**Biochemical and Molecular Roles of Nutrients**

**Reduction in the Quantity of the Polymeric Immunoglobulin Receptor Is Sufficient to Account for the Low Concentration of Intestinal Secretory Immunoglobulin A in a Weanling Mouse Model of Wasting Protein-Energy Malnutrition**

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**ABSTRACT**  
The main objective of this investigation was to determine the influence of protein-energy malnutrition (PEN) in weanling mice on the expression of the hepatic and intestinal polymeric immunoglobulin receptor (pIgR), a molecule that transports mucosal immunoglobulin A (IgA) into the intestinal lumen. An experimental system was used that produces systemic wasting (loss of approximately 1.9% of initial body weight per day) and that exhibits fidelity to human PEM in its influence on the concentration of IgA in critical biological fluids as well as in its influence on lymphoid involution and thymus-dependent immunocompetence. Male C57BL/6J mice were allocated to a zero-time control group (19 d of age) or to groups fed for 14 d as follows: free access to a complete purified diet (19% crude protein, 17 kJ/g gross energy) or free access to a low protein diet (0.5% crude protein). The concentration and total quantity per organ of the pIgR were assessed in the liver and intestine by Western immunoblotting using an antisera against the secretory component portion of rat pIgR. Malnourished mice exhibited low quantities of hepatic and intestinal pIgR relative to well-nourished controls (0.4% and 36% of control, respectively) and also exhibited a low concentration (soluble-protein basis) of hepatic pIgR (2% of control). The concentration of biliary secretory component also was low in the malnourished mice (4% of the value for well-nourished controls). Finally, Western blotting revealed an eightfold increase in serum concentration of dimeric IgA in the malnourished group relative to well-nourished mice, whereas the levels of the monomeric form and of the higher order polymers of IgA were elevated by factors of three and two, respectively. In this experimental system, decreased expression of the pIgR is sufficient to account for the low concentration of IgA that is maintained in the mucous secretions of the intestine.  


**KEY WORDS:**  
protein-energy malnutrition  
polymeric immunoglobulin receptor  
serum IgA  
secretory component  
mice

Wasting, pre-pubescent protein-energy malnutrition (PEM) consistently depresses the ability to generate antibody-mediated protection for the wet mucosa (Chandra 1991). In contrast, the influence of PEM on antibody responses within the deep tissues, i.e., systemic humoral immunity, seems much less predictable (Chandra 1991). No clear explanation is available in relation to the apparent difference in sensitivity to wasting PEM on the part of mucosal and systemic immunoglobulin (Ig)-producing systems. Recent evidence reveals that, up to the terminal differentiation of Ig-containing cells, the systemic and mucosal Ig-producing systems exhibit similarly remarkable resistance to wasting PEM (Ha et al. 1996). These results focus attention on the translocation of mucosal Ig into mucous secretions as a process unique to the mucosal antibody response, which may prove particularly sensitive to PEM (Ha et al. 1996). Others have suggested previously, on the basis of blood plasma and mucosal Ig concentrations (McMurray et al. 1977), that PEM may reduce translocation of mucosal Ig onto epithelial surfaces.

Mucosal Ig-secreting cells are located within the subepithelial loose connective tissue, and the majority of these cells produce antibody of IgA class, e.g., at least 80% in the intestinal lamina propria (Ha et al. 1996). Transport of IgA onto mucosal surfaces is mediated by the polymeric immunoglobulin receptor (pIgR), a transmembrane protein found on the basolateral membrane of intestinal and other mucosal epithelial cells in mammalian species as well as on the sinusoidal membrane of the hepatocyte of rodents and, perhaps, of humans (Kerr 1990). The epithelial pIgR is considered important mainly in the transport of locally produced IgA, whereas the

1 Supported by individual operating grants to B.D.W. from the Natural Sciences and Engineering Research Council of Canada and from McKellar Structured Settlements, Inc., Guelph, ON, Canada.

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5 Abbreviations used: d-, m- and pIgA designate dimeric, monomeric and polymeric immunoglobulins of A isotype, respectively; Groups C, LP and B designate well-nourished control, low protein, and zero-time control groups, respectively; Ig, immunoglobulin; MW, molecular weight; pIgR, polymeric immunoglobulin receptor; PEM, protein-energy malnutrition; SC, secretory component.
hepatic plgR is considered to function primarily in the transport of IgA from the blood (Kerr 1990). Important species differences exist in the relative contribution of epithelial and hepatic transport to the total quantity of IgA found in intestinal mucus. In humans, epithelial transport is thought to be quantitatively the more important (Daniels and Schmucker 1987, Kerr 1990), whereas rats represent the opposite extreme (Lemaître-Coelho et al. 1978), and mice are an intermediate case more similar to humans (Delacoix et al. 1985).

Uniquely among plasma membrane receptors, the plgR is neither recycled nor degraded intracellularly after binding to its ligand (IgA), but a portion of the receptor is released in a complex with the ligand (Kerr 1990). The ligand-bound fragment is derived from the extracellular domain of the plgR and is designated secretory component (SC), and the molecular complex released onto the mucosal surface is referred to as secretory IgA (Kerr 1990). In addition, both hepatocytes and extrahepatic epithelial cells seem to release unbound SC constitutively (Kerr 1990).

Watson et al. (1985) found low concentrations of unbound SC in lacrimal secretions of wasting children, and Sullivan et al. (1993) reported corroborating results in a study of lacrimal, salivary and intestinal fluids of malarial patients. Following directly from these results, the primary objective of the present investigation was to determine the concentration and total quantity of the intestinal and hepatic plgR in mice subjected to a protocol of experimental PEM that produces low concentrations of secretory IgA in intestinal mucosal secretions. The broad hypothesis emanating from this investigation, when considered together with a related study of the mucosal IgA-producing effector compartment in wasting disease (Ha et al. 1996), is that expression of the plgR is a particularly sensitive aspect of mucosal humoral immunity in PEM. The focus of this study was on PEM in its most debilitating forms because of the need to improve understanding of critical features of the human disease (Ha et al. 1996).

MATERIALS AND METHODS

Animals and experimental diets. C57BL/6 mice originally from the Jackson Laboratory (Bar Harbor, ME) were used. Weaning males, 18 d of age, were housed individually in a windowless room maintained at 23–27°C with a photoperiod (fluorescent lighting) of 14 h light and 10 h darkness. Mice were acclimated for 1 d to a complete, egg white–based purified diet described in detail elsewhere (Filteau and Woodward 1982) and were then allocated to experimental groups. A low protein diet was prepared by substituting cornstarch with the sample buffer containing 10 g/L Triton X-100. Bovine serum albumin (fraction V, Sigma Chemical) was used as the protein standard. This assay was used because of its compatibility with the Bio-Rad DC (detergent compatible) protein assay (Bio-Rad Co., Hercules, CA) used to determine protein concentration according to the method of Perez et al. (1989) with modification. Briefly, the organ was homogenized using a Caframo homogenizer (Wiarton, Ont.) at a setting of 7 (Stirrer type R2R1-64). Either 10 volumes (Groups B and C) or 20 volumes (Group LP) of cold (4°C) buffer, pH 7.4, was used containing 10 mmol/L TES [N-Tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid, Sigma Chemical], 0.2 mol/L sucrose, 1 mmol/L MgCl₂, 12.5 mmol/L benzamidine and 21 mmol/L leupeptin. A small sample of liver homogenate was reserved for assay of protein concentration. The remainder of the homogenate was centrifuged at 100,000 × g for 10 min at 4°C, after which the supernatant was ultracentrifuged at 100,000 × g for 60 min at 4°C. The resulting pellet was resuspended in TES-sucrose buffer containing 10 mL Triton X-100, 12.5 mmol/L benzamidine, 21 mmol/L leupeptin and 1 mmol/L phenylmethylsulfonyl fluoride (Boehringer Mannheim Canada, Laval). This procedure yielded a crude particulate fraction, rather than a pure membrane fraction, but was adopted in order to maximize recovery of plgR. The crude particulate fraction was stored at −80°C.

Preparation of samples for Western immunoblotting analysis. Bile was collected from the gall bladder with a 27-gauge needle and immediately placed on ice. Cold (4°C) non-reducing Laemmli buffer (62.5 mmol/L Tris-HCl, pH 6.8, containing 20 g/L SDS and 100 mL/L glycerol) containing 12.5 mmol/L benzamidine and 21 mmol/L leupeptin (Sigma Chemical, St Louis, MO) was added to the bile (9 volumes of buffer to 1 volume of bile), and the resulting mixture was stored at −80°C.

The liver was weighed, cut into small pieces and prepared according to the method of Perez et al. (1989) with modification. Briefly, the organ was homogenized using a Caframo homogenizer (Wiarton, ON) at a setting of 7 (Stirrer type R2R1-64). Either 10 volumes (Groups B and C) or 20 volumes (Group LP) of cold (4°C) buffer, pH 7.4, was used containing 10 mmol/L TES [N-Tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid, Sigma Chemical], 0.2 mol/L sucrose, 1 mmol/L MgCl₂, 12.5 mmol/L benzamidine and 21 mmol/L leupeptin. A small sample of liver homogenate was reserved for assay of protein concentration. The remainder of the homogenate was centrifuged at 100,000 × g for 10 min at 4°C, after which the supernatant was ultracentrifuged at 100,000 × g for 60 min at 4°C. The resulting pellet was resuspended in TES-sucrose buffer containing 10 mL Triton X-100, 12.5 mmol/L benzamidine, 21 mmol/L leupeptin and 1 mmol/L phenylmethylsulfonyl fluoride (Boehringer Mannheim Canada, Laval). This procedure yielded a crude particulate fraction, rather than a pure membrane fraction, but was adopted in order to maximize recovery of plgR. The crude particulate fraction was stored at −80°C.

Intestinal tissue, washed free of contents using cold (4°C) PBS, was blotted on filter paper and weighed. A crude particulate fraction of intestinal content was prepared and stored as described for the liver samples, except that the intestine was initially homogenized for 20 s by means of a homogenizing apparatus (Ultra-Turrax®) while being homogenized in the Caframo apparatus. A bioassay for the Bio-Rad DC (detergent compatible) protein assay (Bio-Rad Co., Hercules, CA) was used to determine protein concentration according to the manufacturer's instructions. Bovine serum albumin (fraction V, Sigma Chemical) was used as the protein standard. This assay was used because of its compatibility with the sample buffer containing 10 g/L Triton X-100.

Electrophoresis. Samples of bile and of hepatic and intestinal crude particulate fractions were diluted in non-reducing Laemmli buffer. Polyacrylamide gradient slab gels (40–150 g/L, Mini-PROTEAN® II Ready Gels, Bio-Rad Co.) prepared in 375 mmol/L Tris-HCl buffer permitted achievement of protein separations at room temperature within 35 min at 200 V. Serum samples were subjected to similar conditions of electrophoresis except that 1.5-mm slab gels were prepared with a 30 g/L acrylamide stacking gel and a 50 g/L acrylamide separation gel. Molecular weight (MW) standards including α-µ-macroglobulin from horse plasma (MW 340,000; Boehringer Mannheim Canada, Laval) and a mixture of protein standards purchased from Sigma Chemical (myosin from rabbit muscle, MW 205,000; mouse IgG, MW 150,000; β-galactosidase from E. coli, MW 116,000; phosphorylase b from rabbit muscle, MW 97,400; bovine albumin, MW 66,000; egg albumin, MW 45,000; carbonic anhydrase from bovine erythrocytes, MW 29,000) were used in order to permit estimation of the MW of immunoreactive target bands. Lanes containing 180, 360, 540 and 720 pg of rat free SC standard were used in order to permit estimation of the MW of immunoreactive target bands. Lanes containing 180, 360, 540 and 720 pg of rat free SC standard were used in order to permit estimation of the MW of immunoreactive target bands. A crude particulate of intestinal and hepatic pIgR in mice was ultracentrifuged at 100,000 × g for 60 min at 4°C. The resulting pellet was resuspended in TES-sucrose buffer containing 10 mL Triton X-100, 12.5 mmol/L benzamidine, 21 mmol/L leupeptin and 1 mmol/L phenylmethylsulfonyl fluoride (Boehringer Mannheim Canada, Laval). This procedure yielded a crude particulate fraction, rather than a pure membrane fraction, but was adopted in order to maximize recovery of plgR. The crude particulate fraction was stored at −80°C.
MA) using a semi-dry transfer apparatus (Electrophoretic Transfer System ET10 or ET20, Tyler Research Instruments, Edmonton, AB). Complete transfer was achieved in 2 h at settings of 100 or 150 mA in a buffer, pH 8.3, containing 200 mL/L methanol, 150 mL/L glycine, 25 mmol/L Tris and 1 g/L sodium dodecyl sulfate. Complete transfer of target protein(s) was confirmed by staining the gel with Coomassie blue. In addition, based on the linearity of the rat SC standard curve, the quantity of target protein(s) transferred to the PVDF membrane was always within the protein-binding capacity of the membrane. The MW markers proteins on the membrane were revealed by fast green staining (1 g/L fast green, 200 mL/L methanol, 50 mL/L acetic acid). After destaining with methanol, the membrane was blocked by incubation for 1 h in 20 mL/L Tris-buffered saline, pH 7.6, containing 0.5 mL/L Tween 20 (Sigma Chemical) and 5 g/L gelatin (275 Bloom, Fisher Scientific, Fair Lawn, NJ). The membrane was then incubated for 2 h in a 1:10,000 dilution of rabbit anti-rat SC (gift from B. Underdown, McMaster University) followed by four washes (10 min each) with 20 mL/L Tris-buffered saline, pH 7.6, containing 0.5 mL/L Tween 20 (TBST). The membrane was subsequently incubated for 1 h in a 1:50,000 dilution of peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad Co.) and subjected to five washes (10 min each) with TBST.

Western immunoblotting procedures for serum IgA were the same as for the pIgR and SC, except for the use of peroxidase-conjugated goat anti-mouse IgA antibody (a chain specific, Nordic Immunological Laboratories, Maidenhead, England). The PVDF membrane was incubated for 90 min in a 1:80,000 dilution of this reagent.

Bound peroxidase activity was revealed in the washed PVDF membranes by incubation in enhanced chemiluminescence reagent (Dual-wavelength Flying-spot Scanner, Shimadzu, Kyoto, Japan). The PVDF membrane was exposed to X-ray film for as long as 5 min. Carcass compositional analysis was conducted on pooled samples of hepatic, intestinal and biliary material, except that 70 g/L polyacrylamide gels were used and 4.3 mmol/L Tris and 1 g/L sodium dodecyl sulfate. Complete transfer of target protein(s) was confirmed by staining the gel with Coomassie blue. In addition, based on the linearity of the rat SC standard curve, the quantity of target protein(s) transferred to the PVDF membrane was always within the protein-binding capacity of the membrane. The MW markers proteins on the membrane were revealed by fast green staining (1 g/L fast green, 200 mL/L methanol, 50 mL/L acetic acid). After destaining with methanol, the membrane was blocked by incubation for 1 h in 20 mL/L Tris-buffered saline, pH 7.6, containing 0.5 mL/L Tween 20 (Sigma Chemical) and 5 g/L gelatin (275 Bloom, Fisher Scientific, Fair Lawn, NJ). The membrane was then incubated for 2 h in a 1:10,000 dilution of rabbit anti-rat SC (gift from B. Underdown, McMaster University) followed by four washes (10 min each) with 20 mL/L Tris-buffered saline, pH 7.6, containing 0.5 mL/L Tween 20 (TBST). The membrane was subsequently incubated for 1 h in a 1:50,000 dilution of peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad Co.) and subjected to five washes (10 min each) with TBST.

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The specificity of the peroxidase-conjugated goat anti-mouse IgA was verified using preparations of purified mouse myeloma IgA and IgM (TEPC 15 and TEPC 183, respectively, Sigma Chemical) and of purified mouse IgG (Sigma Chemical) as described elsewhere (Ha et al. 1996). Moreover, the reagent yielded a reaction product when used immunohistochemically with intestinal sections rich in IgA-containing cells but failed to yield a reaction product with negative control tissue sections from mouse kidney (Ha et al. 1996).

**Carcass analyses.** Dry matter content of carcasses was determined by freeze-drying followed by oven-drying under vacuum for 16 h at 90°C. Crude protein and total lipid concentrations were measured, using samples of dried carcasses, as described elsewhere (Woods and Woodward 1991).

**Statistical analyses.** Results were subjected to two-tailed Student’s t test or to ANOVA followed, if justified, by Tukey’s Studentized range test, unless a different statistical procedure is indicated.

**RESULTS**

**Growth indices.** Weight loss within the malnourished group averaged 27% of initial body weight, whereas the well-nourished control animals gained an average of 140% of their initial weight during the 14-d experimental period (Table 1). Carcass compositional analysis was conducted on pooled samples, only, thereby precluding statistical analysis of the data. Dry matter contents (g/100 g) were 29.4, 28.0 and 26.5 in Groups B, C and LP, respectively; crude protein contents (g/100 g) were 14.4, 16.4 and 16.2 in Groups B, C and LP, respectively; lipid contents (g/100 g) were 11.7, 8.2 and 4.5 in Groups B, C and LP, respectively. These analyses are similar to results obtained previously in relation to this experimental system in which weight loss on the part of the LP group was attributable to decrements in both lean and fat tissue (Ha et al. 1996, Woods and Woodward 1991). The wasting disease produced in the present investigation was similar to the condition shown in previous studies of the same experimental system (Woods and Woodward 1991) to be associated with profound depression in cell-mediated and humoral acquired immunity.

The cumulative food intake of the malnourished group (i.e., over the 14-d experimental period) was 43% of that exhibited by the well-nourished controls (Table 1). On a daily basis, however, this represented an intake of dietary energy and micronutrients comparable, on a body weight basis, to that of

<table>
<thead>
<tr>
<th>Index</th>
<th>Dietary group</th>
<th>B</th>
<th>C</th>
<th>LP</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight, g/mouse</td>
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<td>8.8</td>
<td>8.9</td>
<td>0.14</td>
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<tr>
<td>Final weight, g/mouse</td>
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<td>21.1b</td>
<td>6.5a</td>
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<tr>
<td>Food intake, g/(mouse · 14 d)</td>
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<td>42.6b</td>
<td>18.5a</td>
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<tr>
<td>Liver weight, g</td>
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<td>1.1c</td>
<td>0.2a</td>
<td>0.02</td>
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<tr>
<td>Liver protein, mg/g</td>
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<td>182.9b</td>
<td>153.5a</td>
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<tr>
<td>Intestine weight, g</td>
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<td>1.3c</td>
<td>0.4a</td>
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<tr>
<td>Intestine protein, mg/g</td>
<td>49.6ab</td>
<td>52.6b</td>
<td>42.0a</td>
<td>2.32</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means, n = 8 per group. Within a row, values not sharing a superscript letter are different (P < 0.05) according to Tukey’s Studentized range test, unless a different statistical procedure is indicated.

2 B: zero-time control, i.e., 19 d of age; C: group given free access to complete diet; LP: group given free access to low protein diet. Experimental period extended from 19 to 33 d of age.

3 Values not sharing a superscript letter are different (P < 0.05) according to Wilcoxon two-sample test (rank sums: C, 100; LP, 36).

4 Values not sharing a superscript letter are different (P < 0.05) according to two-tailed Student’s t test.

5 Values expressed on a wet weight basis. “Intestine” refers to small and large intestine plus cecum.

6 Protein concentration was determined on the 1000 x g supernatant of intestinal homogenates.

Dentified range test (Steel and Torrie 1960). Several data sets were subjected to logarithmic transformation to meet the requirement that a normal distribution be achieved. In one case involving a comparison of two groups (final body weight), normality could not be achieved by either logarithmic or square root transformation. In this case, the Wilcoxon two-sample rank sums test (Steel and Torrie 1960) was applied. The predetermined upper limit of probability for significance throughout this investigation was P < 0.05.
the well-nourished group (results not shown). This outcome is consistent with previous findings pertaining to the low protein system used in this investigation, viz., that serum triiodothyronine concentration remains comparable to that of well-nourished weanling mice (Filteau and Woodward 1987, Woods and Woodward 1991), whereas animals malnourished by means of restricted intake of a complete diet exhibit rapid and profound depression in the serum concentration of this hormone (Filteau and Woodward 1987). The wasting disease of the low protein group, therefore, resulted from dietary imbalance rather than from an insufficient intake of energy or of nutrients other than protein.

On the basis of comparison with the zero-time control group, both the liver and the intestine increased in weight in the well-nourished control group during the 14-d experimental period, although protein concentration was not affected in either organ in this group (Table 1). In the malnourished mice, both organs exhibited weight loss (in comparison with the zero-time control group) and a low protein concentration (relative to well-nourished controls).

**Quantification of hepatic and intestinal plgR and of biliary secretory component.** A representative Western immunoblot of the hepatic plgR of Groups B, C and LP together with the rat SC standard is shown in Figure 1. The position of the band of the SC standard revealed a MW of approximately 80 kDa, similar to the MW reported for rat SC by Musil and Baenziger (1988). The MW of the main hepatic band revealed by immunoblotting was ~120 kDa and hence was similar to the MW of rat hepatic plgR (Sztul et al. 1985). Nonspecific binding of antibody reagents to diverse proteins in the samples of Group B (lanes 5 and 8) and LP (lanes 7 and 10) mice resulted from the overloading of sample, which was necessary in order to detect an anti-rat SC immunoreactive band of appropriate MW in these groups.

A representative Western immunoblot of the intestinal plgR is shown in Figure 2 together with lanes of rat SC standard. Mice of each group exhibited one or both of two bands with MW close to 97 and 116 kDa. Molecular heterogeneity has been demonstrated by Kuhn et al. (1983) in the plgR of the mammary gland and the liver in rabbits and by Kloppel and Brown (1984) in the biliary SC of rats. Heterogeneity in the plgR is therefore an established phenomenon, although it apparently has not been reported previously in connection with the intestine. The mammalian plgR is heavily N-glycosylated (Piskurich et al. 1995), and heterogeneity in the molecule could derive from variations in this post-translational modification.

A representative Western immunoblot of biliary SC, including lanes of rat SC standard, is shown in Figure 3. Two immunoreactive bands were revealed in the bile and exhibited MW between 80 and 97 kDa (Fig. 3), a result similar to findings reported in a study of rats (Kloppel and Brown 1984). Bile from Group C mice also yielded a band (Fig. 3, lanes 6 and 9) at a MW of approximately 340 kDa. A band was also revealed in the same position by immunoblotting with peroxidase-conjugated anti-mouse IgA (results not shown). No such band was detected in the bile of Group LP (lanes 7 and 10) or Group B (lanes 5 and 8) mice even when the volumes of
groups of mice (Fig. 4). Because of this finding, a supplementary immunohistological study was conducted using methods described elsewhere (Ha et al. 1996). Dilutions of rabbit anti-rat SC and of peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) antibody were 1:1000 and 1:2000, respectively. Duodenal and jejunal sections exhibited an intense reaction product distributed throughout the epithelial cells in both Group C (n = 4) and Group LP (n = 2) mice (results not shown). These immunohistological results were therefore consistent with the outcome of the Western immunoblotting study of the intestinal crude particulate fraction. Duodenal sections did not reveal a reaction product when stained with only the peroxidase-conjugated second antibody. Spleen sections stained with both primary and secondary antibodies served as additional negative controls, and failed to exhibit peroxidase reaction product (result not shown).

Hepatic and intestinal plgR (i.e., anti-rat SC immunoreactive material), expressed on a per organ basis, are shown in Figure 5. A significantly greater total amount of plgR in both organs was found in Group C mice relative to the zero-time control group. In contrast, a profound reduction of the total amount of hepatic plgR, to 9.6% of the quantity in Group B liver (and only 0.4% of the quantity in Group C), was found in Group LP mice. Although the concentration of intestinal plgR was not different in Groups C and LP (Fig. 4), the total intestinal plgR of Group C mice was about threefold higher than in Group LP animals because of the intestinal growth achieved by the former group (Table 1). Group LP mice maintained intestinal plgR, on a per organ basis, relative to Group B mice (Fig. 5).

Distribution of serum immunoglobulin A among monomeric, dimeric and polymeric forms. Three main bands reactive with anti-mouse IgA were found in the serum from mice of Groups LP and C (Fig. 6) and exhibited MW of ~150, 340 and >340 kDa. In contrast, Group B animals exhibited only the band corresponding to a MW exceeding 340 kDa. A fourth band within the MW range between 150 and 205 kDa was
inconsistently apparent in samples from animals of Groups C and LP. Some samples of serum were electrophoresed in reducing conditions. For this purpose, samples were diluted with reducing Laemmli buffer (containing 50 mL/L 2-mercaptoethanol, Sigma Chemical) and were boiled for 5 min prior to loading onto 40–150 g/L gradient gels for electrophoresis and subsequent Western immunoblotting. Following application of this procedure, only one anti-mouse IgA-reactive band was found. This band exhibited a MW of ~65 kDa (results not shown), a MW comparable to that of the human α-heavy chain (Kerr 1990). All bands identified in serum samples by Western immunoblotting were therefore IgA-related proteins.

On the basis of the foregoing, the bands of MW ~150, 340 and >340 kDa were designated as monomeric (m), dimeric (d) and polymeric (p) IgA, respectively. The sum of the densitometer readings of the three bands in immunoblots of serum from Group LP mice (arbitrary units, mean = 125.7 × 10^3) was 3.1-fold the value obtained for Group C mice (arbitrary units, mean = 37.1 × 10^3), a finding that is consistent with a previous report that the low protein model produces an elevation in serum IgA concentration as is also common in malnourished humans (Ha et al. 1996). Each of these three presumptive forms of IgA was found in high concentration in the serum of Group LP mice compared with the serum of Group C animals (Fig. 7). This was particularly the case for the dimeric form, which was found at a concentration 7.6-fold that of Group C, whereas the monomeric and polymeric forms were found at concentrations that were 2.7- and 2.2-fold, respectively, the levels in Group C.

DISCUSSION

The experimental system used in the present investigation duplicates numerous physiological features of human PEM, including depression in acquired immunocompetence and well-known effects on the concentration of the mucosal immunoglobulin isotype, IgA, in critical biological fluids including intestinal mucous secretions (Ha et al. 1996). The present investigation therefore permits the conclusion that the quantity of the plgR is low in diverse anatomical sites in weanling PEM and that an organ-specific response may be anticipated with respect to the magnitude of this phenomenon. In addition, and perhaps in consequence, the distribution of serum IgA among monomeric, dimeric and polymeric forms is affected by PEM. Interpretation of the results depends on the specificity with which anti-rat SC antiserum detects mouse plgR and SC as well as on the extent to which rat SC can serve as a standard for the corresponding protein in mice. In this regard, verification is provided in the present investigation as to the specificity of the antiserum when applied to extracts from mouse tissues. Furthermore, a previous report documents extensive immunological cross-reactivity between the SC of mice and of rats (Delacroix et al. 1984), a finding consistent with the recent demonstration of 86% homology between the plgR, as well as the SC portions of this molecule, in the two species (Piskurich et al. 1995). At the same time, use of a rat SC standard may have resulted in underestimation of the concentrations of murine plgR and SC, because these proteins inevitably fail to express all the antigenic determinants found on rat SC. Finally, the present investigation illustrates, as shown in a related study (Ha et al. 1996), the importance of including a zero-time control group in studies of the immune system when PEM is imposed during the weaning stage of development. This design feature eliminates the confounding influence of ontogenetic change but is seldom employed in work with experimental animals and is often either unethical or difficult to put in place in studies of human subjects. The following paragraphs expand on these points whereby the present results represent a meaningful extension of available information pertaining to the plgR in weanling PEM.

Results from diverse experimental systems point to the quantity of plgR as a determining factor in relation to the rate of transcytosis of IgA both in vitro (Kaelzer et al. 1991, Phillips et al. 1990) and in vivo (Prabhala and Wira 1991). The present
results demonstrate reduction in the quantity of intestinal and hepatic plgR in a model of wasting disease in which biliary IgA concentration and the quantity of IgA within the contents of the intestinal lumen both were low despite a high serum IgA concentration (Ha et al. 1996). The low mucosal concentrations of secretory IgA in this experimental system are therefore attributable substantially to a depressed expression of the plgR. Others have proposed that PEM exerts a depressive influence on the transport of IgA to its mucosal sites of action (Chandra and Wadhwa 1993, McMurray et al. 1977). The present results are consistent with this proposition, and they initiate an understanding of the mechanism whereby PEM exerts a consistent depressive influence on the concentration of mucosal secretory IgA (Chandra 1991).

Low concentrations of free SC have been reported in tears of wasted children (Watson et al. 1985) and in tears, saliva and intestinal washings of weanling rats subjected to PEM by way of a low protein diet regimen (Sullivan et al. 1993). The low concentration of biliary SC associated with PEM in the present investigation is therefore consistent with previous information relating to this molecule in other external secretions in PEM. Functional inefficiency on the part of the hepatic plgR in PEM may be inferred from the discovery of free SC, albeit in low concentration, in the bile of Group LP mice (as in Group C mice) in the apparent absence of secretory IgA which was detectable only in the well-nourished controls. The mechanism whereby the low protein protocol may impair the function of the plgR remains to be investigated. In this regard, however, the G protein subunit, Gaα, stimulates transcytosis of the plgR by way of cAMP and protein kinase A (Han and Casanova 1994), whereas desensitization of the protein kinase A response to cAMP was reported in the liver of weanling rats subjected to wasting PEM (Rozwadowski et al. 1995). In addition, disruption of microtubules impairs transcytosis of IgA (Breitfeld et al. 1990), and such a disturbance in cellular structure seems probable in diverse tissues, e.g., the cerebral cortex (de Mattos et al. 1994) and the thymic epithelium (Mittal and Woodward 1986), in PEM. Alternatively, production of dIgA lacking the J chain peptide would also reduce the efficiency of plgR-mediated transcytosis of IgA by the liver (Hendrickson et al. 1995). Low mucosal IgA concentrations such as occur in the present system of experimental PEM (Ha et al. 1996) may therefore result both from reduced expression of the plgR and from functional inefficiency on the part of the small quantity of receptor that is expressed.

In the present investigation, the intestine and liver responded differently to PEM in terms of the cellular plgR concentration. This outcome is easily reconciled with existing information demonstrating that the neuroendocrine regulation of plgR synthesis is site specific (Lambert et al. 1994). Moreover, the hepatic and intestinal plgR exhibited different developmental kinetics in the present experimental system. The hepatic plgR increased 9.5-fold in concentration in well-nourished mice between 19 and 33 d of age. In contrast, the intestinal plgR concentration did not differ between zero-time control and well-nourished mice. The present study therefore seems to have been conducted at a stage of murine development at which the hepatic plgR is more physiologically labile than the intestinal plgR. A clear understanding is lacking as to the neuroendocrine mechanisms whereby site-specific control of plgR synthesis is achieved.

The magnitude of PEM-associated depression in hepatic plgR concentration in the present experimental system implicates an influence on the synthesis of this protein. Depression of hepatic protein synthesis, however, is not a general phenomenon in PEM (Straus et al. 1994). In a model of wasting malnutrition involving weanling rats fed a low protein diet, the hepatic messenger RNA levels for some proteins (albumin, transferrin, carbamyl phosphate synthetase and alcohol dehydrogenase) were low relative to levels in well-nourished controls, whereas the messenger RNA levels for several other proteins (hypoxanthine-guanine phosphoribosyl transferase, ubiquitin, H-ferritin and insulin-like growth factor binding protein-4) were either unaffected or high (Straus et al. 1994). The plgR therefore seems likely to be among those hepatic proteins that are most sensitive to PEM.

The distribution of molecular forms of serum IgA in PEM has not been reported prior to this investigation, and was studied as an index related to the quantity of functional hepatic plgR. The present results pertaining to well-nourished control animals (59% mlgA and 41% in the combined dimeric and polymeric IgA fraction) are comparable, quantitatively, to those reported by Delacroix et al. (1985) in relation to adult mice. Presuming that PEM does not increase the rate of synthesis of IgA, the high serum level of this Ig (all forms) in the LP group focuses attention on mechanisms whereby this molecule is removed from the blood. The main point pertains to the disproportionately high concentration of dIgA found in the serum of the LP group. In mice, mlgA is removed from the blood plasma by a hepatic asialoglycoprotein receptor (Moldoveanu et al. 1988), whereas dIgA is thought to be removed mainly by way of plgR-mediated hepatobiliary transport (Kerr 1990). The abundance of dIgA relative to mlgA in the blood of the Group LP mice is therefore easily reconciled with the low expression of the hepatic plgR in this group. Like dIgA, higher MW forms of this Ig are also thought to be removed from the blood by the hepatic plgR (Kerr 1990). The mechanism whereby the low protein protocol induced an overabundance of dIgA relative to mlgA in the blood is therefore not clear. Song et al. (1995), however, showed that conditions that restrict the number of cellular IgA receptor sites will limit the plgR-mediated transcytosis of dIgA more severely than of plgR (tetrameric form). It is unlikely that a shift toward dIgA in the blood is significant, of itself, to the immunopathology of PEM. This finding, however, is consistent with the profound decrease in hepatic plgR in the LP group and thus provides independent support for the conclusion that the effect of the low protein protocol on the hepatic plgR bears functional importance.

In summary, we investigated a murine model of weanling PEM that mimics the human condition in terms of important aspects of IgA concentration (Ha et al. 1996, this study) and systemic acquired immunity (Woods and Woodward 1991). Use of this experimental system highlights the resistance of the systemic and mucosal humoral effector compartments to the influence of wasting PEM (Ha et al. 1996) but, at the same time, provides evidence that the unique mucosal requirement for epithelial transport of IgA is sensitive to PEM (this investigation). In PEM, therefore, expression of functionally efficient plgR may limit IgA concentrations in external secretions independently of any influence on IgA-containing cell numbers (Fig. 8). The result is a deficiency of the blocking antibody action of IgA that counteracts the propensity of disease-causing microorganisms to adhere to, and sometimes to penetrate, mucosal epithelia. In addition, an important implication of these results relates to the therapeutic enhancement of mucosal immunity in wasted subjects. An intervention directed only toward increasing IgA-containing cell numbers would be unlikely to enhance mucosal humoral immunity in PEM, whereas stimulation of plgR synthesis and function might promote a meaningful increase in the mucosal IgA concentration. Polymeric immunoglobulin receptor synthesis is...
The authors gratefully acknowledge the technical assistance of Lyn Hillyer in conducting the carcass analyses and assisting with computer-generated figures. Brian Underdown (McMaster University, Hamilton, ON) kindly provided the rat secretory component standard and rabbit anti-rat secretory component antibody.


