

## Malnutrition Modifies Pig Small Intestinal Inflammatory Responses to Rotavirus

Ruurd T. Zijlstra,<sup>\*1</sup> Barbara A. McCracken,<sup>\*</sup> Jack Odle,<sup>\*†2</sup> Sharon M. Donovan,<sup>†\*\*</sup> Howard B. Gelberg,<sup>‡</sup> Bryon W. Petschow,<sup>††</sup> Federico A. Zuckermann<sup>‡</sup> and H. Rex Gaskins,<sup>\*†‡3</sup>

<sup>†</sup>Division of Nutritional Sciences, Departments of <sup>\*</sup>Animal Sciences, <sup>\*\*</sup>Food Science and Human Nutrition, and <sup>‡</sup>Veterinary Pathobiology, University of Illinois at Urbana-Champaign and <sup>††</sup>Mead Johnson Research Center, Evansville, IN

**ABSTRACT** Infectious diarrheal diseases and malnutrition are major causes of child morbidity and mortality. In this study, malnutrition was superimposed on rotavirus infection in neonatal piglets to simulate the combined intestinal stress of viral enteritis in malnourished infants. Two-day-old piglets were assigned to three treatment groups as follows: 1) noninfected, fully nourished; 2) infected, fully nourished; and 3) infected, malnourished. Intestinal indices of inflammation were monitored over the subsequent 2-wk period. Intestinal damage and diarrhea were observed within 2 d of rotavirus infection and began to subside in nourished piglets by d 9 but persisted through d 16 postinfection in malnourished piglets. Rotavirus upregulated small intestinal expression of major histocompatibility complex (MHC) class I and class II genes; malnutrition intensified MHC class I gene expression and suppressed MHC class II expression. Jejunal CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocyte numbers were elevated for infected, nourished piglets on d 2, 9 and 16 postinfection. Malnutrition did not significantly affect the local expansion of T cell subsets in response to rotavirus. Intestinal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) concentrations were elevated early after rotavirus infection independent of nutritional state. By d 9, PGE<sub>2</sub> concentrations returned to baseline in infected, nourished piglets but remained elevated in malnourished piglets, corresponding to diarrhea observations. Together, the results identify intestinal indices of inflammation that are modulated by malnutrition and prompt reconsideration of current models of rotavirus pathophysiology. *J. Nutr.* 129: 838–843, 1999.

**KEY WORDS:** • rotavirus • inflammation • malnutrition • pigs • small intestine

It has now been more than two decades since rotaviruses were determined to be the most common cause of severe dehydrating diarrhea (Bishop et al. 1973, Kapikian and Chanock 1996). Extensive child morbidity and mortality continue nonetheless. In developing countries, in which nutritional status is often compromised, rotaviruses cause >850,000 deaths each year. Worldwide, rotaviruses account for >25% of diarrheal-related deaths and 6% of all deaths in children <5 y old (Matsui and Angel 1997). Considerable promise arises from recent efforts to develop an antirotavirus vaccine (Glass et al. 1996). In fact, progress with vaccine strategies exceeds our understanding of mechanisms underlying rotavirus pathophysiology. Knowledge of the molecular and cellular basis of host responsiveness to rotavirus infection remains crucial, particularly in the context of malnutrition, which exacerbates disease severity and will likely affect the efficacy of antirotavirus vaccine strategies.

Rotavirus infections are characterized by viral replication in

small intestinal enterocytes (Estes 1990), with subsequent cell lysis and attendant villous blunting (Theil et al. 1978), depressed levels of mucosal disaccharidases (Bishop et al. 1973), watery diarrhea (Theil et al. 1978) and dehydration. It is generally accepted that the destruction of villous epithelial cells reduces enzymatic and absorptive capacity in the small intestine, resulting in a malabsorptive-type diarrhea (Graham et al. 1984). With a neonatal pig model, we recently identified nutritionally responsive indices of structural repair that remain operative despite epithelial damage in rotavirus-infected intestine (Zijlstra et al. 1997). Those findings bring into question the extent to which malabsorption contributes to rotaviral diarrhea and challenge the common nutritional management protocol of "bowel rest," i.e., reduction of luminal nutrients during the diarrheal episode (American Academy of Pediatrics 1996, Lieberman 1994, Zijlstra et al. 1997). We have now extended our observations and provide evidence that rotavirus induces an attendant intestinal inflammatory response that may contribute to a secretory-type diarrhea. Also identified are molecular and biochemical indices of rotavirus infection that are responsive to malnutrition, enabling opportunities to understand how individual macronutrients contribute at the intestinal level to host clearance of rotavirus.

<sup>1</sup> Current address: Prairie Swine Centre Inc., Box 21057, Saskatoon, SK, Canada S7H 5N9.

<sup>2</sup> Current address: North Carolina State University, Box 7621, Raleigh, NC 27695-7621.

<sup>3</sup> To whom correspondence should be addressed.

## MATERIALS AND METHODS

**Animals and experimental design.** Animal protocol was approved by the University of Illinois Laboratory Animal Care Advisory Committee and followed principles established by the NIH (NRC 1985). Experimental design, diet considerations, and infection protocol were described in more detail in a previous report of other data from this study (Zijlstra et al. 1997). Two-d-old cesarean-delivered, colostrum-deprived piglets ( $n = 39$ ) were randomly assigned to three treatment groups as follows: 1) noninfected, fully nourished; 2) infected, fully nourished; and 3) infected, malnourished. Pigs in groups 2 and 3 were infected with porcine rotavirus at 2 d of age.

All piglets were fed a liquid diet formulated to meet the nutritional requirements for growing piglets from birth through 3 wk of age (McClead et al. 1990) and prepared by the Mead Johnson Nutritional Group (Evansville, IN) as a dry powder. Pigs in groups 1 and 2 were fed complete reconstituted formula (180 g/L) and were pair-fed according to daily intake of the infected, fully nourished group. For the malnourished, infected group, formula was diluted 50% with water plus electrolytes and also supplied according to daily volume intake of infected, fully nourished piglets (group 2) (Butzner et al. 1985, Zijlstra et al. 1997).

Diarrhea was scored daily based on consistency of feces (0, no diarrhea; 1, stiff flowing feces; 2, easy flowing feces; 3, severe, watery diarrhea). On d 2, 9 and 16 postinfection, four piglets per treatment were killed by electrocution followed by exsanguination. The small intestine was dissected free of mesentery and arranged in six parts of equal length to enable collection of tissue at seven equidistant sites from the duodenum (segment 1) and proximal jejunum (segment 2) to distal jejunum (segment 6) and distal ileum (segment 7). Small intestine tissue was collected immediately and fixed for immunocytochemistry or frozen at  $-80^{\circ}\text{C}$  for other analyses as described below.

**Intestinal MHC class I and II expression.** Intestinal major histocompatibility complex (MHC)<sup>4</sup> RNA expression was monitored as a general barometer of inflammation (Abbas et al. 1991). Total RNA was extracted from frozen jejunal tissue (segment 6) using the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi 1987). Total RNA samples (10  $\mu\text{g}$  each) were size-separated in 1.25% agarose/3% formaldehyde gels and immobilized onto nylon membranes (Magna Graph, Westborough, MA) by using standard Northern blotting techniques (Sambrook et al. 1989). Blots were probed with  $\alpha^{32}\text{P}$ -labeled cDNA probes specific for porcine MHC class I and II genes (kindly provided by Dr. L. B. Schook, University of Minnesota) with the use of conventional hybridization technique (Sambrook et al. 1989). To verify equivalent RNA loading in agarose gels, blots were probed with an  $\alpha^{32}\text{P}$ -labeled cDNA specific for human  $\beta$ -actin. Autoradiographic exposure of the membranes to Kodak (Rochester, NY) X-Omat AR film was carried out at  $-80^{\circ}\text{C}$  with intensifying screens. Relative signal intensities were determined by laser densitometry using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

**Lymphocyte immunocytochemistry.** Intestinal CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes, which participate in effector mechanisms underlying rotavirus immunity (Bruce et al. 1995, Matsui and Angel 1997), were monitored in this study by quantitative immunocytochemistry. Jejunal tissues (segment 5) were fixed in Bouin's solution (Carson 1990) for 24 h, soaked in four changes of 70% ethanol to remove excess picric acid, then embedded in paraffin and sectioned onto glass slides. Slides were deparaffinized in xylene and then rehydrated through a series of washes in alcohol, water and Tris buffer. Fluorescent anti-pig CD4 (clone 74-12-4) or biotinylated anti-pig CD8 (clone 76-2-11) antibodies were used according to standard immunofluorescence or immunocytochemical techniques (Carson 1990, Saalmuller 1996). Tissue sections were blocked with 20% mouse serum before primary antibody incubations for 2 h at  $37^{\circ}\text{C}$ . Biotinylated anti-CD8 positive cells were developed using a Histomark Red kit (Kirkegaard & Perry Laboratories, Gaithersburg, MD) according to the manufacturer's protocol. Electronic images were captured using a Sony (New York, NY) PowerHAD color video camera attached to a Nikon (Melville,

NY) OPTIPHOT-2 microscope. CD4<sup>+</sup> or CD8<sup>+</sup> lymphocytes were counted in one entire jejunal section per pig at a magnification of X 100 (Olympus BH-2-RFCA, Lake Success, NY). To standardize lymphocyte counts, entire jejunal cross-sectional areas were measured using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD).

**Intestinal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) analysis.** Approximately 1 g of frozen jejunal tissue (segment 4) was homogenized on ice in 2 mL of 95% methanol for 30 s. Homogenates were centrifuged at  $3000 \times g$  for 15 min at  $4^{\circ}\text{C}$  for supernatant recovery. PGE<sub>2</sub> was measured with an enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's protocol. EIA plates were read on a Thermo<sub>max</sub> microplate reader (Molecular Devices, Menlo Park, CA). To normalize jejunal PGE<sub>2</sub>, total protein concentrations in the methanol-insoluble fraction were determined by the modified Lowry method (Hartree 1972) with bovine serum albumin as a standard.

**Statistical analyses.** Data were analyzed using the General Linear Models procedure of the SAS statistical package (SAS 1985). Infected, fully nourished piglets (group 2: d 2,  $n = 5$ ; d 9,  $n = 5$ ; d 16,  $n = 4$ ) were compared by preplanned contrasts with either noninfected piglets (group 1: d 2,  $n = 4$ ; d 9,  $n = 4$ ; d 16,  $n = 4$ ) to determine the effect of rotavirus infection, or to infected, malnourished piglets (group 3: d 2,  $n = 4$ ; d 9,  $n = 5$ ; d 16,  $n = 4$ ) to determine the effect of malnutrition within infected piglets (Steel and Torrie 1980). Results are presented as least-square means  $\pm$  pooled SEM. Differences were considered significant when  $P < 0.05$ . Instances in which  $P < 0.1$  are discussed as trends. To consider possible mechanisms underlying intestinal responses to rotavirus alone, or to the combined effects of rotavirus and malnutrition, relationships among diarrhea observations and inflammatory variables were evaluated by Spearman correlation analysis (SAS 1985).

## RESULTS

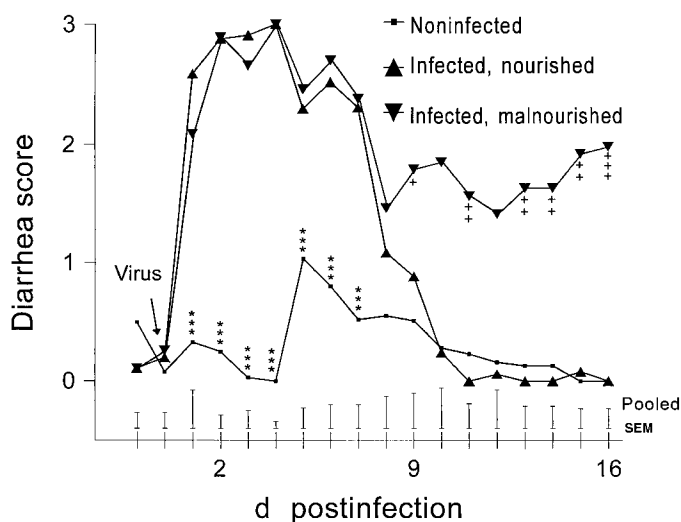
**Animal observations.** Nutrient intake and weight gain data were presented in a previous report of this study that focused on animal growth and metabolic and small intestinal parameters of recovery from rotavirus (Zijlstra et al. 1997).

Diarrhea was not observed in any treatment group before inoculation with rotavirus (Fig. 1). Rotavirus infection resulted in severe, watery diarrhea within 24 h, which lasted for 1 wk for all infected piglets, regardless of nutritional regimen (Fig. 1). By 8 d postinfection, diarrhea began to subside in both nourished and malnourished piglets. Diarrhea cleared completely by 10 d postinfection in infected, nourished piglets (Fig. 1). In contrast, diarrhea continued through d 16 postinfection for infected, malnourished pigs, although the physical appearance of excreted material changed from a liquid consistency to a more paste-like, but easy flowing consistency (Fig. 1).

Corresponding to diarrhea observations, rotavirus infection resulted in weight loss in both nourished and malnourished, infected piglets during the first 48 h postinfection (Zijlstra et al. 1997). Nourished, infected piglets began to regain body weight by 5 d postinfection, with rate of body weight gain similar to that of noninfected piglets at 11 d postinfection (Zijlstra et al. 1997). In contrast, after the initial 48-h period of weight loss, body weights of infected, malnourished piglets remained essentially static throughout the postinfection period.

**Intestinal MHC class I and II RNA expression.** At 2 d postinfection, the level of MHC class I RNA expression doubled in the distal jejunum of rotavirus-infected, nourished piglets relative to noninfected piglets (Fig. 2;  $P < 0.01$ ). A similar increase in intestinal MHC class I RNA expression was observed for rotavirus-infected, malnourished piglets (Fig. 2;  $P < 0.1$  vs. infected, nourished). At 9 d postinfection, MHC

<sup>4</sup> Abbreviations used: EIA, enzyme immunoassay; MHC, major histocompatibility complex; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>.



**FIGURE 1** Daily diarrhea scores for 1) noninfected, 2) infected, nourished and 3) infected, malnourished piglets during the 16 d after rotavirus infection at 2 d of age (0, no diarrhea; 1, stiff flowing feces; 2, easy flowing feces; 3, severe, watery diarrhea). Values are least-square means ( $n = 4-5$ ); pooled SEM per day is symbolized as a single error bar; +, infected, malnourished differs from infected, nourished,  $P < 0.05$ ; ++, infected, malnourished differs from infected, nourished,  $P < 0.01$ ; +++, infected, malnourished differs from infected, nourished,  $P < 0.001$ ; \*\*\*, noninfected differs from infected, nourished,  $P < 0.001$ . Reprinted from Zijlstra et al. (1997) with permission of the American Society for Nutritional Sciences.

class I RNA expression increased further for both nutritional regimens. The level of MHC class I RNA expression in the jejunum of infected, nourished piglets was three times that observed for noninfected pigs (Fig. 2;  $P < 0.001$ ). Among rotavirus-infected piglets on d 9, malnutrition resulted in an additional 26% increase in intestinal MHC class I RNA expression (Fig. 2;  $P < 0.01$ ). At 16 d postinfection, jejunal MHC class I RNA expression was not different ( $P > 0.1$ ) among treatment groups; however the level of expression was numerically greater in infected, malnourished than in infected, nourished piglets (Fig. 2).

Intestinal MHC class II RNA expression was generally affected less than MHC class I by both rotavirus infection and superimposed malnutrition (Fig. 2). Rotavirus infection doubled distal jejunal MHC class II RNA expression 2 d postinfection in nourished piglets ( $P < 0.05$ ). In contrast, malnutrition prevented the apparent normal increase in intestinal MHC class II expression in response to rotavirus 2 d postinfection. At 9 d postinfection, a comparable level of constitutive MHC class II RNA expression was observed for the three treatment groups ( $P > 0.10$ ). Similarly, significant differences were not observed for intestinal MHC class II among treatments on d 16 postinfection; however, constitutive MHC class II expression was numerically lower for infected, malnourished than for infected, nourished piglets (Fig. 2).

To gain additional perspective on the activation of MHC class I vs. class II gene expression in response to both rotavirus and superimposed malnutrition, the ratios of intestinal expression of MHC class I to class II were calculated. In response to rotavirus, the MHC class I/II expression ratio increased on d 2 and 9 postinfection for both nutritional regimens. The intestinal MHC class I/II ratios did not differ between noninfected and infected, nourished animals on d 16 postinfection. Because the level of MHC class I expression generally increased

in response to malnutrition and the level of MHC class II was generally suppressed by malnutrition, the MHC class I/II ratio was numerically greater for infected, malnourished piglets relative to infected, nourished piglets throughout the study.

**Intestinal  $CD4^+$  and  $CD8^+$  T lymphocytes.** The number of jejunal  $CD4^+$  T lymphocytes in infected, nourished piglets was more than twice that of noninfected piglets on d 2, three times greater on d 9 ( $P < 0.05$ ), and approximately doubled on d 16 (Fig. 3). Generally, malnutrition did not affect the temporal pattern of rotavirus-induced  $CD4^+$  T-lymphocyte expansion (Fig. 3).

Jejunal  $CD8^+$  T-lymphocyte numbers doubled in response to rotavirus on d 2 ( $P < 0.05$ ; Fig. 3). A numerical ( $P > 0.1$ ) increase in jejunal  $CD8^+$  T-lymphocyte numbers was also observed 9 and 16 d postinfection (Fig. 3). Similar to observations for  $CD4^+$  T lymphocytes, malnutrition generally did not affect the number of  $CD8^+$  T cells in the jejunum of rotavirus-infected piglets (Fig. 3). The ratio of  $CD4^+$  to  $CD8^+$  T lymphocytes was similar on each of the days examined postinfection.

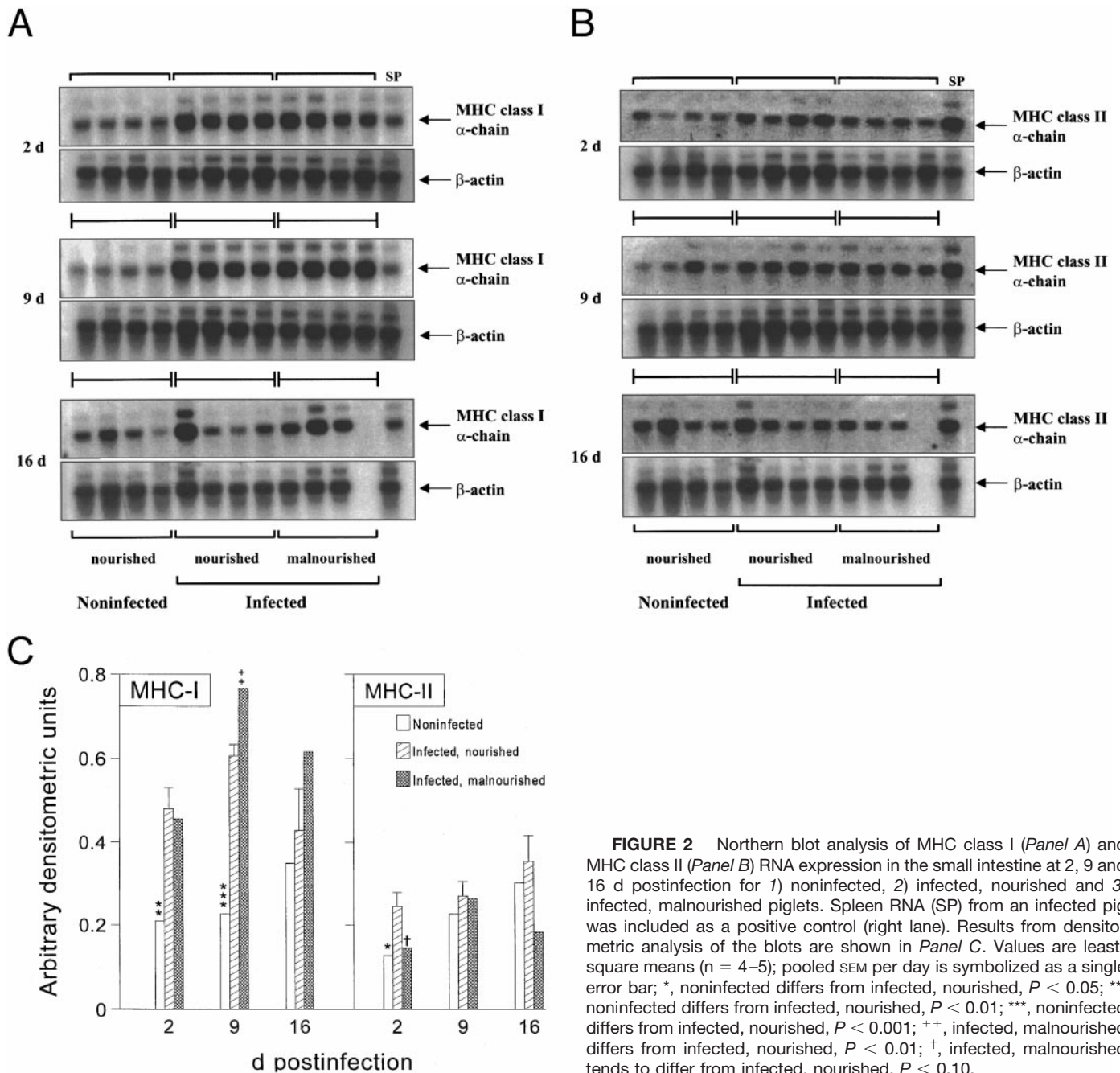
**Intestinal  $PGE_2$ .** Rotavirus infection increased  $PGE_2$  concentrations 10-fold in the mid-jejunum of nourished piglets by 2 d postinfection ( $P < 0.05$ ; Fig. 4). A sixfold increase in jejunal  $PGE_2$  concentrations was observed for infected, malnourished piglets (Fig. 4). At 9 d postinfection,  $PGE_2$  concentrations in infected, nourished piglets were not different from noninfected animals and thus had returned to baseline. In contrast, intestinal  $PGE_2$  concentrations in infected, malnourished piglets remained elevated relative to infected, nourished piglets ( $P < 0.05$ ; Fig. 4). Intestinal  $PGE_2$  concentrations tended to be elevated in infected, nourished piglets relative to noninfected piglets again on d 16 postinfection ( $P < 0.10$ ; Fig. 4). Intestinal  $PGE_2$  concentrations remained elevated for infected, malnourished piglets on d 16, with concentrations similar to those of the infected, nourished group (Fig. 4).

**Correlation analysis.** Diarrhea observations were positively related to  $CD4^+$  T-lymphocyte numbers ( $r = 0.31$ ;  $P < 0.10$ ),  $CD8^+$  T-lymphocyte numbers ( $r = 0.46$ ;  $P < 0.01$ ), MHC class I expression ( $r = 0.53$ ;  $P < 0.01$ ) and  $PGE_2$  concentrations ( $r = 0.53$ ;  $P < 0.001$ ) (Table 1). The number of jejunal  $CD4^+$  T lymphocytes was positively correlated with  $CD8^+$  T-lymphocyte numbers ( $r = 0.42$ ;  $P < 0.01$ ), MHC class I expression ( $r = 0.32$ ;  $P < 0.10$ ) and  $PGE_2$  concentrations ( $r = 0.33$ ;  $P < 0.05$ ). The number of jejunal  $CD8^+$  T lymphocytes was positively correlated with MHC class I expression ( $r = 0.58$ ;  $P < 0.001$ ) and  $PGE_2$  concentrations ( $r = 0.39$ ;  $P < 0.05$ ). Jejunal MHC class I expression was positively correlated with MHC class II expression ( $r = 0.33$ ;  $P < 0.10$ ) and  $PGE_2$  concentrations ( $r = 0.48$ ;  $P < 0.01$ ).

## DISCUSSION

Rotavirus infection of the small intestine of neonatal piglets rapidly activated local cellular and molecular components known to mediate both inflammation and immunity. Moreover, the additional insult of malnutrition prolonged inflammatory responses to rotavirus and thereby intensified and temporally extended the diarrheal episode. Convalescent dietary management of infants after acute rotavirus infection has been debated for many years (AAP 1996). This study suggests that the time-honored treatment method of "bowel rest" after rotavirus infection may delay mucosal recovery.

Rotavirus infection is characterized by viral replication in small intestinal enterocytes with subsequent cell lysis and attendant villous blunting, depressed levels of mucosal disac-



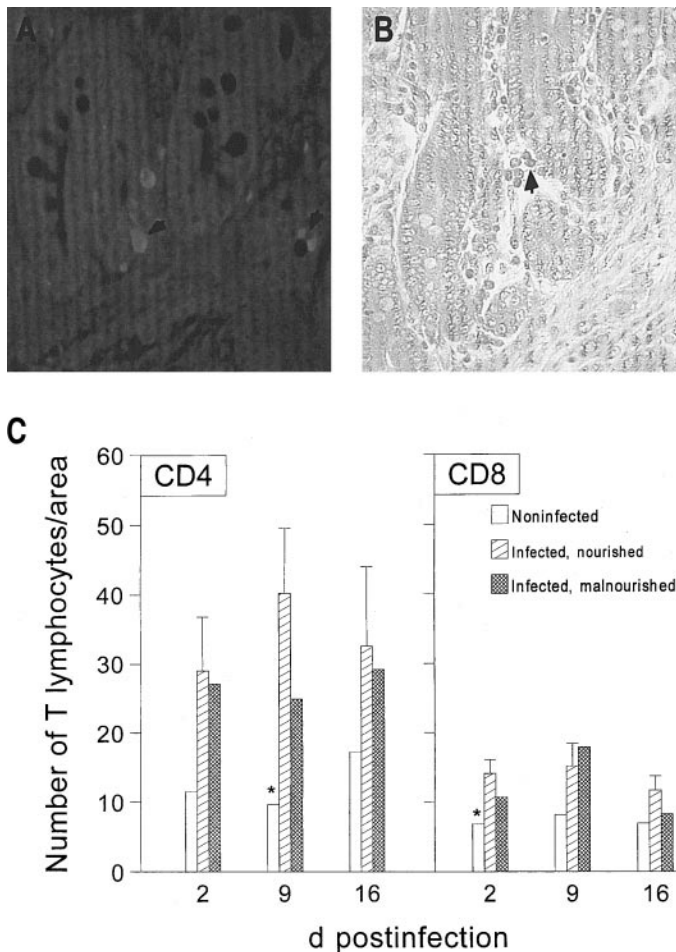
**FIGURE 2** Northern blot analysis of MHC class I (Panel A) and MHC class II (Panel B) RNA expression in the small intestine at 2, 9 and 16 d postinfection for 1) noninfected, 2) infected, nourished and 3) infected, malnourished piglets. Spleen RNA (SP) from an infected pig was included as a positive control (right lane). Results from densitometric analysis of the blots are shown in Panel C. Values are least-square means ( $n = 4-5$ ); pooled SEM per day is symbolized as a single error bar; \*, noninfected differs from infected, nourished,  $P < 0.05$ ; \*\*, noninfected differs from infected, nourished,  $P < 0.01$ ; \*\*\*, noninfected differs from infected, nourished,  $P < 0.001$ ; +, infected, malnourished differs from infected, nourished,  $P < 0.01$ ; †, infected, malnourished tends to differ from infected, nourished,  $P < 0.10$ .

charidases, watery diarrhea and dehydration (Bishop et al. 1973, Estes 1990, Theil et al. 1978). Because of the reduced enzymatic and absorptive capacity in the small intestine, it is generally accepted that rotavirus elicits a malabsorptive-type of diarrhea (Graham 1984, Rhoads et al. 1991). On the basis of clear evidence of local T-lymphocyte expansion, enhanced intestinal MHC class I and class II gene expression and elevated tissue concentrations of  $PGE_2$ , we propose that intestinal inflammatory responses to rotavirus may contribute to a secretory-type of diarrhea.

In a previous report on this piglet model, we demonstrated that the onset of rotaviral diarrhea coincided with villous destruction and reduction of mucosal disaccharidase activities (Zijlstra et al. 1997). Those observations agree with reports on other experimental models of rotavirus infection and are consistent with the possibility that malabsorption contributes to

rotaviral diarrhea (Zijlstra et al. 1997). However, a clear distinction between intestinal damage and diarrhea was established from the observation that diarrhea subsided by 9 d postinfection in nourished but not in malnourished piglets, whereas the degree of intestinal structure damage was comparable between these nutritional groups at that time postinfection (Zijlstra et al. 1997). Thus, it is unlikely that malabsorption resulting from epithelial damage is the sole explanation for rotaviral diarrhea.

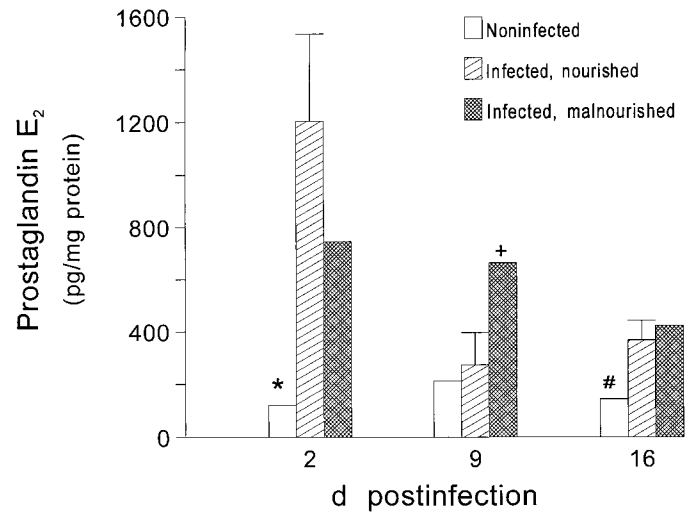
Results from other model systems and limited observations from human studies also invoke reconsideration of the pathophysiology of rotavirus-induced diarrhea. At least three other reports on a piglet model demonstrate rotavirus-induced diarrhea before extensive damage to the intestinal epithelium (McAdaragh et al. 1980, Theil et al. 1978). For example, piglets inoculated with porcine rotavirus developed watery



**FIGURE 3** Representative photomicrographs of piglet jejunal sections showing (arrows) CD4<sup>+</sup> (Panel A, X600) or CD8<sup>+</sup> (Panel B, X400) T lymphocytes. CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes were detected by immunofluorescence or immunocytochemistry as described in the text. Shown in Panel C are numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes per jejunal cross-sectional area for 1) noninfected, 2) infected, nourished and 3) infected, malnourished piglets at 2, 9 and 16 d postinfection. Values are least-square means (*n* = 4–5); pooled SEM per day is symbolized as a single error bar; \*, noninfected differs from infected, nourished, *P* < 0.05.

diarrhea 8 h after infection, whereas minor damage to jejunal segments was not observed until 48 h postinfection (Vellenga et al. 1992). Similarly, for a mouse model, there was no correlation between the number of rotavirus infected cells and the severity of diarrhea (Bass and Greenberg 1995). Studies with a heterologous mouse model demonstrate that chemically inactivated rhesus monkey rotavirus induces diarrhea in the absence of epithelial attachment, cellular entry or viral replication (Shaw et al. 1995). It has also been reported that diarrhea preceded obvious cell damage in human infants infected with rotavirus (Bass and Greenberg 1995). Among intestinal biopsy specimens from 40 rotavirus-infected infants, only 5% exhibited histologic evidence of damage (Kohler et al. 1990).

New concepts of rotaviral pathogenesis are evoked most convincingly from the recent identification of a rotaviral enterotoxin (Ball et al. 1996, Dong et al. 1997, Glass et al. 1996). While making an antiserum to the nonstructural rotaviral glycoprotein, NSP4, Estes and co-workers fortuitously discovered that intraperitoneal delivery of purified NSP4 induced



**FIGURE 4** Jejunal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) concentrations at 2, 9 and 16 d postinfection for 1) noninfected, 2) infected, nourished and 3) infected, malnourished piglets. Values are least-square means (*n* = 4–5); pooled SEM per day is symbolized as a single error bar; \*, noninfected differs from infected, nourished, *P* < 0.05; #, noninfected tends to differ from infected, nourished, *P* < 0.10; +, infected, malnourished differs from infected, nourished, *P* < 0.05.

diarrhea in a mouse model (Ball et al. 1996). Subsequent studies from this group with several model systems clearly demonstrate that NSP4 alters Ca<sup>2+</sup> homeostasis in host cells through receptor-mediated phospholipase C activation and inositol 1,4,5-triphosphate production (Dong et al. 1997). That finding is consistent with numerous other examples of the mediation of infectious diarrhea through altered intracellular Ca<sup>2+</sup> homeostasis, leading to Ca<sup>2+</sup>-dependent fluid secretion across the mucosa toward the lumen (Argenzio 1996). Local prostaglandins can also serve as mediators of intestinal ion imbalances, resulting in epithelial chloride secretion (Kandil et al. 1994). The possible contributions of PGE<sub>2</sub> to rotaviral and specifically NSP4-induced diarrhea should be defined, considering the present evidence that malnutrition exacerbates PGE<sub>2</sub> responses to rotavirus, together with clear evidence that tissue PGE<sub>2</sub> concentrations can be altered

**TABLE 1**

*Spearman correlation coefficients between diarrhea observations and small intestinal variables indicative of an inflammatory response<sup>1</sup>*

	Diarrhea	CD4	CD8	MHCI	MHCII	PGE <sub>2</sub>
Diarrhea	x					
CD4	0.31†	x				
CD8	0.46**	0.42**	x			
MHCI	0.53**	0.32†	0.58***	x		
MHCII	-0.06	-0.07	0.10	0.33†	x	
PGE <sub>2</sub>	0.53***	0.33*	0.39*	0.48**	0.15	x

<sup>1</sup> Correlation coefficients (*r*) were calculated by using observations from the 16-d experimental period. CD4, jejunal CD4<sup>+</sup> T-lymphocyte numbers; CD8, jejunal CD8<sup>+</sup> T-lymphocyte numbers; MHC I, major histocompatibility complex (MHC) class I RNA expression; MHC II, MHC class II RNA expression; PGE<sub>2</sub>, jejunal prostaglandin E<sub>2</sub> concentrations. Superscripts in body of the table indicate level of significance: \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001; †, *P* < 0.10.

through manipulation of diet fatty acid composition (Fritsche et al. 1993).

Prolonged diarrhea in infected, malnourished piglets was associated with more intense and prolonged expression of local mediators or markers of intestinal inflammation. Relative to infected, nourished piglets, intestinal PGE<sub>2</sub> concentrations were greater and remained elevated longer in malnourished piglets also infected with rotavirus. Similarly, patterns of intestinal MHC class I gene expression, an acutely sensitive barometer of local inflammation, were positively and significantly correlated with the pattern of PGE<sub>2</sub> expression and with prolonged diarrhea. Those results are consistent with substantial evidence that undernutrition typically intensifies viral enteritis and results in greater morbidity and mortality from infections (Cunningham-Rundles 1994). The identification of specific indices of rotavirus infection that are responsive to malnutrition enables further studies to determine how individual macronutrients contribute at the intestinal level to host clearance of rotavirus. That information may facilitate the design of rational nutritional therapies to enhance recovery from rotavirus infection, or to maximize responses to rotavirus immunization protocols in geographic regions subject to malnutrition.

#### LITERATURE CITED

- Abbas, A. K., Lichtman, A. H. & Pober, J. S. (1991) Cellular and Molecular Immunology. W. B. Saunders Company, Philadelphia, PA.
- American Academy of Pediatrics (1996) Provisional Committee on Quality Improvement, Subcommittee on Acute Gastroenteritis. Practice parameters: the management of acute gastroenteritis in young children. *Pediatrics* 97: 424-433.
- Argenzio, R. A. (1996) The pig as a model for studying the pathobiology of intestinal transport in infectious enteric disease. In: *Advances in Swine in Biomedical Research* (Tumbleson, M. E. & Schook, L. B., eds.), pp. 45-58. Plenum Press, New York, NY.
- Ball, J. M., Tian, C.Q.Y., Morris, A. P. & Estes, M. K. (1996) Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. *Science* (Washington, DC) 272: 101-104.
- Bass, D. M. & Greenberg, H. B. (1995) Group A rotaviruses. In: *Infections of the Gastrointestinal Tract* (Blaser, M. J., Smith, P. D., Ravdin, J. I., Greenberg, H. B. & Guerrant, R. L., eds.), pp. 967-982. Raven Press, New York, NY.
- Bishop, R. F., Davidson, G. P., Holmes, I. H. & Ruck, B. J. (1973) Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. *Lancet* II: 1281-1283.
- Bruce, M. G., Campbell, I. C., van Pinxteren, L. & Snodgrass, D. R. (1995) Intestinal cellular immunity after primary rotavirus infection. *J. Comp. Pathol.* 113: 115-164.
- Butzner, J. D., Butler, D. G., Miniats, O. P. & Hamilton, J. R. (1985) Impact of chronic protein-calorie malnutrition on small intestinal repair after acute viral enteritis: a study in gnotobiotic piglets. *Pediatr. Res.* 19: 476-481.
- Carson, F. L. (1990) *Histotechnology. A Self-Instructional Text*. ASCP Press, Chicago, IL.
- Chomczynski, P. & Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156-159.
- Cunningham-Rundles, S. (1994) Malnutrition and gut immune function. *Curr. Opin. Gastroenterol.* 10: 664-670.
- Dong, Y. J., Zeng, C.Q.Y., Ball, J. M., Estes, M. K. & Morris, A. P. (1997) The rotavirus enterotoxin NSP4 mobilizes intracellular calcium in human intestinal cells by stimulating phospholipase C mediated inositol 1, 4,5 triphosphate production. *Proc. Natl. Acad. Sci. U.S.A.* 94: 3960-3965.
- Estes, M. K. (1990) Rotaviruses and their replication. In: *Fields Virology* (Fields, B. N. & Knipe, D. M., eds), pp. 1329-1352. Raven Press, New York, NY.
- Fritsche, K. L., Alexander, D. W., Cassity, N. A. & Huang, S.-C. (1993) Maternally supplied fish oil alters piglet immune cell fatty acid profile and eicosanoid production. *Lipids* 28: 677-682.
- Glass, R. I., Gentsch, J. R. & Ivanoff, B. (1996) *New lessons for rotavirus vaccines*. Science (Washington, DC) 272: 46-48.
- Graham, D. Y., Sackman, J. W. & Estes, M. K. (1984) Pathogenesis of rotavirus-induced diarrhea. *Dig. Dis. Sci.* 29: 1028-1035.
- Hartree, E. F. (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.* 48: 422-427.
- Kandil, H. M., Berschneider, H. M. & Argenzio, R. A. (1994) Tumor necrosis factor a changes porcine intestinal ion transport through a paracrine mechanism involving prostaglandins. *Gut* 35: 934-940.
- Kapikian, A. Z. & Chanock, R. M. (1996) Rotaviruses. In: *Fields Virology* (Fields, B. N., Knipe, D. M. & Howley, P. M., eds.), pp. 1657-1708. Lippincott-Raven, Philadelphia, PA.
- Kohler, V. T., Erben, U., Wiedersberg, H. & Bannert, N. (1990) Histologische Befunde der dunndarmschleimhaut bei rotavirus-infektionen im sauglings- und kleinkindalter. *Kinderarztl. Prax.* 58: 323-327.
- Lieberman, J. M. (1994) Rotavirus and other viral causes of gastroenteritis. *Pediatr. Ann.* 23: 529-535.
- Matsui, S. M. & Angel, J. (1997) Viral infections of the gastrointestinal tract. *Curr. Opin. Gastroenterol.* 13: 57-63.
- McAdaragh, J. P., Bergeland, M. E., Meyer, R. C., Johnshoy, M. W., Stotz, I. J., Benfield, D. A. & Hammer, R. (1980) Pathogenesis of rotaviral enteritis in gnotobiotic pigs: a microscopic study. *Am. J. Vet. Res.* 41: 1572-1581.
- McCleod, R. E., Jr., Lentz, M. E. & Vieth, R. (1990) A simple technique to feed newborn piglets. *J. Pediatr. Gastroenterol. Nutr.* 10: 107-110.
- National Research Council (1985) *Guide for the Care and Use of Laboratory Animals*. Publication no. 85-23 (rev.), National Institutes of Health, Bethesda, MD.
- Rhoads, J. M., Keku, E. O., Quinn, J., Woosely, J. & Lecce, J. G. (1991) L-Glutamine stimulates jejunal sodium and chloride absorption in pig rotavirus enteritis. *Gastroenterology* 100: 683-691.
- Saalmuller, A. (1996) Characterization of swine leukocyte differentiation antigens. *Immunol. Today* 17: 352-354.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Plainview, NY.
- SAS Institute Inc. (1985) *SAS Users's Guide: Statistics*. SAS Institute, Cary, NC.
- Shaw, R. D., Hempson, S. J. & Mackow, E. R. (1995) Rotavirus diarrhea is caused by nonreplicating viral particles. *J. Virol.* 69: 5946-5950.
- Steel, R. G. D & Torrie, J. H. (1980) *Principles and Procedures of Statistics*. McGraw-Hill, New York, NY.
- Theil, K. W., Bohl, E. H., Cross, R. F., Kohler, E. M. & Agnes, A. G. (1978) Pathogenesis of porcine rotaviral infection in experimentally inoculated gnotobiotic pigs. *Am. J. Vet. Res.* 39: 213-220.
- Vellenga, L., Egberts, H.J.A., Wensing, T., Van Dijk, J. E., Mouwen, J.M.V.M. & Breukink, H. J. (1992) Intestinal permeability in pigs during rotavirus infection. *Am. J. Vet. Res.* 53: 1180-1183.
- Zijlstra, R. T., Donovan, S. M., Odle, J., Gelberg, H. B., Petschow, B. W. & Gaskins, H. R. (1997) Protein-energy malnutrition delays small-intestinal recovery in neonatal pigs infected with rotavirus. *J. Nutr.* 127: 1118-1127.