rat SFBL)</td>
<td>Normal</td>
<td>18.8 ± 8.3 (11)</td>
<td>2.8 ± 1.8 (11)</td>
</tr>
<tr>
<td></td>
<td>Restricted</td>
<td>23.2 ± 7.8 (9)</td>
<td>2.5 ± 1.4 (9)</td>
</tr>
<tr>
<td><em>Bacteroides vulgatus</em></td>
<td>Normal</td>
<td>8.4 ± 1.4 (11)</td>
<td>2.1 ± 1.0 (11)</td>
</tr>
<tr>
<td>(rat SFBL)</td>
<td>Restricted</td>
<td>12.5 ± 4.6 (8)</td>
<td>3.7 ± 1.1 (8)</td>
</tr>
<tr>
<td>Mixed aerobes</td>
<td>Normal</td>
<td>3.2 ± 0.7 (6)</td>
<td>1.0 ± 0.3 (6)</td>
</tr>
<tr>
<td></td>
<td>Restricted</td>
<td>3.2 ± 0.7 (6)</td>
<td>1.5 ± 0.8 (5)</td>
</tr>
</tbody>
</table>

* Samples were taken from both 1-week and 2-week groups. Numbers within parentheses indicate numbers of animals examined.
TABLE 4. Absorption of sIgA from 100,000 × g supernatant by bacterial antigena

<table>
<thead>
<tr>
<th>Antigen (source)</th>
<th>No. of animals</th>
<th>sIgA binding (ng/μg ± SD)</th>
<th>% Bacterium-specific sIgA absorbed ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (rat SFBL)</td>
<td>7</td>
<td>1.58 ± 0.64</td>
<td>0.92 ± 0.31</td>
<td>36.3 ± 23.0</td>
</tr>
<tr>
<td>B. vulgatus (rat SFBL)</td>
<td>6</td>
<td>1.91 ± 0.81</td>
<td>0.65 ± 0.36</td>
<td>59.5 ± 32.0</td>
</tr>
<tr>
<td>Mixed aerobes (human feces)</td>
<td>8</td>
<td>0.97 ± 0.43</td>
<td>0.45 ± 0.48</td>
<td>51.5 ± 36.8</td>
</tr>
</tbody>
</table>

a The 100,000 × g supernatant from the blind loop lumen was absorbed with bacterial sonicate suspension (see Materials and Methods). The specific binding capacity of the sIgA for bacterial sonicates was determined pre- and postabsorption.

LITERATURE CITED


