41. Analysis of these spectra can be complicated by proton transfer between added phenol and the nitrophenolate, which depletes the total amount of nitrophenolate available for H bonding and causes spectral shift of the nitrophenolate beyond that from H bonding. To avoid this problem, we used phenols whose pK_a values were considerably higher than those of the nitrophenolate, the proton acceptor. However, at high phenol concentrations, phenol can self-associate and lose a proton to give the phenol•phenolate complex, thereby allowing protonation of the nitrophenolate. For this reason, K_f values were only obtained in the region $\Delta pK_a^{water} > 2$, where the proton transfers occur at much higher phenol concentrations than are required for complex formation with the nitrophenolate. Control experiments with sterically hindered phenols (2,6-di-*tert*-butylphenol and 2,6-di-*tert*-butyl-4-nitrophenol) did not produce the spectral shift described in (*23*), even at concentrations up to 1 M. This finding ruled out complications from general solvent effects by addition of phenols to the phenolate solution and suggested that H bonding is responsible for complexation. Addition of increasing concentrations of a nonconjugate phenol to a solution containing a preformed homoconjugate nitrophenol-nitrophenolate complex leads to loss of the homoconjugate complex and formation of the heteroconjugate complex, as judged by modest spectral shifts. The concentration dependence of these changes was consistent with the relative stability of the H-bonded species obtained from the direct measurements described in (*23*).
42. The pK_a scales are substantially expanded in low-dielectric solvents. For example, for benzoic acids and phenols, ΔpK_a's of 1 in water correspond to ΔpK_a's of ~2.4 in DMSO (*40*). For THF, the increase of the ΔpK_a scales is expected to be somewhat greater (*43*).
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44. Isotope fractionation factors (the D/H isotopic ratio for the H-bonded proton relative to the ratio for the water protons) were measured by ^1H nuclear magnetic resonance (NMR) spectroscopy. A small amount (~0.2 to 0.3%) of a D_2O/H_2O mixture was added to 0.2 M PA^- solutions in DMSO. The deuterons in the added isotope-containing water were allowed to exchange and reach equilibrium with the COOH protons. The slowly exchanging water and PA^- proton peaks were integrated and normalized to that of the nonexchangeable benzylic protons. The ratio of the two normalized peak areas, in comparison with that in a control sample where the same amount of H_2O was added, yielded the fractionation factors [M. Saunders, S. Saunders, C. Johnson, *J. Am. Chem. Soc.* **106**, 3098 (1984)]. To improve the accuracy of data, we performed multiple measurements using different D_2O/H_2O ratios (1/9 → 9/1). The small amount of water added did not affect the observed downfield chemical shift of the H-bonded proton. Values are reported as mean ± 2 SD.
45. ΔpK_a values are defined as the difference between the intrinsic pK_a's (pK_a^{int}) (*18*) of the H-bond donor and acceptor. For X-PAs, this reduces to $\Delta pK_a = \rho(|\sigma_{X,para} - \sigma_{X,meta}|)$.
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Age-Dependent Diarrhea Induced by a Rotaviral Nonstructural Glycoprotein

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The rotavirus nonstructural glycoprotein NSP4 is an intracellular receptor that mediates the acquisition of a transient membrane envelope as subviral particles bud into the endoplasmic reticulum. NSP4 also causes an increase in intracellular calcium in insect cells. Purified NSP4 or a peptide corresponding to NSP4 residues 114 to 135 induced diarrhea in young (6 to 10 days old) CD1 mice. This disease response was age-dependent, dose-dependent, and specific. Electrophysiologic data from intestinal mucosa showed that the NSP4 114-135 peptide potentiates chloride secretion by a calcium-dependent signaling pathway. Diarrhea is induced when NSP4, acting as a viral enterotoxin, triggers a signal transduction pathway.

Rotaviruses are the leading cause of severe, life-threatening viral gastroenteritis in infants and animals (*1*) and are associated with sporadic outbreaks of diarrhea in elderly (*2*) and immunocompromised patients (*3*). These viruses have a limited tissue tropism, with infection primarily being restricted to cells of the small intestine (*4*). Moreover, the outcome of infection is age-related; although rotaviruses may infect individuals and animals of all ages, symptomatic infection (that is, diarrhea) generally occurs in the young (6 months to 2 years in children and up to 14 days in mice).

Despite the prevalence of rotavirus infections and extensive studies in animal models, rotavirus pathogenesis remains poorly understood. Proposed pathophysiologic mechanisms by which rotaviruses induce diarrhea after virus replication include malabsorption secondary to the destruction of enterocytes (*5*), alterations in transepithelial fluid balance (*6*), and local villus ischemia leading to vascular damage and diarrhea (*7*). These mechanisms do not explain cases of rotavirus-induced diarrhea observed before, or in the absence of, histopathologic changes (*4*, *8*).

While making an antiserum to a nonstructural glycoprotein, NSP4, we made the fortuitous discovery that intraperitoneal (ip) delivery of purified NSP4 induces diarrhea in a mouse model (Fig. 1). Whether administration was ip or intraileal (il), diarrhea was observed within 1 to 4 hours after inoculation. It typically continued for up to 8 hours, but occasionally persisted for 24 hours (*9*). Purified NSP4 (0.1 to 5 nmol) was administered by the ip route to CD1 mouse pups 6 to 7 and 8 to 9 days old. No diarrhea was induced with 0.1 nmol of protein in the 8- to 9-day-old mice, whereas 60% of the 6- to 7-day-old pups had diarrhea. Intraperitoneal administration of 1 nmol of NSP4 resulted in diarrhea in 100% of the 6- to 7-day-old mice, and in 60% of the older animals. Intraileal delivery of 0.5 nmol of protein induced diarrhea in 100% of the young (8 to 9 days) mice, whereas no diarrhea was observed in the 17- to 18-day-old pups. Thus, the response to NSP4 was age- and dose-dependent in CD1 pups. In addition, the induction of diarrhea by NSP4 was specific, as administration of the same concentration of purified rotavirus VP6 or the same volume of buffer had no effect (Fig. 1). Intramuscular (im) inoculation of 1 nmol of purified NSP4 produced no ill effects (*10*).

We next tested the effect of a synthetic peptide corresponding to NSP4 residues 114 to 135 (NSP4 114-135) delivered by the ip or il route to mice of different ages (Fig. 2) (*9*, *11*). Diarrhea was observed in the 6- to 7-day-old mice within 1 to 3 hours after inoculation [60% (ip), 71% (il)], whereas diarrhea was not seen in animals older than 11 days, even when a two- or fourfold greater dose of peptide was administered ip (Fig. 2). Intraileal delivery of the peptide to pups 11 to 12 or 17 to 18 days old caused diarrhea in 25 or 0% of the animals, respectively. These data indicate an increased sensitivity to the peptide when delivered directly into the lumen of the intestine, and reveal an age-dependent disease response to the NSP4 114-135 peptide that is similar to that seen in natural rotavirus infections or after inoculation of purified NSP4. Regardless of the dose or the route of administration of the peptide, the kinetics of diarrhea induction were similar to those observed with purified NSP4. However, as compared with NSP4, the effective dose of NSP4 114-135 peptide was considerably

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higher. This may not be surprising, as the peptide may represent only a portion of the active domain or may not fold into the native conformation.

The NSP4 114-135 peptide is predicted to fold as an amphipathic helix (*11*), is localized in the cytoplasmic domain of NSP4, and mobilizes intracellular calcium in eukaryotic cells (*12*). Specificity of the diarrhea induction by the NSP4 114-135 peptide was confirmed by the administration of a panel of control peptides to young mouse pups (*13*). A mutated NSP4 114-135 peptide in which the tyrosine at position 131 was replaced with a lysine residue (mNSP4 131K) did not induce diarrhea (0 out of 11 pups), which indicates the importance of this tyrosine residue in the induction of diarrhea. A longer peptide, NSP4 90-123, which overlaps the 114-135 peptide by nine residues, induced diarrhea in only 20% (2 out of 10) of the mice tested. Thus, the response in CD1 mice appears to be directed to a region of NSP4 that is inclusive of residues 114 to 135. The reduced response to the longer NSP4 90-123 peptide may be due to the absence of amino acids 124 to 135. A peptide corresponding to the COOH-terminus of the capsid protein of another gastroenteritis virus, Norwalk virus (NV 464-483), was selected as a control peptide because it has a calculated amphipathic score (AS) similar to that of NSP4 114-135 (AS = 41) and a centrally located tyrosine residue (*13*); NV 464-483 did not induce diarrhea (0 out of 10) in any mouse pups.

To determine if the response to the NSP4 114-135 peptide was dose-dependent, 0.1 to 500 nmol of peptide was administered ip to 84 CD1 pups [6 to 7 days old (*11*)]. The disease response to the NSP4 114-135 peptide was dose-dependent ($\chi^2_{trend} = 9.98$, $P = 0.0016$), with a DD_{50} (50% diarrheal dose) of 79 nmol (*14*).

We also evaluated whether antiserum made to the NSP4 114-135 peptide was able to block the induction of diarrhea (*15*). In the absence of antibody, ip delivery of 50 to 100 nmol of NSP4 114-135 peptide induced diarrhea in 67% of the mice. Intraperitoneal inoculation of NSP4 114-135 peptide–specific antiserum 5 min before ip delivery of peptide (50 to 100 nmol) resulted in a 90% reduction of disease. Intraperitoneal administration of normal rabbit serum before peptide delivery did not block the diarrhea.

The potential of NSP4 antibodies to protect against virus-induced disease was tested by challenging pups born to dams that were immunized with the NSP4 114-135 peptide or a control peptide with a high dose of infectious simian agent 11 (SA11) virus (*16*). Diarrheal disease in pups born to dams immunized with the NSP4 114-135 peptide was significantly (Fisher's exact test) reduced in severity and duration, and in the number of pups with diarrhea. In another experiment, young mouse pups were infected with SA11 virus, and NSP4 or control antiserum was orally administered every 4 to 6 hours for 60 hours. The pups given NSP4-specific antibody had significantly reduced diarrheal disease as compared with animals given control antisera (*16*). These data show the potential of NSP4 antibodies to block rotavirus-induced disease.

The above data suggest that NSP4 causes diarrhea by acting as an enterotoxin. Because enterotoxins stimulate net secretion in ligated intestinal segments without histological alterations, or stimulate secretion in Ussing chambers, the effects of the peptide and of known Ca^{2+}- and cyclic adenosine monophosphate (cAMP)–elevating agonists were tested on unstripped mouse intestinal mucosal sheets in modified Ussing chambers (*17*). Addition of forskolin (FSK, a cAMP agonist) and carbachol (Cch, a cholinergic agonist that mobilizes Ca^{2+}) to normal mouse ileal mucosa resulted in measurable elevations in Cl^- secretory current (I_{sc}, Table 1). Addition of either 5 μM (200 nmol) of NSP4 114-135 peptide (cross-linked to itself for enhanced stability) or 5 μM of Cch to mucosal sheets of CD1 mice 19 to 22 days old induced small (3 or 9 $\mu A/cm^2$, respectively) and transient (1- to 2-min) increases in I_{sc}. When the mucosal sheets were exposed to 5 μM of the cAMP-mobilizing agonist FSK, larger increases in I_{sc} (44 $\mu A/cm^2$) were elicited that reached sustained levels within 2 to 3 min. After FSK pretreatment, challenge of the mucosa with either peptide or Cch resulted in much larger increases in mucosal I_{sc} (64 or 63 $\mu A/cm^2$, respectively); both the peptide and Cch potentiated the response to FSK. All of the responses to agonists were sensitive to bumetamide, and treatment of ileal mucosal sheets with cross-linked control NSP4 2-22 peptide did not induce a response. Addition of Cch to 19- to 22-day-old mouse mucosal sheets that had been pretreated with peptide alone or with peptide in combination with FSK had minimal or no additional effect on I_{sc}. This subsequent loss of sensitivity to the Ca^{2+}-elevating agonist (Cch) after peptide pretreatment suggests that the NSP4 peptide increases I_{sc} through changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$). Addition of Cch to mucosa from a 35-day-old mouse again elicited a small (14 $\mu A/cm^2$) and transient (1- to 2-min) response that potentiated the effect of FSK (64 $\mu A/cm^2$), whereas there was no or a minimal increase in I_{sc} when the NSP4 114-135 peptide was added alone or with FSK to the 35-day-old mouse mucosal sheets (Table 1).

The electrophysiological responses from 19-day-old mice initially seem paradoxical when compared with the biological data, because measurable secretion was not observed as diarrhea in animals of this age. Diarrhea likely was not seen in these older animals because of fluid reabsorption by the colon. This hypothesis was tested by il administration of 200 nmol of NSP4 114-135 or of control peptide to 19-day-old pups. At 4 hours after inoculation, the mice were killed and the intestines were tied off, removed, and

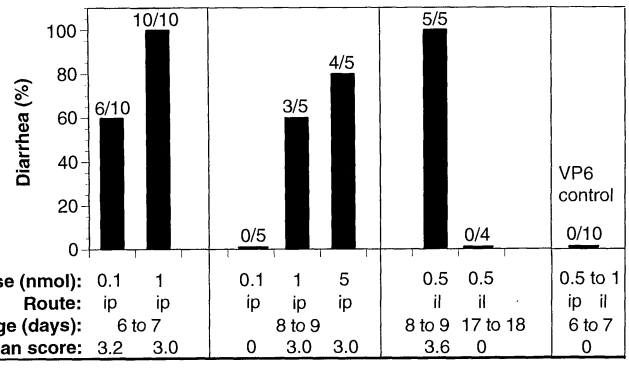

Fig. 1. Rotavirus NSP4 protein induces diarrhea in CD1 mice. NSP4 was purified from recombinant baculovirus pAC461-G10–infected *Spodoptera frugiperda* (Sf9) cells expressing gene 10 by fast protein liquid chromatography on a QMA anion exchange column as previously described (*12*) and with an additional affinity purification step on a column containing antibodies to NSP4. Different NSP4 preparations of ≥70% and 90% purity gave the same biologic results. The protein was sterile based on results of bacteriologic culturing in LB broth incubated at 37°C for 1 week, and lacked endotoxin (*26*). VP6 was purified to >95% purity from recombinant baculovirus pAc461–SA11-G6 infected Sf9 cells by gradient centrifugation as previously described (*27*). Both proteins were diluted in sterile phosphate-buffered saline (PBS) to a final volume of 50 μl per dose, regardless of the route of administration. For the surgical introduction of protein into the ileum, animals were anesthetized with isofurane (Anaquest), a small incision was made below the stomach, the protein inoculum was injected into the upper ileum, and the incision was sealed with polypropylene sutures (PROLENE 6-0). The pups were isolated, kept warm, and closely monitored for a minimum of 2 hours. Animals with diarrhea also displayed lethargy and coldness to the touch. The dose and route of the proteins, age of the animals, and mean diarrhea score (mean score) are indicated on the bottom of the graph. Above each column is the number of responders (mice with diarrheal disease) over the total number of animals tested.

Table 1. Electrophysiological analyses of ileal mucosa of CD1 mice. The change in short-circuit current (ΔI_{sc}) was calculated by subtracting the stimulated I_{sc} measurement from the I_{sc} measured immediately before the addition of agonist. All agonist-stimulated values were significantly different ($P < 0.001$, unpaired t test).

Agonist treatment*	Age of mice	
	19 to 22 days [ΔI_{sc} (μA/cm^2)]	35 days [ΔI_{sc} (μA/cm^2)]
Forskolin (FSK) (5 μM)	44 ± 0.7 (n = 8)	41 ± 7 (n = 5)
Carbochol (Cch) (5 μM)	9 ± 2 (n = 8)	14 ± 4 (n = 5)
NSP4 114-135 peptide (5 μM)†	3 ± 0.2 (n = 4)	0.4 ± 0.4 (n = 4)‡
FSK (5 μM) + Cch (5 μM)	63 ± 10 (n = 5)	64 ± 9 (n = 6)
FSK (5 μM) + NSP4 114-135 peptide (5 μM)	64 ± 5 (n = 7)	43 ± 9 (n = 5)

*The mean resting conductance for the ileal mucosal sheets before agonist treatment was 10.4 ± 4.8 mS/cm^2 (n = 32) for the 19- to 22-day-old mice and 12.3 ± 3.8 mS/cm^2 (n = 25) for the 35-day-old mice. †NSP4 114-135 peptide is active when added to either surface of the mucosa. ‡For n = 3, there was no response with peptide; for n = 1, the response was 2 μA/cm^2.

weighed, and their length was measured. The pups given NSP4 114-135 peptide showed substantial fluid accumulation when compared with the control pups, although no diarrhea was seen in any animals.

We anticipate that younger mice would show a greater increase in I_{sc} than that seen in the 19-day-old mucosa. However, intestinal mucosa from younger mice (<19 days) could not be mounted efficiently into the Ussing chambers because of their small size; such experiments in very young mice will require the development of new methods to measure Cl^- secretion in vitro. Nonetheless, the NSP4 114-135 peptide did not augment secretion in 35-day-old mice, correlating the age dependence seen in vivo.

On the basis of our results, we propose a model in which two intestinal receptors are required for symptomatic rotavirus infection. One receptor binds rotavirus particles, resulting in virus entry and gene expression but not necessarily in disease, whereas the second receptor is NSP4-specific. NSP4 expressed in infected cells would be released into the lumen and would interact with the second receptor on adjacent cells. This interaction would trigger a signal transduction pathway, thereby increasing $[Ca^{2+}]_i$ levels and augmenting endogenous intestinal secretory pathways. NSP4 has been detected in diarrheal stools of rotavirus-infected mice at concentrations sufficient to cause diarrhea (*18*).

This model fits available data on rotavirus-induced diarrhea. In young mice, homologous and heterologous rotaviruses cause diarrheal disease. For example, in young mice infected with the simian virus SA11, infectious virus is not produced and histopathologic blunting of the villi is not observed, but diarrhea is induced (*19*). In other animals, diarrhea is seen before histologic changes (*8*). In our model, the intestines of young mice would possess a NSP4-specific receptor that decreases in number as the mouse ages, and interactions with this receptor would stimulate Cl^- secretion, resulting in the observed diarrheal disease. Adult mice are readily infected by murine rotaviruses in that virus can be isolated from fecal samples and virus replication can be demonstrated in intestinal cells (*20*). However, these older animals do not display diarrhea or other symptoms (*21*). Our model predicts that the concentration of NSP4 receptors is substantially reduced in adult animals, so that the colon can accommodate the increase in fluid secretion. The adult mouse can replicate and excrete virus but no disease is observed. Another possibility is that the adult mouse lacks an apical membrane Ca^{2+}-dependent Cl^- channel.

These results offer a new concept of rotavirus pathogenesis but do not exclude other mechanisms of diarrhea such as malabsorption due to villus blunting secondary to cell death. This latter effect caused by highly virulent viruses, alone or in combination with lowered immunity, may explain the rare cases or outbreaks of rotavirus-induced diarrhea in adults. Our results showing that NSP4 induces diarrhea offer a mechanistic explanation for why the gene encoding NSP4 is a virulence gene (*22*).

The pathophysiology of bacterially induced diarrhea based on interactions with intestinal receptors and bacterial enterotoxins is well understood (*23*). The heat-stable toxin A and the heat-labile toxin of *Escherichia coli*, as well as guanylin (an endogenous, 15–amino acid intestinal ligand originally isolated from the rat jejunum), induce diarrhea by binding a specific intestinal receptor, increasing cAMP or cGMP and activating a cyclic nucleotide signal transduction pathway (*24*). The net effect of these bacterial toxins is to increase Cl^- secretion and decrease Na^+ and water absorption. Our previous work with insect cells shows that a receptor-mediated phospholipase C pathway is associated with increases in $[Ca^{2+}]_i$ after exogenous treatment of cells with NSP4 or NSP4 114-135 peptide (*12*). We have shown that NSP4 114-135 promotes and augments cAMP-dependent Cl^- secretion in mouse intestinal mucosa and induces diarrhea in rodents in a time frame similar to that of heat-stable toxin B of *E. coli* (about 3 hours). We speculate that NSP4 stimulates a Ca^{2+}-dependent signal transduction pathway that alters intestinal epithelial transport. On the basis of the enteropathogenic similarities of its effect on intestinal secretion with those reported for guanylin and the heat-stable enterotoxins, NSP4 can be considered a viral enterotoxin.

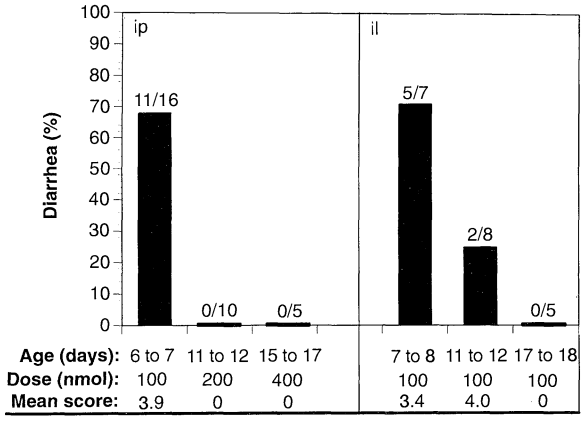

Fig. 2. Intraperitoneal and il delivery of NSP4 114-135 peptide induce age-dependent diarrhea in CD1 mice. Mice of different ages were inoculated (ip or il) with NSP4 114-135 peptide and evaluated for disease (*9*). The age of the pups, dose of the synthetic peptide, and mean diarrhea score (mean score) are indicated on the bottom of the graph. The dose of the ip-delivered peptide varied with the age of the animals—older animals received a higher dose to control for the differences in body weight. Above each column is the number of responders over the total number of animals inoculated. The peptide was diluted in sterile PBS and evaluated for sterility as in Fig. 1. A final volume of 50 μl per dose was used. As was analogous to the effects of purified NSP4, additional symptoms included lethargy and coldness to the touch.

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9. To determine the presence of diarrhea, we examined each mouse pup every 1 to 2 hours for the first 8 hours and at 24 hours after inoculation by gently pressing the abdomen. Diarrhea was noted and scored from 1 to 4, with a score of 1 reflecting unusually loose yellow stool and a score of 4 indicating completely liquid stool. A score of 2 (mucous with liquid stool, some loose but solid stool) and above was considered diarrhea. A score of 1 was noted but was not considered as diarrhea. The scoring was done by a single person and the pups were coded during analysis of diarrhea. Other symptoms monitored included lethargy, coldness to the touch, and ruffled coats in older animals.
10. J. M. Ball, P. Tian, C. Q.-Y. Zeng, A. P. Morris, M. K. Estes, data not shown.
11. Synthetic peptides used in this study were originally selected on the basis of algorithms that predict surface potential [J. M. R. Parker, D. Guo, R. S. Hodges, *Biochemistry* **25**, 5425 (1986)], turn potential (Pt) [P. Y. Chou and G. D. Fassman, *Adv. Enzymol.* **47**, 45 (1978)], and amphipathic structure [H. Margolit *et al.*, *J. Immunol.* **138**, 2213 (1987)]. A block length of 11 was used and an amphipathic score (AS) of 4 was considered significant. The NSP4 114-135 peptide has an AS of 35. All peptides were synthesized by the University of Pittsburgh Peptide Core Facility with the use of a 9-fluoroenyl methyloxycarbonyl chemical strategy and standard protocols [L. A. Carpino and G. H. Han, *J. Org. Chem.* **37**, 5748 (1970)]. Coupling and deblocking efficiencies were monitored by the ninhydrin colorimetric reaction [E. Kaiser, R. L. Colescott, C. D. Bosinger, P. I. Cook, *Anal. Biochem.* **34**, 595 (1970)]. Peptides were cleaved from their solid resin support and separated from organic contaminants by multiple cold ether extractions and conventional gel filtration chromatography (Sephadex G-25). The final peptide product was characterized by reversed-phase high-performance liquid chromatography (HPLC) (Deltapak C4, Waters) and plasma desorption mass spectroscopy [G. P. Jonnson *et al.*, *Anal. Chem.* **58**, 1084 (1986)]. Only those peptides with the correct theoretical mass and 90% or greater full-length product were used in these studies. Peptides were purified either by HPLC on a semipreparative, reversed-phase C18 column (uBondapak, Waters) or by multiple elutions from a conventional gel filtration column (1.5 mm by 40 mm). Peptide purity and sterility were confirmed before inoculations.
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17. Short-circuit currents (I_{sc}) were measured with an automatic voltage clamp across unstripped intestinal mucosal sheets from CD1 mice 19 to 22 and 35 days old (Bioengineering, Univ. of Iowa) as described [C. L. Sears, R. L. Guerrant, J. B. Kaper, in *Infections of the Gastrointestinal Tract*, M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, R. L. Guerrant, Eds. (Raven, New York, 1995), chap. 44; A. P. Morris, S. A. Cunningham, A. Tousson, D. J. Benos, R. A. Frizzell, *Am. J. Physiol.* **266**, C254 (1994)]. The midileum of the mouse intestines was used. The mucosal sheets taken from the intestine were placed into modified Ussing chambers with 0.12-cm^2 apertures (machine shop, Univ. of Texas Health Science Center) and transepithelial potential (V_t) was registered by 3 M KCl agar bridges connected to balanced calomel half-cells. The transepithelial current required to clamp V_t to 0 was passed through Ag-AgCl electrodes connected to the 3 M KCl bridges. All experiments were performed at 37°C in bicarbonate Ringer solution gassed with 95% O_2 and 5% CO_2 by airlift circulators as previously described (*ibid.*). The mucosal bath contained sodium-free (*N*-methyl-D-glutamine) substituted Ringer solution to minimize the effects on I_{sc} of cAMP-stimulated electrogenic Na^+-glucose co-transport across the small bowel [B. R. Grubb, *Am. J. Physiol.* **268**, G505 (1995)]. After temperature and ionic equilibration, basal I_{sc} measurements were taken and intestinal mucosal sheets were challenged with peptide, Cch, or FSK. Bumetamide sensitivity was tested and confirmed the chloride secretory response.
18. Using a newly established ELISA that is sensitive enough to detect 31.3 ng or 0.02 nmol of NSP4, we have detected NSP4 in the stools of mice with diarrhea at concentrations necessary to induce disease. NSP4 was not present in stools from animals without diarrhea.
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25. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Protective Effect of Rotavirus VP6–Specific IgA Monoclonal Antibodies That Lack Neutralizing Activity

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Rotaviruses are the leading cause of severe gastroenteritis and dehydrating diarrhea in young children and animals worldwide. A murine model and "backpack tumor" transplantation were used to determine the protective effect of antibodies against VP4 (an outer capsid viral protein) and VP6 (a major inner capsid viral protein). Only two non-neutralizing immunoglobulin A (IgA) antibodies to VP6 were capable of preventing primary and resolving chronic murine rotavirus infections. These antibodies were not active, however, when presented directly to the luminal side of the intestinal tract. These findings support the hypothesis that in vivo intracellular viral inactivation by secretory IgA during transcytosis is a mechanism of host defense against rotavirus infection.

Mucosal IgA is a secretory antibody that forms a first line of defense against many pathogens. It is synthesized as an oligomeric molecule that can be transported via transcytosis across certain epithelial cell types lining mucosal surfaces and then released into the mucosal environment (*1*). Several mechanisms by which secretory IgA provides protection have been proposed (*2*). Recently, Mazanec *et al.* described an in vitro model in which transcytosing IgA molecules form complexes with certain viruses that have entered the cell and thereby inhibit viral replication intracellularly (*3*). To determine whether this can occur in vivo and whether non-neutralizing antibodies can mediate this intracellular effect, we studied the effects of IgA monoclonal antibodies (mAbs) on rotavirus infection in mice.

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