Rotavirus-Specific Protein Synthesis Is Not Necessary for Recognition of Infected Cells by Virus-Specific Cytotoxic T Lymphocytes

PAUL A. OFFIT,^{1,2*} HARRY B. GREENBERG,³ AND KARLA I. DUDZIK^{1,2}

Division of Infectious Diseases, The Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard,^{1} and The Wistar Institute of Anatomy and Biology,² Philadelphia, Pennsylvania 19104, and Departments of Medicine and Medical Microbiology, Stanford University School of Medicine, Stanford, California 94305³*

Received 8 November 1988/Accepted 25 April 1989

We found that rotavirus-specific protein synthesis was not necessary for recognition by virus-specific cytotoxic T lymphocytes (CTLs). In addition, CTLs lysed rotavirus-infected target cells prior to production of infectious virus. Target cell processing of rotavirus antigens for presentation to CTLs was enhanced by treatment of rotavirus with trypsin prior to infection; trypsin-induced cleavage of the viral hemagglutinin (vp4) has previously been found to facilitate rotavirus entry into target cells by direct penetration of virions through the plasma membrane. We conclude that sufficient quantities of exogenous viral proteins may be introduced into the cytoplasm for processing by target cells. The mechanism by which rotavirus proteins are processed for presentation to the target cell surface remains to be determined.

Infections of the intestinal tract are among the most prevalent causes of infant disease and death worldwide. In Asia, Africa, and Latin America, between 1978 and 1979, 3 to 5 billion cases of diarrhea accounted for 5 to 10 million deaths (30). Since their initial identification as a human pathogen in 1973, rotaviruses have been found to be the most important cause of acute gastroenteritis of infants and young children both in the United States and in developing countries (2, 12). Because of the worldwide impact of this virus, there is a great deal of interest in disease prevention by vaccine. To develop a successful vaccine, it may be important to understand both humoral and cellular immunologic determinants of protection against challenge.

There are few studies examining the host cellular immune response to rotavirus infection (13, 24, 29). We recently examined the rotavirus-specific cytotoxic T lymphocyte (CTL) response in mice inoculated with simian strain RRV (21); RRV is currently used by researchers at the National Institutes of Health in large-scale trials of protective efficacy in infants and young children (11). We found that splenocytes from adult C57BL/6 mice orally inoculated with RRV lysed syngeneic rotavirus-infected target cells (21). Cytotoxic activity was (i) specific for rotavirus-infected target cells, (ii) eliminated by treatment of splenocytes with Thy1.2-specific immunoglobulin M and complement, (iii) restricted at *H-2D^b*, and (iv) not detected in uninoculated animals.

To determine the importance of rotavirus replication in target cells for recognition by virus-specific CTLs, we exposed target cells to infectious and noninfectious preparations of rotavirus. In addition, we examined the effect of inhibition of cellular and viral protein synthesis on CTL recognition of virus-infected target cells. We found that rotavirus-specific CTLs recognized RRV-infected target cells prior to production of infectious virus and that rotavirus-specific protein synthesis was not necessary for recognition of virus-infected cells by CTLs. Lysis of rotavirus-infected intestinal epithelial cells by CTLs prior to

production of infectious virus particles may be important in either amelioration of acute infection or prevention of reinfection.

MATERIALS AND METHODS

Animals. C57BL/6 (*H-2^b*) female mice, 8 to 12 weeks old, were obtained from either Charles River Breeding Laboratories (Portage, Mich.) or Taconic Laboratories (German-town, N.Y.).

Cells. Simian virus 40-transformed C57BL/6 mouse embryo fibroblast cells (B6/WT-3), grown as previously described (21), were provided by Steven Jennings (Louisiana State University, Shreveport, La.). Fetal rhesus monkey kidney cells (MA-104) were grown as described previously (21).

Virus. Simian rotavirus RRV strain 2 (MMU 18006) was obtained from Nathalie Schmidt (Viral and Rickettsial Disease Laboratory, Berkeley, Calif.). Plaque-purified stocks of virus for use in these studies were prepared in MA-104 cells. Viral growth and infectivity titration by plaque assay were performed as described previously (20).

Trypsin-free RRV was grown as described previously (20) except that trypsin was omitted from the overlay medium. Trypsin (type IX; Sigma Chemical Co., St. Louis, Mo.) was added to stocks of trypsin-free RRV at 37°C 30 min before infection of B6/WT-3 cells.

Tissue culture stocks of RRV mixed with various concentrations of β -propiolactone (BPL) were maintained at 4°C for 72 h. BPL activity was eliminated by exposure of virus to 37°C for 3 h. BPL-treated RRV was tested for viral infectivity (20) and hemagglutination activity (9) as described previously.

Cytotoxicity assays. (i) Immunization of animals. Mice were inoculated intraperitoneally with 10⁷ PFU of tissue culture-derived RRV.

(ii) Preparation of effector cells. Mice were sacrificed 6 days after intraperitoneal inoculation with RRV, and spleens were removed. Splenic lymphocytes treated as described previously (21) were suspended in RPMI 1640 containing 10% fetal bovine serum, 10 mM HEPES (*N*-2-hydroxyeth-

* Corresponding author.

ylpiperazine-*N'*-2-ethanesulfonic acid), 0.03% glutamine, and 3×10^{-5} M 2-mercaptoethanol (RPMI CM).

(iii) **Preparation of target cells.** B6/WT-3 cells, grown to confluency in 96-well plates, were washed twice with phosphate-buffered saline and incubated for 30 min at 37°C with 25 μ l of RRV, BPL-treated RRV, or supernatant fluids from mock-infected MA-104 cells per well. Cells were washed once with phosphate-buffered saline, 100 μ l of serum-free K1 medium (Dulbecco modified Eagle medium, Ham's F-12, 10 mM HEPES, 10 mM selenium salts, 1.1 g of NaHCO₃ per liter, 25 ng of prostaglandin E1 per ml, 50 nM hydrocortisone, 10 μ g of bovine pancreas insulin per ml, 5 μ g of transferrin per ml, 5 pM triiodothyronine) was added per well, and cells were placed at 37°C. For CTL recognition time course experiments, 2.5 μ Ci of ⁵¹Cr (Amersham Corp., Arlington Heights, Ill.) was added to individual wells of a 96-well plate (2.5 μ Ci per 3×10^4 cells) 45 min before infection, and target cells were washed twice with phosphate-buffered saline. For experiments using BPL-treated rotavirus preparations, cells were labeled 3 h after infection. For experiments using emetine, RRV infection and ⁵¹Cr labeling were performed in the presence or absence of 10^{-5} M emetine as previously described (19), and cells were labeled 3 h after infection.

(iv) **⁵¹Cr-release assays.** For time course experiments, effector cells were added to target cells for 2 h. For experiments using BPL-inactivated rotaviruses, effector cells were added to target cells for 4 h. For experiments using emetine, effector cells were added to target cells for 4 h in the absence of emetine as described previously (19). All assays were performed in triplicate. After incubation of effector cells and target cells for 2 or 4 h at 37°C in 5% CO₂, 50 μ l of supernatant fluids were removed and assayed for radioactivity in a gamma radiation counter. Target cells incubated with RPMI CM only (spontaneous release) or RPMI CM containing 1% sodium dodecyl sulfate (total release) were included in each assay. (Spontaneous-to-total-release ratios were between 20 and 25%.) Percent ⁵¹Cr release was defined as follows: (experimental release [in counts per minute] – spontaneous release [in counts per minute]) / (total release [in counts per minute] – spontaneous release [in counts per minute]) $\times$ 100.

RESULTS

Growth of RRV in B6/WT-3 cells. B6/WT-3 cells were infected with 100 PFU of RRV per cell. Cells and culture fluids were harvested together by freezing whole cultures at various times after infection, and viral infectivities were determined by plaque assay (Fig. 1). Infectious virus progeny was first detected between 4 and 8 h after infection. The logarithmic phase of growth occurred between 8 and 12 h after infection, and the peak concentration of infectious virus was detected at the end of that period.

CTL recognition of rotavirus-infected target cells at various intervals after infection. To determine whether RRV-specific CTLs recognized infected target cells prior to the onset of infectious virus production, B6/WT-3 cells were infected with 100 PFU of RRV per cell and the percentage of ⁵¹Cr released by RRV-specific CTLs was determined at various 2-h intervals after infection (Table 1). We found that RRV-specific cytotoxic activity was detected by 2 h after infection; the level of virus-specific cytotoxic activity first detected by 2 h after infection remained constant up to 8 h after infection. We were unable to detect virus-specific cytotoxic activity after incubation of effector cells and target cells for 1 h.

FIG. 1. Viral infectivities of B6/WT-3 cells infected with 100 PFU of RRV per cell. RRV was grown in the presence of trypsin. Cells and culture fluids were harvested at various times after infection, and viral infectivities were determined by plaque assay.

CTL recognition of target cells exposed to infectious or noninfectious rotavirus. To determine whether noninfectious rotaviruses were processed by target cells for CTL recognition, B6/WT-3 cells were exposed to RRV treated with 0.05, 0.1, 0.15, or 0.2% BPL. We determined viral infectivities and hemagglutination activities of BPL-treated RRV, as well as the percentage of ⁵¹Cr released by RRV-specific CTLs from target cells exposed to BPL-treated virus (Table 2). Treatment of RRV with 0.15 or 0.20% BPL eliminated viral infectivity and decreased viral hemagglutination activity by 16-fold. However, target cells exposed to preparations of noninfectious RRV were lysed by RRV-specific CTLs. Treatment of RRV with 0.05 or 0.10% BPL reduced viral infectivities by 27-fold and 4,000-fold, respectively. Target cells exposed to these BPL-treated, partially inactivated preparations of RRV were lysed by RRV-specific CTLs with efficiencies greater than those of target cells exposed to untreated preparations of RRV at identical multiplicities of infection.

CTL recognition of target cells exposed to RRV in the presence of a protein synthesis inhibitor. To determine

TABLE 1. CTL recognition of rotavirus-infected target cells at various intervals after infection^a

Target cell treatment ^b	% Specific ⁵¹ Cr release at various time intervals (h) after infection ^c			
	0-2	1-3	4-6	6-8
RRV	28	44	45	40
Mock	4	6	7	6

^a Splenic lymphocytes obtained 6 days after intraperitoneal inoculation of mice with 10^7 PFU of RRV were tested against RRV- or mock-infected target cells at various intervals after target cell infection.

^b B6/WT-3 cells were infected with 100 PFU of RRV per cell or treated with supernatant fluids from mock-infected MA-104 cells (Mock). RRV was grown in the presence of trypsin.

^c CTL assays were performed at an effector-to-target-cell ratio of 50:1.

TABLE 2. CTL recognition of target cells exposed to infectious or noninfectious rotavirus^a

% BPL ^b	Infectivity (MOI) ^c	HA ^d	% Specific ⁵¹ Cr release at effector-to-target-cell ratio of:	
			100:1	33:1
0	40	64	47	39
0.05	1.5	8	33	25
0	1.5	0	6	3
0.10	0.01	4	18	10
0	0.01	0	0	0
0.15	0	4	12	5
0.20	0	4	10	6
	Mock		0	0

^a Splenic lymphocytes obtained 6 days after intraperitoneal inoculation of mice with 10⁷ PFU of RRV were tested against target cells exposed to BPL-treated or untreated preparations of RRV at various multiplicities of infection.

^b Tissue culture stock of RRV were treated with various concentrations of BPL.

^c Target cells were exposed to RRV, BPL-treated RRV, or supernatant fluids from mock-infected MA-104 cells (Mock). Included as controls were target cells infected with RRV at the same multiplicity of infection (MOI) as RRV preparations which were not completely inactivated by BPL treatment (i.e., 0.05 and 0.10% BPL). RRV was grown in the presence of trypsin.

^d Reciprocal of hemagglutination (HA) titer.

whether RRV was processed by target cells exposed to a protein synthesis inhibitor for recognition by RRV-specific CTLs, B6/WT-3 cells were both infected with RRV and labeled with ⁵¹Cr in the presence or absence of emetine (Table 3). We found that RRV-specific CTLs recognized target cells exposed to RRV and labeled with ⁵¹Cr in the presence or absence of emetine.

CTL recognition of target cells infected with RRV in the presence of various concentrations of trypsin. Trypsin-induced cleavage of the outer capsid protein vp4 has been found to facilitate rotavirus entry into target cells (4, 10). To determine whether the presence of trypsin during RRV infection enhanced CTL recognition of infected target cells, B6/WT-3 cells were infected with RRV in the presence of 5.0, 0.5, 0.05, 0.005, or 0 µg of trypsin per ml. The percentage of ⁵¹Cr released by splenic lymphocytes from RRV-infected and mock-infected animals was determined (Table 4). RRV-specific CTLs failed to recognize target cells exposed to RRV in the absence of trypsin. Between 0.05 and 0.5 µg of trypsin per ml was required during RRV infection for recognition of target cells by RRV-specific CTLs.

TABLE 3. CTL recognition of target cells infected with RRV and labeled with ⁵¹Cr in the presence or absence of a protein synthesis inhibitor (emetine)^a

Infection and labeling of target cells	Infection of target cells with ^b	% Specific ⁵¹ Cr release at effector-to-target-cell ratio of:		
		33:1	11:1	4:1
With emetine	RRV	35	25	18
	Mock	3	1	1
Without emetine	RRV	42	30	12
	Mock	1	0	1

^a Splenic lymphocytes obtained 6 days after intraperitoneal inoculation of mice with 10⁷ PFU of RRV were tested against ⁵¹Cr-labeled, RRV- or mock-infected target cells. Infection and labeling were performed in the presence or absence of 10⁻⁵ M emetine. RRV was grown in the presence of trypsin.

^b Cells were exposed to RRV or supernatant fluids from mock-infected MA-104 cells (Mock).

TABLE 4. CTL recognition of target cells infected with RRV in the presence of various concentrations of trypsin^a

Trypsin concn ^b (µg/ml)	% ⁵¹ Cr release by lymphocytes from mice at effector-to-target-cell ratio			
	RRV infected		Mock infected	
	100:1	33:1	100:1	33:1
5.0	40	26	7	5
0.5	38	32	3	1
0.05	9	3	0	0
0.005	2	1	0	0
0	3	5	3	2
Mock	3	3	3	2

^a Splenic lymphocytes obtained 6 days after intraperitoneal inoculation of mice with 10⁷ pfu of RRV or with supernatant fluids from MA-104 cells were tested against target cells exposed to RRV treated with various concentrations of trypsin.

^b Various concentrations of trypsin were added to RRV at 37°C 30 min before infection of B6/WT-3 cells. Target cells exposed to supernatant fluids from mock-infected MA-104 cells (Mock) were included as controls.

DISCUSSION

Rotaviruses cause disease by replicating in small intestine villus epithelial cells (27). For virus-specific CTLs to play a role in either amelioration of acute viral infection or prevention of reinfection, lysis of virus-infected intestinal epithelial cells would optimally occur prior to onset of infectious virus production. To determine the time between target cell infection and infectious virus production after a single cycle of virus growth, we infected mouse embryo fibroblast cells (B6/WT-3) with 100 PFU of RRV per cell. We found that infectious virus progeny was first detected between 4 and 8 h after a single cycle of virus replication (Fig. 1). This finding is similar to that reported for growth of bovine (18) and human (20) rotaviruses in simian kidney cells under conditions of single-cycle replication.

To determine whether rotavirus-specific CTLs lysed infected cells prior to onset of infectious virus production after a single cycle of replication, splenic lymphocytes from mice parenterally inoculated with RRV were incubated with RRV-infected, ⁵¹Cr-labeled B6/WT-3 cells and the percentage of ⁵¹Cr released was determined at various 2-h intervals after infection (Table 1). We found that RRV-specific cytotoxic activity was detected by 2 h after infection, prior to the onset of infectious virus production. Our experiments involved a single cycle of RRV replication in fibroblast cells in vitro. However, the logarithmic phase of viral growth in mice inoculated with homologous host rotaviruses involves multiple cycles of viral replication and usually occurs 36 to 48 h after infection (15, 27). Because CTL effector cells can be generated from CTL precursors within 48 h of stimulation in vivo (22), rotavirus-specific CTLs in vivo could lyse virus-infected cells prior to the logarithmic phase of infectious virus production. Herpes simplex virus-specific (23) and ectromelia virus-specific (8) CTLs have also been found to lyse infected target cells in vitro prior to detection of infectious virus progeny.

Rotavirus-specific proteins are first detected 2 h after target cell infection (18). To determine whether viral protein synthesis was necessary for CTL recognition of infected target cells, we exposed target cells to infectious and noninfectious preparations of rotavirus. In addition, target cells were exposed to RRV and labeled with ⁵¹Cr in the presence of a protein synthesis inhibitor (emetine). We found that viral protein synthesis was not necessary for target cell

recognition by virus-specific CTLs (Tables 2 and 3). These findings are consistent with those for paramyxoviruses, orthomyxoviruses, and poxviruses. Target cells exposed to intact, noninfectious preparations of paramyxoviruses (1, 14) or orthomyxoviruses (7, 32) are recognized by virus-specific CTLs. In addition, CTL recognition of vaccinia virus-infected target cells occurs in the absence of viral or cellular protein synthesis (6, 17).

The mechanism by which viral proteins are processed by target cells for recognition by virus-specific CTLs remains uncertain. For paramyxoviruses and orthomyxoviruses, CTL recognition occurs if sufficient quantities of viral proteins enter the cytoplasm (32). For Sendai virus and respiratory syncytial virus, cytoplasmic entry occurs after fusion of the viral envelope to the target cell plasma membrane (5, 28); fusion is mediated at neutral pH by a strong fusion protein (F protein), a structural characteristic shared among this group of viruses. For influenza virus, cytoplasmic entry occurs only after fusion of the viral envelope with intracytoplasmic endosomes at acid pH (32). However, rotaviruses, unlike paramyxoviruses and orthomyxoviruses, are nonenveloped and do not enter cells by either plasma membrane or lysosomal membrane fusion (10). Rather, rotaviruses enter the cytoplasm by direct penetration through the plasma membrane (4, 10). Endocytosis inhibitors and lysosomotropic agents have a limited effect on rotavirus entry into cells (10). Penetration of virions directly through the plasma membrane may be another means by which viral proteins are introduced into the cytoplasm for target cell processing. The mechanism by which rotavirus proteins are then processed for presentation to the target cell surface remains to be determined.

We found that viral infectivity and hemagglutination activity was reduced by BPL treatment (Table 2). BPL inactivates viral RNA by alkylation of guanine to form 7-(2-carboxyethyl)guanine (25). However, BPL has also been found to alter viral surface proteins associated with hemagglutination and target cell attachment (31). The rotavirus outer capsid is composed of two major proteins, vp4 and vp7 (3, 16). Rotavirus entry into the cytoplasm is a function of vp4; entry is facilitated by trypsin-induced cleavage of vp4 to vp5 and vp8 (4, 10). Attachment of rotavirus to target cells, on the other hand, is mediated by vp7 (4, 26). The decreased recognition of target cells exposed to RRV treated with 0.15 or 0.20% BPL (Table 2) was most likely due to decreased virus attachment or entry associated with an alteration of vp4 and vp7. Alteration of rotavirus surface proteins by BPL was suggested by a decrease in rotavirus hemagglutinating activity (Table 2).

We found that target cells exposed to RRV grown in the absence of trypsin were not lysed by RRV-specific CTLs (Table 3). Recognition of RRV-infected target cells by RRV-specific CTLs was dependent upon the presence of at least 0.5 µg of trypsin per ml during infection. The cleavage products of vp4 (vp5 and vp8) were not detected in preparations of RRV grown in the absence of trypsin (data not shown). Enhanced recognition by RRV-specific CTLs was probably associated with facilitation of viral entry into the cell cytoplasm by trypsin cleavage of vp4 during target cell infection.

ACKNOWLEDGMENTS

We thank H. Fred Clark for helpful suggestions and critical review of the manuscript and Susan L. Cunningham for technical assistance.

This work was supported in part by The Thomas B. McCabe and Jeannette E. Laws McCabe Fund (to P.A.O.), The Lederle Biologicals Young Investigator Award in Vaccine Development (to P.A.O.), The University of Pennsylvania Research Foundation Award (to P.A.O.), and Public Health Service grants 2 S07 RR05506, 1 KO4 4100889 (to P.A.O.), and R22AI21362 (to H.B.G.) from the National Institutes of Health.

LITERATURE CITED

1. Bangham, C. R. M., M. J. Cannon, D. T. Karzon, and B. A. Askonas. 1985. Cytotoxic T-cell response to respiratory syncytial virus in mice. *J. Virol.* **56**:55–59.
2. Black, R. E. M., M. H. Merson, A. S. S. M. Rahman, M. Yunis, A. R. M. A. Alim, I. Huq, R. H. Yolken, and G. T. Curlin. 1980. A two-year study of bacterial, viral and parasitic agents associated with diarrhea in rural Bangladesh. *J. Infect. Dis.* **142**: 660–664.
3. Estes, M. K., E. L. Palmer, and J. F. Obijeski. 1983. Rotaviruses: a review. *Curr. Top. Microbiol. Immunol.* **195**:124–184.
4. Fukuhara, N., O. Yoshie, S. Kitaoka, and T. Konno. 1988. Role of vp3 in human rotavirus internalization after target cell attachment via vp7. *J. Virol.* **62**:2209–2218.
5. Gething, M. J., U. Koszinowski, and M. Waterfield. 1978. Fusion of Sendai virus with the target cell membrane is required for T cell cytotoxicity. *Nature (London)* **274**:689–691.
6. Hapel, A. J., R. Bablanian, and G. A. Cole. 1978. Inductive requirements for the generation of virus-specific T lymphocytes. I. The nature of the host cell-virus interaction that triggers secondary poxvirus-specific cytotoxic T lymphocyte induction. *J. Immunol.* **121**:736–743.
7. Hosaka, Y., F. Sasao, and R. Ohara. 1985. Cell-mediated lysis of heat-inactivated influenza virus-coated murine targets. *Vaccine* **3**:245–251.
8. Jackson, D. C., G. L. Ada, and R. Tha Hla. 1976. Cytotoxic T cells recognize very early, minor changes in ectromelia virus-infected target cells. *Aust. J. Exp. Biol. Med. Sci.* **54**:349–366.
9. Kalica, A. R., H. D. James, Jr., and A. Z. Kapikian. 1978. Hemagglutination by simian rotavirus. *J. Clin. Microbiol.* **7**: 314–315.
10. Kaljot, K. T., R. D. Shaw, D. H. Rubin, and H. B. Greenberg. 1988. Infectious rotavirus enter cells by direct cell membrane penetration, not by endocytosis. *J. Virol.* **62**:1136–1144.
11. Kapikian, A. Z., J. Flores, Y. Hoshino, R. I. Glass, K. Midthun, M. Gorziglia, and R. M. Chanock. 1986. Rotavirus: the major etiologic agent of severe infantile diarrhea may be controllable by a "Jennerian" approach to vaccination. *J. Infect. Dis.* **153**:815–822.
12. Kapikian, A. Z., H. W. Kim, R. G. Wyatt, W. L. Cline, J. O. Arrobio, C. D. Brandt, W. J. Rodriguez, D. A. Sack, R. M. Chanock, and R. H. Parrott. 1976. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. *N. Engl. J. Med.* **294**:965–972.
13. Kohl, S., M. W. Harmon, and J. Ping Tang. 1983. Cytokine-stimulated human natural killer cytotoxicity: response to rotavirus-infected cells. *Pediatr. Res.* **17**:868–872.
14. Koszinowski, U., M. J. Gething, and M. Waterfield. 1977. T-cell cytotoxicity in the absence of viral protein synthesis in target cells. *Nature (London)* **267**:160–163.
15. Little, L. M., and J. A. Shaddock. 1982. Pathogenesis of rotavirus infection in mice. *Infect. Immun.* **38**:755–763.
16. Liu, M., P. A. Offit, and M. K. Estes. 1988. Identification of the simian virus SA11 genome segment 3 product. *Virology* **163**: 26–32.
17. Mallon, V. R., E. A. Domber, and J. A. Holowczak. 1985. Vaccinia virus proteins on the plasma membranes of infected cells. II. Expression of viral antigens and killing of infected cells by vaccinia virus-specific cytotoxic T cells. *Virology* **145**:1–23.
18. McCrae, M. A., and G. P. Faulkner-Valle. 1981. Molecular biology of rotaviruses. I. Characterization of basic growth parameters and pattern of macromolecular synthesis. *J. Virol.* **39**:490–496.
19. Morrison, L. A., A. E. Lukacher, V. L. Braciale, D. P. Fan, and

- T. J. Braciale. 1986. Differences in antigen presentation to MHC class I- and class II-restricted influenza virus-specific cytolytic T lymphocyte clones. *J. Exp. Med.* **163**:903–921.
20. Offit, P. A., H. F. Clark, W. G. Stroop, E. M. Twist, and S. A. Plotkin. 1983. The cultivation of human rotavirus, strain 'Wa', to high titer in cell culture and characterization of the viral structural polypeptides. *J. Virol. Methods* **7**:29–40.
21. Offit, P. A., and K. I. Dudzik. 1988. Rotavirus-specific cytotoxic T lymphocytes cross-react with target cells infected with different rotavirus serotypes. *J. Virol.* **62**:127–131.
22. Owen, J. A., K. I. Dudzik, L. Klein, and D. R. Dorer. 1988. The kinetics of generation of influenza-specific cytotoxic T lymphocyte precursor cells. *Cell. Immunol.* **111**:247–252.
23. Pfizenmaier, K., H. Jung, A. Starzinski-Powitz, M. Rollinghoff, and H. Wagner. 1977. The role of T cells in anti-herpes simplex virus immunity. I. Induction of antigen-specific cytotoxic T lymphocytes. *J. Immunol.* **119**:939–944.
24. Riepenhoff-Talty, M., H. Suzuki, and P. L. Ogra. 1983. Characteristics of the cell-mediated immune response to rotavirus in suckling mice. *Dev. Biol. Stand.* **53**:263–268.
25. Roberts, J. J., and G. P. Warwick. 1963. The reaction of β -propiolactone with guanosine, deoxyguanic acid, and RNA. *Biochem. Pharmacol.* **12**:1441–1442.
26. Sabara, M., J. E. Gilchrist, G. R. Hudson, and L. A. Babiuk. 1985. Preliminary characterization of an epitope involved in neutralization and cell attachment that is located on the major bovine rotavirus glycoprotein. *J. Virol.* **53**:58–66.
27. Starkey, W. G., J. Collins, T. S. Wallis, G. J. Clarke, A. J. Spencer, S. J. Haddon, M. P. Osborne, D. C. A. Candy, and J. Stephen. 1986. Kinetics, tissue specificity and pathological changes in murine rotavirus infection of mice. *J. Gen. Virol.* **67**:2625–2634.
28. Sugamura, K., K. Shimizu, D. A. Zarling, and F. H. Bach. 1977. Role of Sendai virus fusion-glycoprotein in target cell susceptibility to cytotoxic T cells. *Nature (London)* **270**:251–253.
29. Totterdell, B. M., J. E. Banatvala, I. L. Chrystie, G. Ball, and W. D. Cubitt. 1988. Systemic lymphoproliferative responses to rotavirus. *J. Med. Virol.* **25**:37–44.
30. Walsh, J. A., and K. S. Warren. 1979. Selective primary health care: an interim strategy for disease control in developing countries. *N. Engl. J. Med.* **301**:967–974.
31. Wiktor, T. J., H. G. Aaslestad, and M. M. Kaplan. 1972. Immunogenicity of rabies virus inactivated by β -propiolactone, acetyleneimine, and ionizing radiation. *Appl. Microbiol.* **23**:914–918.
32. Yewdell, J. W., J. R. Bennink, and Y. Hosaka. 1988. Cells process exogenous proteins for recognition by cytotoxic T lymphocytes. *Science* **239**:637–640.