Differences in the Capacity of Reovirus Strains To Induce Apoptosis Are Determined by the Viral Attachment Protein $\sigma 1$ [†]

KENNETH L. TYLER,^{1,2,3,4,5*} MARGARET K. T. SQUIER,⁴ STEVEN E. RODGERS,^{6,7}
BENJAMIN E. SCHNEIDER,¹ STEPHANIE M. OBERHAUS,¹ THERESA A. GRDINA,⁵
J. JOHN COHEN,⁴ AND TERENCE S. DERMODY^{6,7,8}

Departments of Neurology,¹ Medicine,² Microbiology,³ and Immunology,⁴ University of Colorado Health Sciences Center, and Neurology Service, Denver Veterans Affairs Medical Center,⁵ Denver, Colorado 80220, and Departments of Pediatrics⁸ and Microbiology & Immunology⁶ and Elizabeth B. Lamb Center for Pediatric Research,⁷ Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Received 30 May 1995/Accepted 25 July 1995

Reoviruses are important models for studies of viral pathogenesis; however, the mechanisms by which these viruses produce cytopathic effects in infected cells have not been defined. In this report, we show that murine L929 (L) cells infected with prototype reovirus strains type 1 Lang (T1L) and type 3 Dearing (T3D) undergo apoptosis and that T3D induces apoptosis to a substantially greater extent than T1L. Using T1L × T3D reassortant viruses, we found that differences in the capacity of T1L and T3D to induce apoptosis are determined by the viral S1 gene segment, which encodes the viral attachment protein $\sigma 1$ and the non-virion-associated protein $\sigma 1s$. Apoptosis was induced by UV-inactivated, replication-incompetent reovirus virions, which do not contain $\sigma 1s$ and do not mediate its synthesis in infected cells. Additionally, T3D-induced apoptosis was inhibited by anti-reovirus monoclonal antibodies that inhibit T3D cell attachment and disassembly. These results indicate that $\sigma 1$, rather than $\sigma 1s$, is required for induction of apoptosis by the reovirus and suggest that interaction of virions with cell surface receptors is an essential step in this mechanism of cell killing.

The mechanisms used by viruses to produce cytopathic effects (CPE) in their host cells are not well understood. Such knowledge is essential to an understanding of viral pathogenesis and development of novel antiviral therapies. Viruses can cause CPE by disrupting a variety of cellular processes, including synthesis of nucleic acids and proteins, maintenance of cytoskeletal architecture, and preservation of membrane integrity (32). Several viruses, including herpes simplex virus (11), human immunodeficiency virus (27, 35, 42, 65), influenza virus (28, 64), and measles virus (23), cause CPE by induction of apoptosis of target cells. Apoptosis is distinguished from necrosis by the presence of distinctive morphological changes and cleavage of cellular DNA into oligonucleosome-length fragments (80).

Reoviruses infect a variety of mammalian hosts and serve as an important experimental system for studying functions of individual viral proteins at distinct stages of the virus-host interaction (69). Reoviruses are nonenveloped viruses that contain a genome of segmented, double-stranded RNA (48). Prototype reovirus strains type 1 Lang (T1L) and type 3 Dearing (T3D) infect the same host species but spread within the infected host via different routes and produce distinct patterns of tissue injury (69). Reovirus replication occurs in the cytoplasm, and progeny virions are released upon cell lysis (30, 48, 60, 81). Reovirus infection results in inhibition of cellular DNA (26, 51, 55), RNA (33, 56), and protein synthesis (17, 21, 34, 44, 56), alteration of cytoskeletal structure (54), and changes in the number of growth factor re-

ceptors and their signaling capacity (43, 62, 70). It is not clear which, if any, of these effects are responsible for reovirus-induced cell injury in culture and in vivo.

To determine whether reovirus-induced CPE is associated with apoptosis, we assessed morphological changes and fragmentation of cellular DNA using murine L929 (L) cells infected with T1L and T3D. We found that although both T1L and T3D induce apoptosis in L cells, T3D does so to a significantly greater degree than T1L. We used T1L × T3D reassortant viruses to identify viral genes associated with strain-specific differences in apoptosis induction, and we investigated whether apoptosis can be triggered in the absence of viral replication using UV-inactivated reovirus virions. The results indicate that apoptosis is an important mechanism of reovirus-induced CPE and that reovirus attachment to cells in the absence of productive viral infection is sufficient to induce apoptosis.

MATERIALS AND METHODS

Cells, viruses, and antibodies. Spinner-adapted L cells were grown in either suspension or monolayer cultures in Joklik's modified Eagle's minimal essential medium (MEM) (Irvine Scientific, Santa Ana, Calif.) that was supplemented to contain 5% fetal bovine serum (Gibco BRL, Gaithersburg, Md.) and 2 mM L-glutamine (complete MEM). The medium used in viral growth studies and ultrastructural studies of L cells was also supplemented, per milliliter, with 100 U of penicillin, 100 μ g of streptomycin, and 250 ng of amphotericin (Irvine).

Reovirus strains T1L and T3D are laboratory stocks. T1L × T3D reassortant viruses were grown from stocks originally obtained from Kevin Coombs, Bernard Fields, and Max Nibert (5, 14, 77). The electrophoretotypes of these viruses are shown in Table 1. Viral strains were plaque purified twice and passaged twice in L cells as previously described (67) to generate working stocks. Purified virion preparations were made with second-passage L-cell lysate stocks of twice-plaque-purified reovirus as previously described (25, 71). Concentrations of virions in purified preparations were determined from the equivalence 1 OD₂₆₀ = 2.1 × 10¹² virions per ml (61). The particle-to-PFU ratio in these preparations was approximately 100 to 1.

Monoclonal antibody 9BG5 (ATCC HB 167) is a mouse IgG2a antibody specific for the T3D $\sigma 1$ protein (6). Monoclonal antibody 5C6 is a mouse IgG2a antibody specific for the T1L $\sigma 1$ protein (72). Reovirus $\sigma 3$ -specific monoclonal

* Correspondent Footnote. Mailing address: Department of Neurology (B-182), University of Colorado Health Sciences Center, 4200 E. 9th Ave., Denver, CO 80262. Phone: (303) 393-2874. Fax: (303) 393-4686. Electronic mail address: tylerk@essex.UCHSC.edu.

[†] Dedicated to the memory of our friend and mentor, Bernard N. Fields.

TABLE 1. Capacities of T1L × T3D reassortant viruses to induce apoptosis detected by AO staining and fragmentation of [³H]thymidine-labeled DNA

Virus strain	Genome segment ^a										% of AO-stained cells ^b	Rank ^c	% of [³ H]thymidine DNA fragmentation ^d	Rank
	L1	L2	L3	M1	M2	M3	S1	S2	S3	S4				
T1L	1L	1L	1L	1L	1L	1L	1L	1L	1L	1L	3		21	
T3D	3D	3D	3D	3D	3D	3D	3D	3D	3D	3D	48		100	
KC150	3D	1L	1L	1L	3D	1L	3D	3D	1L	3D	98	1	66	9
1.HA3	1L	1L	1L	1L	1L	1L	3D	1L	1L	1L	97	2	— ^e	—
EB86	1L	3D	3D	3D	3D	1L	3D	3D	3D	1L	96	3	106	2
H15	1L	3D	3D	1L	3D	3D	3D	3D	3D	1L	95	4	—	—
EB62	3D	3D	3D	3D	3D	3D	3D	1L	3D	1L	94	5.5	101	5
EB13	3D	3D	3D	3D	3D	3D	3D	3D	3D	1L	94	5.5	87	8
EB138	3D	1L	1L	3D	3D	1L	3D	3D	1L	1L	91	7	106	2
EB97	3D	3D	1L	3D	3D	3D	3D	3D	3D	1L	90	8	—	—
EB88	3D	3D	3D	3D	1L	3D	3D	3D	3D	3D	87	9	—	—
EB28	3D	3D	1L	3D	3D	3D	3D	1L	3D	3D	81	10	106	2
EB39	1L	3D	3D	1L	3D	3D	3D	3D	3D	3D	76	11	101	5
EB129	3D	3D	1L	3D	3D	1L	3D	1L	1L	3D	74	12	—	—
EB120	3D	3D	3D	1L	1L	3D	3D	3D	1L	1L	73	13	—	—
EB143	3D	1L	1L	1L	1L	1L	3D	1L	1L	1L	70	14	—	—
E3	3D	3D	3D	3D	1L	3D	3D	3D	3D	3D	69	15	—	—
H9	3D	3D	1L	3D	1L	1L	3D	3D	3D	3D	62	16	101	5
EB144	1L	1L	1L	1L	3D	3D	1L	1L	3D	1L	51	17	—	—
EB109	3D	3D	1L	3D	3D	3D	1L	3D	3D	3D	50	18.5	92	7
G2	1L	3D	1L	1L	1L	1L	3D	1L	1L	1L	50	18.5	—	—
KC9	3D	3D	3D	3D	3D	3D	1L	3D	3D	3D	45	20	—	—
EB18	3D	3D	1L	3D	3D	3D	1L	1L	3D	1L	42	21	—	—
EB132	3D	3D	1L	3D	3D	1L	3D	3D	3D	1L	30	22	—	—
EB68	1L	3D	1L	1L	3D	1L	1L	1L	3D	3D	25	23	—	—
EB67	1L	3D	1L	1L	1L	3D	1L	1L	1L	1L	8	24	—	—
H14	1L	1L	3D	1L	1L	1L	1L	1L	3D	1L	7	25	—	—
EB145	3D	3D	3D	3D	3D	1L	1L	3D	3D	3D	6	26	23	14
EB113	1L	1L	1L	3D	1L	1L	1L	1L	3D	1L	5	27	23	14
EB98	1L	3D	1L	1L	1L	1L	1L	3D	1L	3D	4	29	—	—
EB47	1L	3D	1L	1L	1L	1L	1L	1L	1L	1L	4	29	—	—
EB1	1L	3D	1L	1L	3D	1L	1L	1L	3D	1L	4	29	—	—
H17	3D	3D	3D	1L	3D	3D	1L	3D	3D	1L	3	31	12	16
EB85	1L	1L	1L	1L	1L	3D	1L	3D	1L	1L	2	34	—	—
EB31	1L	1L	1L	3D	1L	1L	1L	3D	3D	1L	2	34	27	11
EB121	3D	3D	1L	3D	1L	3D	1L	3D	3D	3D	2	34	23	14
G16	1L	1L	1L	3D	1L	1L	1L	1L	1L	1L	2	34	26	12
H24	1L	1L	1L	1L	1L	1L	1L	1L	1L	3D	2	34	9	17
H27	1L	3D	3D	1L	3D	1L	1L	1L	1L	1L	1	37.5	33	10
H41	3D	3D	1L	1L	1L	3D	1L	3D	3D	1L	1	37.5	—	—

^a The parental origin of each genome segment in the reassortant strains: 1L, genome segment derived from T1L; 3D, genome segment derived from T3D (5, 14, 76).

^b L cells were infected with viral strains at an MOI of 10 PFU per cell. The percentage of apoptotic cells was determined by AO staining 48 h after infection. The results represent the means for at least two independent experiments for each viral strain.

^c Viruses are ranked from highest to lowest on the basis of their capacities to induce apoptosis.

^d L cells containing [³H]thymidine-labeled DNA were infected with viral strains at an MOI of 10 PFU per cell, and fragmented DNA was detected by scintillation counting 48 h after infection. The extent of DNA fragmentation produced by each reassortant virus is expressed as a percentage of that produced by T3D after subtraction of background fragmentation. The results represent the means of at least two independent experiments for each viral strain.

^e —, reassortant virus was not tested.

antibodies 4F2, 8F12, 10C1, and 10G10 and μ 1-specific monoclonal antibodies 8H6, and 10F6 have been described previously (72, 73). Antibodies were prepared and stored as previously described (72).

Growth of reovirus in L cells. L cells (2×10^5 cells) were infected with T1L or T3D at a multiplicity of infection (MOI) of 2 PFU per cell. After a 1-h adsorption period at 4°C, the inoculum was removed, 1.0 ml of fresh medium was added, and the cells were incubated at 37°C for defined intervals. Cells were frozen and thawed twice prior to being titrated on L-cell monolayers by plaque assay (71).

Generation of UV-inactivated reovirus virions. Reovirus virions were suspended at a concentration of 4×10^8 PFU/ml in MEM supplemented to contain 2 mM glutamine and were exposed to shortwave (254 nm) UV light by using conditions sufficient to generate an intensity of 560 μ W/cm² for 15 min (energy level determined with a Blak-Ray J-225 shortwave UV intensity meter). UV-inactivated virus stocks were devoid of infectious virus as determined by plaque assay (≤ 10 PFU/ml).

Electron microscopy. L cells were infected with T3D at an MOI of 1,000 PFU per cell. After incubation at 37°C for 18 h, cells were harvested, pelleted by low-speed centrifugation ($1,000 \times g$, 10 min), and fixed in phosphate-buffered 2% glutaraldehyde. After primary fixation, cells were pelleted ($1,000 \times g$, 10

min), suspended in 1% osmium tetroxide, dehydrated in 25 to 100% ethanol and propylene oxide, and embedded in epoxy resin. Ultrathin sections were prepared with a Sorvall MT 6000 ultramicrotome, stained with lead citrate uranyl acetate, and examined by using a Philips 300 electron microscope.

Detection of oligonucleosomal DNA fragmentation by Southern blotting. Fragmentation of L-cell DNA into oligonucleosome-length ladders was detected by a Southern blot assay (24). L cells were either mock-infected or infected with T1L or T3D at an MOI of 10 PFU per cell. After incubation at 37°C for 48 h, culture supernatants and trypsinized cells were combined and digested with 0.1 mg of proteinase K per ml at 37°C for 12 h. L-cell DNA was isolated by phenol-chloroform extraction and precipitation with 95% ethanol. Purified L-cell DNA (10 to 20 μ g per lane) was resolved by electrophoresis in a 1.5% agarose gel and transferred to a nylon membrane. The membrane was incubated at 80°C for 1 h, prehybridized in prehybridization buffer (50% formamide, $5 \times$ Denhardt's solution, $5 \times$ SSC [$1 \times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.2% sodium dodecyl sulfate [SDS]), 100 μ g of denatured salmon sperm DNA per ml) at 42°C for 3 h, and then incubated for 16 to 18 h at 42°C with ³²P-labeled nick-translated L-cell genomic DNA probe ($\geq 10^6$ cpm/ml in a mixture of 50% formamide, $5 \times$ SSC, 0.2% SDS, 50 μ g of denatured salmon sperm DNA per ml,

FIG. 1. Electron microscopic appearance of uninfected (A) and T3D-infected (B, C, and D) L cells. L cells were infected with T3D at an MOI of 1,000 PFU per cell and incubated at 37°C for 18 h. Cells then were fixed in phosphate-buffered 2% glutaraldehyde, embedded in epoxy resin, sectioned, and stained with lead citrate uranyl acetate. Infected cells undergo various stages of chromatin condensation: initial condensation (B), margination of chromatin at the nuclear membrane (C), and complete condensation of the nucleus (D). Bars, 5 μ m.

1 $\times$ Denhardt's solution). Membranes were washed for 5 min in 2 $\times$ SSC-0.5% SDS at room temperature with shaking, for 15 min in 2 $\times$ SSC-0.1% SDS at room temperature with shaking, and finally for 2.5 h in three changes of 0.1 $\times$ SSC-0.5% SDS at 52°C. Membranes were air dried and exposed to XAR film (Eastman Kodak Co., Rochester, N.Y.) at -70°C.

Quantitation of apoptosis by fluorescent dye staining. L cells were either mock-infected or infected with reovirus strains at an MOI of 10 PFU per cell. After incubation at 37°C for defined intervals, cells were collected and suspended in incomplete MEM at a concentration of 2×10^5 cells per ml. A 25- μ l aliquot of cells was mixed with 1 μ l of a dye solution containing 100 μ g of acridine orange (AO) (Sigma Chemical Co., St. Louis, Mo.) per ml and 100 μ g of ethidium bromide (Sigma Chemical Co.) per ml in phosphate-buffered saline. A 10- μ l aliquot of the treated cells was placed on a glass slide with a coverslip and examined by using epillumination with a Zeiss microscope equipped with a fluorescein filter. Cells were scored as apoptotic when their nuclei contained uniformly stained condensed or fragmented chromatin in accordance with previously described criteria (19). At least 200 cells were scored for each viral strain.

Quantitation of DNA fragmentation in cells containing [^{3} H]thymidine-labeled DNA. L-cell DNA was radiolabeled by incubating cells in six-well plates (Costar, Cambridge, Mass.) (2×10^5 cells per well) with 1 μ Ci of [^{3} H]thymidine per well (2 Ci/mmol, specific activity; Amersham, Arlington Heights, Ill.) at 37°C for 12 h. Cells were washed three times with incomplete MEM (37°C) and resuspended in complete MEM. Incorporation of [^{3} H]thymidine was determined by using a liquid scintillation (β) counter (model LS3801; Beckman, Fullerton, Calif.). Cell preparations contained approximately 2,000 to 10,000 cpm per 10,000 cells. Radiolabeled L cells were either mock-infected or infected with reovirus strains at an MOI of 10 PFU per cell. After incubation at 37°C for

defined intervals, cells were harvested with trypsin-EDTA, pooled with supernatants, and centrifuged at $148 \times g$ at 4°C for 10 min. The supernatant fraction (S) was removed, an aliquot was precipitated with trichloroacetic acid (12.5% [vol/vol], final concentration), and the radioactivity in the aliquot was determined by scintillation counting. The cell pellet was suspended in 500 μ l of TTE buffer (10 mM Tris, 1 mM EDTA, 0.2% Triton X-100, [pH 7.6]) and centrifuged in an Eppendorf model 5415C microcentrifuge at 13,000 rpm at 4°C for 10 min. The pellet was resuspended in 500 μ l of 1% SDS. The radioactivity in supernatant (T) and pellet (B) fractions was determined by scintillation counting. The percentage of fragmented DNA was determined with the following formula: $[(T+S)/(T+B+S)] \times 100$.

Statistical analysis. AO staining and DNA fragmentation data were analyzed by both nonparametric (Mann-Whitney [MW] test) and parametric (t test) statistical techniques. The contributions of individual reovirus gene segments were assessed further by parametric stepwise linear regression analysis. R^2 (percent variance) values were calculated for regression equations by using all 10 gene segments as predictors, as were R^2 values for regression equations by using only those segments found to be significant ($P < 0.05$ by MW test or t test analysis). Statistical analyses were performed by using Minitab statistical software (Addison-Wesley, Reading, Mass.).

Inhibition of apoptosis by anti-reovirus monoclonal antibodies. T3D (5×10^6 PFU/ml) was incubated at 4°C overnight with 10 μ g of a given monoclonal antibody per ml. A 500- μ l aliquot of the T3D-antibody mixture (2.5×10^6 PFU of T3D) was then adsorbed onto L cells (2.5×10^5 cells; MOI, 10 PFU per cell in six-well plates) at room temperature for 1 h. Following adsorption, 2 ml of complete MEM was added to each well, and the cells were incubated at 37°C for

48 h. The percentage of apoptotic cells was then determined by using the AO-staining assay as described above.

RESULTS

Reovirus induces apoptosis of infected L cells. To determine whether reovirus induces apoptosis, L cells were infected with reovirus strain T3D and analyzed for morphological hallmarks of apoptosis by electron microscopy (Fig. 1). Infected cells showed nuclear condensation, zeiosis (blebbing) of the plasma membrane, and cell shrinkage, all of which are characteristic features of apoptosis (80). Infected cells showed a continuum of nuclear chromatin alterations, including margination of the nuclear chromatin along the inner wall of the nuclear envelope, nuclear collapse and breakage, and, ultimately, cellular fragmentation into apoptotic bodies.

DNA extracted from cells undergoing apoptosis is frequently cleaved into oligonucleosome-length fragments by an endogenous endonuclease (80). This cleavage results in the appearance of oligonucleosomal ladders of DNA fragments of $n \times 180$ to 200 bp in length after agarose gel electrophoresis of cellular DNA. We used Southern blot hybridization to determine whether DNA extracted from L cells infected with strains T1L and T3D was fragmented into oligonucleosome-length ladders (Fig. 2). The results demonstrate that DNA fragments characteristic of apoptosis were generated in both T1L- and T3D-infected cells.

Reovirus strains T1L and T3D differ in their capacities to induce apoptosis. To determine whether T1L and T3D differ in their capacities to induce apoptosis, we used an AO-staining assay to quantitate the percentage of reovirus-infected L cells undergoing apoptosis (19) (Fig. 3A). L cells were infected with either T1L or T3D at an MOI of 10 PFU per cell, and apoptosis was quantitated by AO staining. A substantially greater percentage of cells infected with T3D than those infected with T1L exhibited condensed chromatin when stained with AO. For both strains, maximum apoptosis was detected at 48 h postinfection. When MOIs greater than 10 PFU per cell were used, maximum apoptosis occurred earlier than 48 h postinfection (data not shown). Similar results were obtained when apoptosis was detected by quantitating DNA fragmentation in cells containing [^{3} H]thymidine-labeled DNA (Fig. 3B) or by the diphenylamine reaction (19) (data not shown). Differences in the efficiency with which T1L and T3D produce apoptosis were not the result of differences in the capacity of these viruses to grow in L cells (Fig. 4) or of differences in the percentages of infected cells in culture as determined by immunocytochemical detection of reovirus antigen in infected cells (data not shown).

T1L $\times$ T3D reassortant viruses link differences in apoptosis induction to the S1 and M2 gene segments. Reovirus gene segments can reassort when cells are coinfecting with two or more reovirus strains, and biologic polymorphisms exhibited by different viral strains can be mapped to specific gene segments by screening reassortant viruses in appropriate assays (48, 49, 69). We used 38 T1L $\times$ T3D reassortant viruses (5, 14, 76) and the AO-staining assay to identify viral genes associated with differences in the capacities of T1L and T3D to induce apoptosis (Table 1). The results demonstrate a significant association between the capacity of reassortant viruses to induce apoptosis and the T3D S1 gene (data analyzed by MW test and t test, $P < 0.0001$) and a moderate association between induction of apoptosis and the T3D M2 gene (MW test, $P = 0.024$; t test, $P = 0.027$). No other gene segments were significantly associated with differences in AO staining ($P > 0.05$). Using linear regression analysis, we obtained R^2 values of 88.3% ($P <$

FIG. 2. Autoradiograph of Southern blot hybridization of total cellular DNA extracted from L cells. L cells were either mock-infected or infected with T1L or T3D at an MOI of 10 PFU per cell. After incubation at 37°C for 24 or 48 h, purified cellular DNA was resolved by agarose gel electrophoresis, transferred to a nylon membrane, and probed with [^{32} P]-labeled, nick-translated, L-cell genomic DNA. Size markers (in base pairs) are indicated on the left.

0.001) for the regression equation for all 10 reovirus genes, 80.8% ($P < 0.001$) for the S1 and M2 genes, and 77.4% ($P < 0.001$) for the S1 gene alone. These results indicate that the S1 gene is the principal determinant of the difference in the capacities of T1L and T3D to induce apoptosis; however, a smaller, independent contribution is made by the M2 gene segment.

We confirmed these results by quantitating DNA fragmentation in L cells containing [^{3} H]thymidine-labeled DNA infected with 17 T1L $\times$ T3D reassortants (Table 1). Again, the T3D S1 gene was significantly associated with induction of apoptosis (MW test, $P = 0.0012$; t test, $P < 0.0001$); the T3D M2 gene was less so (MW test, $P = 0.061$; t test, $P = 0.036$). No other reovirus genes were significantly associated with differences in DNA fragmentation ($P > 0.05$). By linear regression analysis, the R^2 value was 92.8% ($P = 0.01$) for all 10 reovirus genes, 74.5% ($P < 0.001$) for the T3D S1 and M2 genes, and 72.4% ($P < 0.001$) for the S1 gene alone. As expected, there

FIG. 3. Percentage of reovirus-infected L cells undergoing apoptosis as detected by either AO staining (A) or fragmentation of [^{3} H]thymidine-labeled DNA (B). L cells were either mock-infected or infected with T1L or T3D at an MOI of 10 PFU per cell. After incubation at 37°C for 24 or 48 h, cells were harvested and processed for AO staining or DNA fragmentation assays. The results are expressed as the means for three independent experiments. Error bars indicate standard deviations.

was a significant correlation between the results obtained by the AO-staining and [^{3} H]thymidine-labeled DNA fragmentation assays ($R^2 = 81.9\%$, $P < 0.0001$ by linear regression analysis).

Apoptosis can be triggered in the absence of productive viral infection by UV-inactivated reovirus virions. The S1 gene encodes two proteins, $\sigma 1$ and $\sigma 1s$, in overlapping reading frames (22, 29, 53). The $\sigma 1$ protein is a minor constituent of the virion outer capsid and serves as the viral attachment protein (37, 74). The $\sigma 1s$ protein is not a virion-associated protein but is detected in reovirus-infected cells (2, 7, 8). To determine which of the S1 gene products is associated with strain-specific differences in reovirus-induced apoptosis, we tested UV-inactivated, replication-incompetent T1L and T3D using the AO-staining assay (Fig. 5). UV-inactivated virions contain $\sigma 1$ and are capable of binding target cells but are not transcriptionally active (48, 57); therefore, these particles lack both $\sigma 1s$ and the capacity to mediate its synthesis. UV-inactivated T3D, and to a lesser extent T1L, induced apoptosis in a concentration-dependent fashion. Apoptosis induced by UV-inactivated T3D was inhibited substantially by preincubation with the anti- $\sigma 1$ monoclonal antibody (6, 73) (Fig. 5). These results indicate that reovirus-induced apoptosis can occur in the absence of viral replication provided that a sufficient number of viral particles are present in the inoculum. Furthermore, since UV-inactivated virions do not contain $\sigma 1s$ and are transcriptionally inactive, these results indicate that the S1 gene product $\sigma 1$, and not $\sigma 1s$, is required for induction of apoptosis.

Viral replication is required for induction of apoptosis by low MOIs of T3D. Experiments using UV-inactivated virions indicated that viral replication is not required for induction of apoptosis when sufficient numbers of viral particles are present in the inoculum. The requirement for high MOIs to induce apoptosis by replication-incompetent virions suggested that apoptosis induced at low MOIs might require viral replication. To test this possibility, we incubated T3D with a panel of anti-reovirus monoclonal antibodies that vary in their capacity to block viral growth in L cells (73). T3D-antibody mixtures were then inoculated onto L-cell monolayers at an MOI of 10 PFU per cell, and the percentage of apoptotic cells was deter-

mined 48 h after inoculation by the AO-staining assay (Fig. 6). Monoclonal antibodies that inhibit growth of T3D in L cells (type 3 $\sigma 1$ -specific antibody 9BG5; $\sigma 3$ -specific antibodies 4F2, 10C1, and 10G10; and $\mu 1$ -specific antibody 8H6) inhibited apoptosis, whereas monoclonal antibodies that do not inhibit T3D growth in L cells (type 1 $\sigma 1$ -specific antibody 5C6, $\sigma 3$ -specific antibody 8F12, and $\mu 1$ -specific antibody 10F6) did not inhibit apoptosis. These results indicate that viral replication is required for induction of apoptosis when low viral inoculums are used to initiate infection.

FIG. 4. Growth of T1L and T3D in L cells. Monolayers of L cells (2×10^5 cells) were infected with either T1L or T3D at an MOI of 2 PFU per cell. After a 1-h adsorption period (4°C), the inoculum was removed, 1.0 ml of fresh medium was added, and the cells were incubated at 37°C for the indicated intervals. Cells were frozen and thawed twice prior to being titrated on L-cell monolayers by plaque assay. The results are presented as the mean viral yields for four independent experiments. Error bars indicate standard deviations.

FIG. 5. Induction of apoptosis by UV-inactivated reovirus virions. Virions of T1L and T3D were inactivated by UV exposure and adsorbed to L cells at the MOIs indicated. For anti- σ 1 antibody (Ab) and control antibody conditions, UV-inactivated T3D was incubated at 4°C for 12 h with the anti-T3D σ 1 monoclonal antibody, 9BG5, or an isotype-matched monoclonal antibody specific for T1L σ 1 (5C6) at a final concentration of 100 μ g/ml. Cells were stained with AO 12 h after adsorption of UV-inactivated virions. The results are expressed as the means for three independent experiments. Error bars indicate standard deviations.

DISCUSSION

Reovirus-receptor interactions are required for apoptosis induction. Experiments described in this report demonstrate that reovirus induces apoptosis in infected L cells. We found that reovirus strains T1L and T3D differ in their capacity to trigger apoptosis, and we used T1L $\times$ T3D reassortant viruses to identify the viral S1 gene as the primary determinant of strain-specific differences in apoptosis induction. The finding that UV-inactivated reovirus is capable of triggering apoptosis suggests that the S1 gene product σ 1 is responsible for determining the observed differences in the efficiency with which T1L and T3D induce apoptosis. This conclusion is supported further by the observation that serotype 3 reovirus strains that have identical predicted σ 1s amino acid sequences, but variable σ 1 sequences (18), differ dramatically in their capacity to induce apoptosis (68). These results, and those obtained with monoclonal anti- σ 1 antibody 9BG5, suggest that binding of reovirus to cellular receptors by viral attachment protein σ 1 is necessary for induction of apoptosis. Virus-receptor interactions also have been postulated to play a role in human immunodeficiency virus-mediated priming of CD4⁺ T cells for apoptosis (1, 36, 38); however, in contrast to human immunodeficiency virus-induced apoptosis, reovirus can trigger apoptosis in the absence of either viral replication or costimulatory molecules.

Although UV-inactivated virions can induce apoptosis, the number of virion particles required for maximal effect is approximately 100-fold greater than that for replication-competent virions. The percentage of AO-stained cells was maximal at 12 h after adsorption of high concentrations of UV-inactivated virions compared with the percentage after lower inocula of replication-competent virions. This finding suggests

that viral replication increases the viral inoculum to a sufficient threshold to trigger apoptosis. If this threshold is achieved by using a high MOI, such as that used in our experiments with UV-inactivated virions, apoptosis may occur with faster kinetics because additional amplification of the viral signal through replication is not required. This also is supported by our finding that monoclonal antibodies that inhibit viral growth also inhibit apoptosis induced by low viral inocula.

The mechanism by which the binding of reovirus to L cells leads to apoptosis has not been defined, although it may be related to its capacity to inhibit cellular DNA synthesis. It has been suggested that reovirus inhibition of cellular DNA synthesis is triggered by activation of a receptor-linked signaling pathway (26). Like apoptosis, inhibition of host-cell DNA synthesis is induced by UV-inactivated, replication-incompetent reovirus virions (26, 55, 57). Reovirus-induced inhibition of DNA synthesis also is associated with alterations in nuclear structure, including clumping and margination of chromatin (9). This phenomenon has striking similarities to reovirus-induced apoptosis, suggesting that the two processes are linked. Strain-specific differences in reovirus-mediated DNA synthesis inhibition also are observed, and these differences are determined by the S1 gene (55, 68). The capacities of T1L $\times$ T3D reassortants and type 3 field isolate strains to induce apoptosis and to inhibit DNA synthesis are closely associated (68), providing further support for a relationship between these two phenomena.

The viral M2 gene also is linked to differences in apoptosis induction by reovirus. The association of the M2 gene segment with differences in the capacity of T1L and T3D to trigger apoptosis suggests that events in viral replication subsequent to

FIG. 6. Effect of anti-reovirus monoclonal antibodies on apoptosis induction. T3D was incubated at 4°C overnight with the monoclonal antibodies indicated at a final antibody concentration of 10 μ g/ml. The virus-antibody mixture was then inoculated onto L cells at an MOI of 10 PFU per cell. Cells were stained with AO 48 h after viral adsorption. The results shown are the mean percentages of apoptotic cells. Error bars indicate standard errors of the means.

engagement of cellular receptors are also necessary for induction of apoptosis. The M2 gene encodes the viral outer-capsid protein $\mu 1/\mu 1c$ (41, 45). During viral disassembly in cellular endosomes, the $\mu 1/\mu 1c$ protein is proteolytically processed (4, 10, 59, 63), and the processed form of the protein is postulated to mediate penetration of infecting virions into the cytosol (39, 47, 66). The M2 gene product $\mu 1/\mu 1c$ may play a role in apoptosis induction by affecting cross-linking of reovirus receptors or altering membrane architecture.

Some T1L $\times$ T3D reassortant viruses were more effective in inducing apoptosis than either of the parental strains. Although the viral S1 and M2 genes were linked statistically with differences in the capacity of reovirus reassortants to induce apoptosis, no other genes or gene combinations were found to be statistically associated with the group of reassortants that induced apoptosis more efficiently than the parental T3D strain. In other studies, some reovirus reassortants were more efficient than the parental strains from which they were derived in such diverse biologic properties as growth in murine intestinal tissue (3), virulence after oral inoculation into neonatal mice (52), transmission of virus between littermates (31), induction of myocarditis (58), and enhancement of hemagglutination after chymotrypsin treatment (46). In our studies, the capacity of certain reassortants to induce apoptosis more efficiently than T3D suggests that certain heterologous combinations of viral proteins can facilitate apoptosis, perhaps by affecting events in the reovirus replication cycle mediated by the S1 and M2 genes.

Apoptosis and viral infection. Apoptosis has been shown to be induced by many viruses; however, it is not clear whether this process functions principally as a host defense mechanism, as a virus-triggered event that facilitates viral replication, or as an inadvertent by-product of the virus-cell interaction. These issues may not be clarified until the role of virus-induced apoptosis in vivo is understood. From the perspective of the host, destruction of virus-infected cells before maximal virus assembly has been completed might serve as a nonspecific defense against viral infection (12, 40). Apoptotic cell death also results in a more subdued inflammatory response in comparison to necrosis (13), which may result in less collateral tissue damage from bystander injury. Such limited injury would be clearly beneficial to the host in organs such as the brain, an important site of reovirus infection in vivo (67, 69, 75, 76), in which critical target cells form a nonrenewable population.

Reoviruses infect transformed cells more efficiently than their normal counterparts (20). Perhaps, as a prerequisite for replication, reovirus infection stimulates unscheduled division of target cells, which in turn triggers these cells to undergo apoptosis. A similar mechanism for apoptosis induction has been proposed for adenovirus (50, 78, 79) and human papilloma virus (16). By binding certain types of cellular receptors, it is possible that reovirus activates cellular factors necessary for its replication that also are capable of inducing apoptosis. This strategy would provide a novel mechanism for a virus to prepare its host cell to support viral replication. Induction of apoptosis would not be expected to limit viral growth if the time required to complete the viral replication program is less than that required to trigger apoptosis, as is the case for reovirus infection of L cells. Understanding the signalling pathways used by viruses, like reovirus, to trigger apoptosis will contribute important information about mechanisms by which viruses produce cellular injury and cause disease.

ACKNOWLEDGMENTS

We are grateful to Joseph Winters for statistical analysis of our reassortant data and for advice concerning the application of appropriate

statistical tests for data analysis. Derall Willis and Virginia Meadow provided expert technical support and assistance. We express our appreciation to Jim Chappell, Patrick Green, Larry Kerr, and Earl Ruley for careful review of the manuscript.

This work was supported by a Merit Review Award from the Department of Veterans Affairs (K.L.T.), the core tissue culture and media facilities of the University of Colorado Cancer Center (K.L.T.), an NIH virology-molecular biology training grant (K.L.T. and S.M.O.), Public Health Service Award AI32539 from the National Institute of Allergy and Infectious Diseases (T.S.D.), and the Elizabeth B. Lamb Center for Pediatric Research (S.E.R. and T.S.D.).

REFERENCES

1. Banda, N. K., J. Bernier, D. K. Kurahara, R. Kurrle, N. Haigwood, R. P. Sekaly, and T. H. Finkel. 1992. Crosslinking CD4 by human immunodeficiency virus gp120 primes T cells for activation-induced apoptosis. *J. Exp. Med.* **176**:1099–1106.
2. Belli, B. A., and C. E. Samuel. 1991. Biosynthesis of reovirus-specified polypeptides: expression of reovirus S1 encoded sigma 1NS protein in transfected and infected cells as measured with serotype specific polyclonal antibody. *Virology* **185**:698–709.
3. Bodkin, D., and B. N. Fields. 1989. Growth and survival of reovirus in intestinal tissue: role of the L2 and S1 genes. *J. Virol.* **63**:1188–1193.
4. Borsa, J., M. D. Sargent, P. A. Lievaart, and T. P. Copps. 1981. Reovirus: evidence for a second step in the intracellular uncoating and transcriptase activation process. *Virology* **111**:191–200.
5. Brown, E. G., M. L. Nibert, and B. N. Fields. 1983. The L2 gene of reovirus serotype 3 controls the capacity to interfere, accumulate deletions and establish persistent infection, p. 275–287. *In* R. W. Compans and D. H. L. Bishop (ed.), *Double-stranded RNA viruses*. Elsevier, New York.
6. Burstin, S. J., D. R. Spriggs, and B. N. Fields. 1982. Evidence for functional domains on the reovirus type 3 hemagglutinin. *Virology* **117**:146–155.
7. Cashdollar, L. W., P. Blair, and S. Van Dyne. 1989. Identification of the sigma 1s protein in reovirus serotype 2-infected cells with antibody prepared against a bacterial fusion protein. *Virology* **168**:183–186.
8. Ceruzzi, M., and A. J. Shatkin. 1986. Expression of reovirus p14 in bacteria and identification in the cytoplasm of infected mouse L cells. *Virology* **153**:35–45.
9. Chaly, N., M. Johnstone, and R. Hand. 1980. Alterations in nuclear structure and function in reovirus-infected cells. *Clin. Invest. Med.* **2**:141–152.
10. Chang, C.-T., and H. J. Zweerink. 1971. Fate of parental reovirus in infected cells. *Virology* **46**:544–555.
11. Chou, J., and B. Roizman. 1992. The gamma1.34.5 gene of herpes simplex virus 1 precludes neuroblastoma cells from triggering total shutoff of protein synthesis characteristic of programmed cell death in neuronal cells. *Proc. Natl. Acad. Sci. USA* **89**:3266–3270.
12. Clouston, W. M., and J. F. Kerr. 1985. Apoptosis, lymphocytotoxicity and the containment of viral infections. *Med. Hypotheses* **18**:399–404.
13. Cohen, J. J. 1991. Programmed cell death in the immune system. *Adv. Immunol.* **50**:55–85.
14. Coombs, K. M., B. N. Fields, and S. C. Harrison. 1990. Crystallization of the reovirus type 3 Dealing core. Crystal packing is determined by the lambda 2 protein. *J. Mol. Biol.* **215**:1–5.
15. Cox, D. C., and J. E. Shaw. 1974. Inhibition of the initiation of cellular DNA synthesis after reovirus infection. *J. Virol.* **13**:760–761.
16. Crook, T., C. Fisher, P. J. Masterson, and K. H. Vousden. 1994. Modulation of transcriptional regulatory properties of p53 by HPV E6. *Oncogene* **9**:1225–1230.
17. Danis, C., T. Mabrouk, S. Garzon, and G. Lemay. 1993. Establishment of persistent reovirus infection in SC1 cells: absence of protein synthesis inhibition and increased levels of double-stranded RNA-activated protein kinase. *Virus Res.* **27**:253–265.
18. Dermody, T. S., M. L. Nibert, R. Bassel-Duby, and B. N. Fields. 1990. Sequence diversity in S1 genes and S1 translation products of 11 serotype 3 reoviruses. *J. Virol.* **64**:4842–4850.
19. Duke, R. C., and J. J. Cohen. 1992. Morphological and biochemical assays of apoptosis, p. 3.17.1–3.17.16. *In* J. E. Coligan (ed.), *Current protocols in immunology*. Wiley, New York.
20. Duncan, M. R., S. M. Stanish, and D. C. Cox. 1978. Differential sensitivity of normal and transformed human cells to reovirus infection. *J. Virol.* **28**:444–449.
21. Ensminger, W. D., and I. Tamm. 1969. Cellular DNA and protein synthesis in reovirus-infected L cells. *Virology* **39**:357–359.
22. Ernst, H., and A. J. Shatkin. 1985. Reovirus hemagglutinin mRNA codes for two polypeptides in overlapping reading frames. *Proc. Natl. Acad. Sci. USA* **82**:48–52.
23. Esolen, L. M., S. W. Park, J. M. Hardwick, and D. E. Griffin. 1995. Apoptosis as a cause of death in measles virus-infected cells. *J. Virol.* **69**:3955–3958.
24. Facchinetti, A., L. Tassarollo, M. Mazzocchi, R. Kingston, D. Collavo, and G. Biasi. 1991. An improved method for the detection of DNA fragmentation. *J. Immunol. Methods* **136**:125–131.
25. Furlong, D. B., M. L. Nibert, and B. N. Fields. 1988. Sigma 1 protein of

- mammalian reoviruses extends from the surfaces of viral particles. *J. Virol.* **62**:246–256.
26. Gaulton, G. N., and M. I. Greene. 1989. Inhibition of cellular DNA synthesis by reovirus occurs through a receptor-linked signaling pathway that is mimicked by antidiabetic, antireceptor antibody. *J. Exp. Med.* **169**:197–211.
  27. Gougeon, M., S. Garcia, J. Heeney, R. Tschopp, H. Lecouer, D. Guetard, V. Rame, C. Dauguet, and L. Montagnier. 1993. Programmed cell death in AIDS-related HIV and SIV infections. *AIDS Res. Hum. Retroviruses* **9**:553–563.
  28. Hinshaw, V. S., C. W. Olsen, N. Dybdahl-Sissoko, and D. Evans. 1994. Apoptosis: a mechanism of cell killing by influenza A and B viruses. *J. Virol.* **68**:3667–3673.
  29. Jacobs, B. L., and C. E. Samuel. 1985. Biosynthesis of reovirus-specified polypeptides: the reovirus s1 mRNA encodes two primary translation products. *Virology* **143**:63–74.
  30. Joklik, W. K. 1985. Recent progress in reovirus research. *Annu. Rev. Genet.* **19**:537–575.
  31. Keroack, M., and B. Fields. 1986. Viral shedding and transmission between hosts determined by reovirus L2 gene. *Science* **232**:1635–1638.
  32. Knipe, D. M. 1996. Virus-host-cell interactions, p. 273–300. *In* B. N. Fields, D. M. Knipe, P. M. Howley, et al. (ed.), *Virology*, 3rd ed. Raven Press, New York.
  33. Kudo, H., and A. F. Graham. 1966. Selective inhibition of reovirus induced RNA in L cells. *Biochim. Biophys. Acta* **24**:150–155.
  34. Lai, M.-H. T., J. J. Wrenne, and W. K. Joklik. 1973. The preparation of reovirus top component and its effect on host DNA and protein synthesis. *Virology* **54**:237–244.
  35. Laurent-Crawford, A. G., G. B. Krust, S. Muller, Y. Riviere, M.-A. Rey-Cuille, J.-M. Bechet, L. Montagnier, and A. G. Hovanessian. 1991. The cytopathic effect of HIV is associated with apoptosis. *Virology* **185**:829–839.
  36. Laurent-Crawford, A. G., B. Krust, Y. Riviere, C. Desgranges, S. Muller, M. P. Kiény, C. Dauguet, and A. G. Hovanessian. 1993. Membrane expression of HIV envelope glycoproteins triggers apoptosis in CD4 cells. *AIDS Res. Hum. Retroviruses* **9**:761–773.
  37. Lee, P. W. K., E. C. Hayes, and W. K. Joklik. 1981. Protein sigma 1 is the reovirus cell attachment protein. *Virology* **108**:156–163.
  38. Lu, Y.-Y., Y. Koga, K. Tanaka, M. Sasaki, G. Kimura, and K. Nomoto. 1994. Apoptosis induced in CD4⁺ cells expressing gp160 of human immunodeficiency virus type 1. *J. Virol.* **68**:390–399.
  39. Lucia-Jandris, P., J. W. Hooper, and B. N. Fields. 1993. Reovirus M2 gene is associated with chromium release from mouse L cells. *J. Virol.* **67**:5339–5345.
  40. Martz, E., and D. M. Howell. 1989. CTL: virus control cells first and cytolytic cells second? DNA fragmentation, apoptosis and the prelytic halt hypothesis. *Immunol. Today* **10**:79–86.
  41. McCrae, M. A., and W. K. Joklik. 1978. The nature of the polypeptide encoded by each of the ten double-stranded RNA segments of reovirus type 3. *Virology* **89**:578–593.
  42. Meyaard, L., S. A. Otto, R. R. Jonker, M. J. Mijster, R. P. M. Keet, and F. Miedema. 1992. Programmed death of T cells in HIV-1 infection. *Science* **257**:217–219.
  43. Montgomery, L. B., C. Y. Kao, E. Verdin, C. Cahill, and E. Maratos-Flier. 1991. Infection of a polarized epithelial cell line with wild-type reovirus leads to virus persistence and altered cellular function. *J. Gen. Virol.* **72**:2939–2946.
  44. Munemitsu, S. M., and C. E. Samuel. 1984. Biosynthesis of reovirus-specified polypeptides. Multiplication rate but not yield of reoviruses serotype 1 and 3 correlates with the level of virus mediated inhibition of cellular protein synthesis. *Virology* **136**:133–143.
  45. Mustoe, T. A., R. F. Ramig, A. H. Sharpe, and B. N. Fields. 1978. Genetics of reovirus: identification of the dsRNA segments encoding the polypeptides of the mu and sigma size classes. *Virology* **89**:594–604.
  46. Nibert, M. L., J. D. Chappell, and T. S. Dermody. 1995. Infectious subviral particles of reovirus type 3 Dearing exhibit a loss in infectivity and contain a cleaved $\sigma 1$ protein. *J. Virol.* **69**:5057–5067.
  47. Nibert, M. L., and B. N. Fields. 1992. A carboxy-terminal fragment of protein $\mu 1/\mu 1C$ is present in infectious subviral particles of mammalian reoviruses and is proposed to have a role in penetration. *J. Virol.* **66**:6408–6418.
  48. Nibert, M. L., L. A. Schiff, and B. N. Fields. 1996. Reoviruses and their replication, p. 1557–1596. *In* B. N. Fields, D. M. Knipe, P. M. Howley, et al. (ed.), *Virology*, 3rd ed. Raven Press, New York.
  49. Ramig, R. F., and R. L. Ward. 1991. Genomic segment reassortment in rotaviruses and other Reoviridae. *Adv. Virus Res.* **39**:163–207.
  50. Rao, L., M. Debbas, P. Sabbatini, D. Hockenbery, S. Korsmeyer, and E. White. 1992. The adenovirus E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins. *Proc. Natl. Acad. Sci. USA* **89**:7742–7746.
  51. Roner, M. R., and D. C. Cox. 1985. Cellular integrity is required for inhibition of initiation of cellular DNA synthesis by reovirus type 3. *J. Virol.* **53**:350–359.
  52. Rubin, D. H., and B. N. Fields. 1980. Molecular basis of reovirus virulence: role of the M2 gene. *J. Exp. Med.* **152**:853–868.
  53. Sarkar, G., J. Pelletier, R. Bassel-Duby, A. Jayasuriya, B. N. Fields, and N. Sonenberg. 1985. Identification of a new polypeptide coded by reovirus gene S1. *J. Virol.* **54**:720–725.
  54. Sharpe, A. H., L. B. Chen, and B. N. Fields. 1982. The interaction of mammalian reoviruses with the cytoskeleton of monkey CV-1 cells. *Virology* **120**:399–411.
  55. Sharpe, A. H., and B. N. Fields. 1981. Reovirus inhibition of cellular DNA synthesis: role of the S1 gene. *J. Virol.* **38**:389–392.
  56. Sharpe, A. H., and B. N. Fields. 1982. Reovirus inhibition of cellular RNA and protein synthesis: role of the S4 gene. *Virology* **122**:381–391.
  57. Shaw, J. E., and D. C. Cox. 1973. Early inhibition of cellular DNA synthesis by high multiplicities of infectious and UV-inactivated reovirus. *J. Virol.* **12**:704–710.
  58. Sherry, B., and M. A. Blum. 1994. Multiple viral core proteins are determinants of reovirus-induced acute myocarditis. *J. Virol.* **68**:8461–8465.
  59. Silverstein, S. C., C. Astell, D. H. Levin, M. Schonberg, and G. Acs. 1972. The mechanisms of reovirus uncoating and gene activation in vivo. *Virology* **47**:797–806.
  60. Silverstein, S. C., J. K. Christman, and G. Acs. 1976. The reovirus replication cycle. *Annu. Rev. Biochem.* **45**:375–408.
  61. Smith, R. E., H. J. Zweerink, and W. K. Joklik. 1969. Polypeptide components of virions, top component, and cores of reovirus type 3. *Virology* **39**:791–810.
  62. Strong, J. E., D. Tang, and P. W. K. Lee. 1993. Evidence that the epidermal growth factor receptor on host cells confers reovirus infection efficiency. *Virology* **197**:405–411.
  63. Sturzenbecker, L. J., M. Nibert, D. Furlong, and B. N. Fields. 1987. Intracellular digestion of reovirus particles requires a low pH and is an essential step in the viral infectious cycle. *J. Virol.* **61**:2351–2361.
  64. Takizawa, T., S. Matsukawa, Y. Higuchi, S. Nakamura, Y. Nakanishi, and R. Fukuda. 1993. Induction of programmed cell death (apoptosis) by influenza virus infection in tissue culture cells. *J. Gen. Virol.* **74**:2347–2355.
  65. Terai, C., R. S. Kornbluth, C. D. Pauza, D. D. Richman, and D. A. Carson. 1991. Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1. *J. Clin. Invest.* **87**:1710–1715.
  66. Tosteson, M. T., M. L. Nibert, and B. N. Fields. 1993. Ion channels induced in lipid bilayers by subviral particles of the nonenveloped mammalian reoviruses. *Proc. Natl. Acad. Sci. USA* **90**:10549–10552.
  67. Tyler, K. L., R. T. Bronson, K. B. Byers, and B. N. Fields. 1985. Molecular basis of viral neurotropism: experimental reovirus infection. *Neurology* **35**:88–92.
  68. Tyler, K. L., and T. S. Dermody. 1995. Unpublished observations.
  69. Tyler, K. L., and B. N. Fields. 1996. Reoviruses, p. 1597–1624. *In* B. N. Fields, D. M. Knipe, P. M. Howley, et al. (ed.), *Virology*, 3rd ed. Raven Press, New York.
  70. Verdin, E. M., E. Maratos-Flier, J. L. Carpentier, and C. R. Kahn. 1986. Persistent infection with a nontransforming RNA virus leads to impaired growth factor receptors and response. *J. Cell. Physiol.* **128**:457–465.
  71. Virgin, H. W., IV, R. Bassel-Duby, B. N. Fields, and K. L. Tyler. 1988. Antibody protects against lethal infection with the neurally spreading reovirus type 3 (Dearing). *J. Virol.* **62**:4594–4604.
  72. Virgin, H. W., M. A. Mann, B. N. Fields, and K. L. Tyler. 1991. Monoclonal antibodies to reovirus reveal structure/function relationships between capsid proteins and genetics of susceptibility to antibody action. *J. Virol.* **65**:6772–6781.
  73. Virgin, H. W., M. A. Mann, and K. L. Tyler. 1994. Protective antibodies inhibit reovirus internalization and uncoating by intracellular proteases. *J. Virol.* **68**:6719–6729.
  74. Weiner, H. L., K. A. Ault, and B. N. Fields. 1980. Interaction of reovirus with cell surface receptors. I. Murine and human lymphocytes have a receptor for the hemagglutinin of reovirus type 3. *J. Immunol.* **124**:2143–2148.
  75. Weiner, H. L., D. Drayna, D. R. Averill, and B. N. Fields. 1977. Molecular basis of reovirus virulence: the role of the S1 gene. *Proc. Natl. Acad. Sci. USA* **74**:5744–5748.
  76. Weiner, H. L., M. L. Powers, and B. N. Fields. 1980. Absolute linkage of virulence with central nervous system cell tropism of reovirus to hemagglutinin. *J. Infect. Dis.* **141**:509–616.
  77. Weiner, H. L., R. F. Ramig, T. A. Mustoe, and B. N. Fields. 1978. Identification of the gene coding for the hemagglutinin of reovirus. *Virology* **86**:581–584.
  78. White, E., R. Cipriani, P. Sabbatini, and A. Denton. 1991. Adenovirus E1B 19-kilodalton protein overcomes the cytotoxicity of E1A proteins. *J. Virol.* **65**:2968–2978.
  79. White, E., P. Sabbatini, M. Debbas, W. S. Wold, D. I. Kusher, and L. R. Gooding. 1992. The 19-kilodalton adenovirus E1B transforming protein inhibits programmed cell death and prevents cytolysis by tumor necrosis factor alpha. *Mol. Cell. Biol.* **12**:2570–2580.
  80. Wyllie, A. H., J. F. Kerr, and A. R. Currie. 1980. Cell death: the significance of apoptosis. *Int. Rev. Cytol.* **68**:251–306.
  81. Zarbl, H., and S. Millward. 1983. The reovirus multiplication cycle, p. 107–196. *In* W. K. Joklik (ed.), *The Reoviridae*. Plenum, New York.