



Reduced apoptosis in human intestinal cells cured of persistent poliovirus infection.

Karine Labadie, Aure Saulnier, Sandra Martin-Latil, Florence Colbère-Garapin

► To cite this version:

Karine Labadie, Aure Saulnier, Sandra Martin-Latil, Florence Colbère-Garapin. Reduced apoptosis in human intestinal cells cured of persistent poliovirus infection.. Journal of Virology, 2007, 81 (6), pp.3033-6. 10.1128/JVI.01960-06 . pasteur-00146511

HAL Id: pasteur-00146511

<https://pasteur.hal.science/pasteur-00146511>

Submitted on 16 Sep 2008

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1

2 **Reduced apoptosis in human intestinal cells**

3 **cured of persistent poliovirus infection**

4

5

6

7 Karine Labadie[†], Aure Saulnier[†], Sandra Martin-Latil and Florence Colbère-Garapin*

8

9 Virus Entérotropes et Stratégies Antivirales, Institut Pasteur, 25 rue du Dr Roux,
10 75724, Paris cedex 15, France.

11

12 Running title: Reduced apoptosis in cells cured of PV infection

13

14

15 Word count for the Abstract: 96

16 Word count for the text (without Acknowledgements): 1219

17 Number of Figures: 3

18

19 [†]**Present address:** Génétique Moléculaire des Virus Respiratoires, Institut Pasteur

20

21 ***Author for correspondence:**

22 Tel: (33) 1 45 68 87 64

23 Fax: (33) 1 40 61 33 67

24 E-mail: fcolbere@pasteur.fr

SUMMARY

Cells cured of persistent virus infection can be used to investigate cellular pathways of resistance to viral cytopathic effects. Persistent poliovirus (PV) infections were established in human intestinal Caco-2 cells and spontaneously cured cell cultures were obtained. Two cell clones, cl6 and b13, cured of type 3 PV mutant infection, and their parental Caco-2 cells were compared for susceptibility to PV infection, PV receptor CD155 expression, capacity to differentiate into polarized enterocytes, and PV-, staurosporine- and actinomycin D-induced apoptosis. Our results strongly suggest that cells partially resistant to apoptosis can be selected during persistent virus infection.

Cells cured of persistent virus infection can be used to investigate cellular pathways of resistance to virus infection (5, 6, 11, 23, 24, 28). The spontaneous cure of persistent virus infection *in vitro* has been described only rarely (5, 9, 15, 22). Poliovirus (PV), the prototype member of the enterovirus genus (25), is composed of a single-stranded positive-sense RNA enclosed in an icosahedral capsid. We have developed several models of persistent PV infection to elucidate the mechanisms of persistent infections and investigate cellular resistance to viral cytopathic effects (7). Mutated forms of the PV receptor, CD155, have been found in persistently PV-infected IMR-32 cells (4, 20). The allelic form with a threonine in position 67 (Thr67) of CD155 conferred on murine L cells a partial resistance to PV-induced apoptosis, with reference to the other form (Ala67) expressed in the parental IMR-32 cell line (13). HEp-2 cells cured of PV infection have a variety of phenotypes, often involving PV-CD155 interactions (5, 6). Persistent infections with PV mutants have also been established in human intestinal Caco-2 cells (15). We used PV type 3 mutant L2-2 (10), and Sabin 3 (S3) mutants H136-11, H442-11 and H637-11, isolated from a chronically infected hypogammaglobulinemic patient (17). Mutations differentiating these PV mutants and S3 have been published (15, 17). The mechanisms of the persistent infection in human intestinal cells involve viral and cellular determinants (15). Caco-2 cultures (20-80%) stopped excreting infectious virus between 1 month and 1 year post-infection (pi) (15).

Clonal cell populations were grown from well-isolated colonies of cured cells, as described (6), and their resistance to *de novo* PV type 3 infection was tested. The absence of PV RNA in the cured clones was first verified by RT-nested PCR, as described (24) (not shown). All cell clones were permissive to PV infection, but 2 of 5 were partially resistant to virus-induced cell lysis. These clones, cl6 and b13, cured of L2-2 and H442-11 infection respectively, were studied in comparison with parental Caco-2 cells. Cells (10^6) were seeded in duplicate in wells of 24-well plates in DMEM medium containing 10% fetal bovine serum,

and incubated overnight at 37 °C. Cells were then mock-infected or infected with a type 3 PV strain: S3, L2-2 or H442-11, at a multiplicity of infection (moi) of 10^{-2} infectious doses (ID_{50}) per cell. The percentage of cells surviving infection was determined 1, 2 and 3 days pi by staining cells with trypan blue. The resistance of the clones to lysis was highly significant 3 days pi: with all type 3 PV strains tested, more than 80% of parental Caco-2 cells were lysed whereas 60-100% of cl6 and 40-80% of b13 cells survived infection (Fig. 1a-1c). Similar results were obtained with infections with Sabin 1 and Sabin 2 PV strains (not shown), indicating that the resistance of cl6 and b13 cells to PV infection was not type-specific. As S3 multiplication was slightly delayed in the clones (Fig. 1d), the resistance of the clones was tested at a higher moi. At an moi of 10 ID_{50} per cell, S3 multiplication in cl6 was delayed by 1-2 h during the exponential phase. However, 24 h pi, viral yields were similar for the three cell types and cl6 cells were much more resistant than Caco-2 cells to S3-induced lysis (Fig. 1e and 1f). At this moi, b13 was more susceptible to infection than cl6, in agreement with data at low moi.

Low levels of CD155 expression may contribute to resistance to virus-induced cell lysis (8). We quantified the expression of CD155 at the surface of Caco-2, cl6 and b13 cells, over a period of 6 days between two cell passages. We used flow cytometry after indirect immunofluorescence labeling with the anti-CD155 monoclonal antibody 404 (16), as described (13, 15). More than 90% of cells of each cell type were brightly stained and the level of CD155 expression was slightly higher on the cured cells than on Caco-2 cells (Fig. 2a). This was confirmed normalizing the results for ICAM-1, detected with the control antibody 8.4A6 (Sigma) (Fig. 2b). Thus, the delay before the cytopathic effects in the clones was not due to a low level of CD155 expression.

We tested whether mutated forms of CD155 had been selected in cl6 and b13, because it has been shown that PV multiplication could occur without cell lysis in cells expressing

mutated CD155 (19). We determined the sequence of CD155 mRNA from these cells and from Caco-2 cells, in the region corresponding to the domain of CD155 that includes the PV-binding site (14, 18), as described (20, 24). Nucleotide positions that were mutated in CD155 mRNA from persistently PV-infected IMR-32 cells were not mutated in Caco-2, cl6 or b13 cells: they were Gly₃₉, Ala₆₇ and Arg₁₀₄. Also, no other mutation was detected, suggesting that the partial resistance of cl6 and b13 cells to PV-induced lysis was not due to the expression of CD155 forms mutated in the PV-binding domain. To exclude the role of other mutations in CD155, cells were infected with coxsackievirus B3 (CVB3), an enterovirus that uses the coxsackievirus and adenovirus receptor (3). Results were similar to those obtained with PV (Fig. 2c), confirming that the resistance of clones to virus-induced lysis did not depend on CD155.

We looked for a correlation between the partial resistance of clones to PV-induced lysis and their differentiation into polarized enterocytes, because enterocytes are more resistant to PV-induced lysis than undifferentiated Caco-2 cells (15, 27). No correlation between these phenotypes could be established (not shown).

PV infection induces apoptosis in several cell types, including Caco-2 cells (1, 2, 12, 21, 26). We therefore tested whether the resistance of clones to PV-induced lysis correlated with partial resistance to PV-induced apoptosis, by comparing the percentage of the three cell types with DNA fragmentation 3 days after infection with S3 at a moi of 10^{-2} ID₅₀/cell: terminal deoxynucleotidyltransferase-mediated dUTP-rhodamine nick end labeling (TUNEL) was used as described (12). In mock-infected cultures, less than 1 % of cells were TUNEL-positive (not shown). In infected cultures, viral antigens were detected by indirect immunofluorescence with a rabbit anti-PV type 3 antiserum (5). The percentages of cells that were viral antigen-positive did not differ significantly between the cell types (Fig. 3a). Interestingly, there were 3-fold fewer viral antigen- and TUNEL-double positive cells in

cultures of cl6 and b13 than in Caco-2 cultures, suggesting that the clones were more resistant to PV-induced apoptosis than Caco-2 cells (Fig. 3a).

To test whether cells cured of persistent infection were also partially resistant to apoptosis induced by non-viral inducers, we treated confluent cultures of each cell type with either actinomycin D (AMD) (8 μ M for 22 h) or staurosporine (2 μ M for 18 h). Like PV, both drugs activate the mitochondrial pathway of apoptosis, but staurosporine has a much more specific effect than AMD. DNA fragmentation was evaluated with a Cell Death Detection ELISA^{plus} kit (Roche). Cells of cl6 and b13 appeared to be about 2- and 3-fold more resistant to AMD- and staurosporine-induced apoptosis, respectively, than Caco-2 cells (Fig 3b and 3c). These results indicate that the partial resistance of the cured clones to apoptosis is not exclusively virus-specific.

In conclusion, our results strongly suggest that cells partially resistant to apoptosis can be selected during persistent virus infection. This may contribute to the mechanism of persistent infection.

We are grateful to I. Pelletier for her help and to J. Estaquier, A. Martin, C. Prehaud, S. Lay, S. Guillot and N. Escriou for helpful suggestions. We thank B. Blondel for critical reading of the manuscript and Marc Lopez for the gift of anti-CD155 monoclonal antibody. KL and AS were supported by fellowships from the Ministry (MENESR) and this work by grants from the Institut Pasteur (PTR 120) and Danone Research Centre Daniel Carasso.

References

1. **Agol, V. I., G. A. Belov, K. Bienz, D. Egger, M. S. Kolesnikova, L. I. Romanova, L. V. Sladkova, and E. A. Tolskaya.** 2000. Competing death programs in poliovirus-infected cells: commitment switch in the middle of the infectious cycle. *J Virol* **74**:5534-41.
2. **Ammendolia, M. G., A. Tinari, A. Calcabrini, and F. Superti.** 1999. Poliovirus infection induces apoptosis in Caco-2 cells. *Journal of Medical Virology* **59**:122-129.
3. **Bergelson, J. M., J. A. Cunningham, G. Droguett, E. A. Kurt-Jones, A. Krithivas, J. S. Hong, M. S. Horwitz, R. L. Crowell, and R. W. Finberg.** 1997. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science* **275**:1320-3.
4. **Blondel, B., F. Colbère-Garapin, T. Couderc, A. Wirotius, and F. Guivel-Benhassine.** 2005. Poliovirus, pathogenesis of poliomyelitis, and apoptosis. *Curr Top Microbiol Immunol* **289**:25-56.
5. **Borzakian, S., T. Couderc, Y. Barbier, G. Attal, I. Pelletier, and F. Colbère-Garapin.** 1992. Persistent poliovirus infection: establishment and maintenance involve distinct mechanisms. *Virology* **186**:398-408.
6. **Calvez, V., I. Pelletier, T. Couderc, N. Pavio-Guedo, B. Blondel, and F. Colbère-Garapin.** 1995. Cell clones cured of persistent poliovirus infection display selective permissivity to the wild-type poliovirus strain Mahoney and partial resistance to the attenuated Sabin 1 strain and Mahoney mutants. *Virology* **212**:309-322.
7. **Colbère-Garapin, F., I. Pelletier, and L. Ouzilou.** 2002. Persistent infections by picornaviruses, p. 437-450. *In* B. L. S. E. Wimmer (ed.), *Molecular Biology of Picornaviruses*. ASM Press, Washington.
8. **Colston, E. M., and V. R. Racaniello.** 1995. Poliovirus variants selected on mutant receptor-expressing cells identify capsid residues that expand receptor recognition. *J Virol* **69**:4823-4829.
9. **de la Torre, J. C., M. Davila, F. Sobrino, J. Ortin, and E. Domingo.** 1985. Establishment of cell lines persistently infected with foot-and-mouth disease virus. *Virology* **145**:24-35.
10. **Duncan, G., I. Pelletier, and F. Colbère-Garapin.** 1998. Two amino acid substitutions in the type 3 poliovirus capsid contribute to the establishment of

persistent infection in HEp-2c cells by modifying virus-receptor interactions. *Virology* **241**:14-29.

11. **Ebert, D. H., S. A. Kopecky-Bromberg, and T. S. Dermody.** 2004. Cathepsin B Is Inhibited in Mutant Cells Selected during Persistent Reovirus Infection. *J Biol Chem* **279**:3837-3851.
12. **Girard, S., T. Couderc, J. Destombes, D. Thiesson, F. Delpeyroux, and B. Blondel.** 1999. Poliovirus induces apoptosis in the mouse central nervous system. *J Virol* **73**:6066-72.
13. **Gosselin, A. S., Y. Simonin, F. Guivel-Benhassine, V. Rincheval, J. L. Vayssiere, B. Mignotte, F. Colbère-Garapin, T. Couderc, and B. Blondel.** 2003. Poliovirus-induced apoptosis is reduced in cells expressing a mutant CD155 selected during persistent poliovirus infection in neuroblastoma cells. *Journal of Virology* **77**:790-798.
14. **Koike, S., H. Horie, I. Ise, A. Okitsu, M. Yoshida, N. Iizuka, K. Takeuchi, T. Takegami, and A. Nomoto.** 1990. The poliovirus receptor protein is produced both as membrane-bound and secreted forms. *EMBO J.* **9**:3217-3224.
15. **Labadie, K., I. Pelletier, A. Saulnier, J. Martin, and F. Colbère-Garapin.** 2004. Poliovirus mutants excreted by a chronically infected hypogammaglobulinemic patient establish persistent infections in human intestinal cells. *Virology* **318**:66-78.
16. **Lopez, M., F. Jordier, F. Bardin, L. Coulombel, C. Chabannon, and P. Dubreuil.** 1999. Identification of a new class of Ig superfamily antigens expressed in hemopoiesis., p. 1081. *In* T. K. Kishimoto, H.; von dem Borne, A. E. G.; Goyert, SM; Mason, D.Y.; Miyasaka, M.; Moretta, M.; Okumura, K.; Shaw, S.; Springer, T. A.; Sugamura, K.; Zola, H. (ed.), *Leucocyte typing VI, White Cell Differentiation Antigens*. Garland.
17. **Martin, J., G. Dunn, R. Hull, V. Patel, and P. D. Minor.** 2000. Evolution of the Sabin strain of type 3 poliovirus in an immunodeficient patient during the entire 637-day period of virus excretion. *Journal of Virology* **74**:3001-3010.
18. **Mendelsohn, C. L., E. Wimmer, and V. R. Racaniello.** 1989. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence and expression of a new member of the immunoglobulin superfamily. *Cell* **56**:855-865.
19. **Morrison, M. E., Y. J. He, M. W. Wien, J. M. Hogle, and V. R. Racaniello.** 1994. Homolog-scanning mutagenesis reveals poliovirus receptor residues important for virus binding and replication. *J Virol* **68**:2578-88.

20. **Pavio, N., T. Couderc, S. Girard, J. Y. Sgro, B. Blondel, and F. Colbère-Garapin.** 2000. Expression of mutated poliovirus receptors in human neuroblastoma cells persistently infected with poliovirus. *Virology* **274**:331-342.
21. **Romanova, L. I., G. A. Belov, P. V. Lidsky, E. A. Tolskaya, M. S. Kolesnikova, A. G. Evstafieva, A. B. Vartapetian, D. Egger, K. Bienz, and V. I. Agol.** 2005. Variability in apoptotic response to poliovirus infection. *Virology* **331**:292-306.
22. **Ron, D., and J. Tal.** 1986. Spontaneous curing of a minute virus of mice carrier state by selection of cells with an intracellular block of viral replication. *J Virol* **58**:26-30.
23. **Sanchez, A. B., M. Perez, T. Cornu, and J. C. de la Torre.** 2005. RNA interference-mediated virus clearance from cells both acutely and chronically infected with the prototypic arenavirus lymphocytic choriomeningitis virus. *J Virol* **79**:11071-11081.
24. **Saulnier, A., I. Pelletier, K. Labadie, and F. Colbère-Garapin.** 2006. Complete cure of persistent virus infections by antiviral siRNAs. *Molecular Therapy* **13**:142-150.
25. **Stanway, G., T. Hovi, N. Knowles, and T. Hyypiä.** 2002. Molecular and biological basis of picornavirus taxonomy, p. 17-26. *In* B. L. Semler and E. Wimmer (ed.), *Molecular biology of picornaviruses*. ASM Press, Washington D.C.
26. **Tolskaya, E. A., L. I. Romanova, M. S. Kolesnikova, T. A. Ivannikova, E. A. Smirnova, N. T. Raikhlin, and V. I. Agol.** 1995. Apoptosis-inducing and apoptosis-preventing functions of poliovirus. *J Virol* **69**:1181-9.
27. **Tucker, S. P., C. L. Thornton, E. Wimmer, and R. W. Compans.** 1993. Vectorial release of poliovirus from polarized human intestinal epithelial cells. *J. Virol.* **67**:4274-4282.
28. **Vallbracht, A., L. Hofmann, K. G. Wurster, and B. Flehmig.** 1984. Persistent infection of human fibroblasts by hepatitis A virus. *J Gen Virol* **65**:609-615.

Legend to Figure 1.

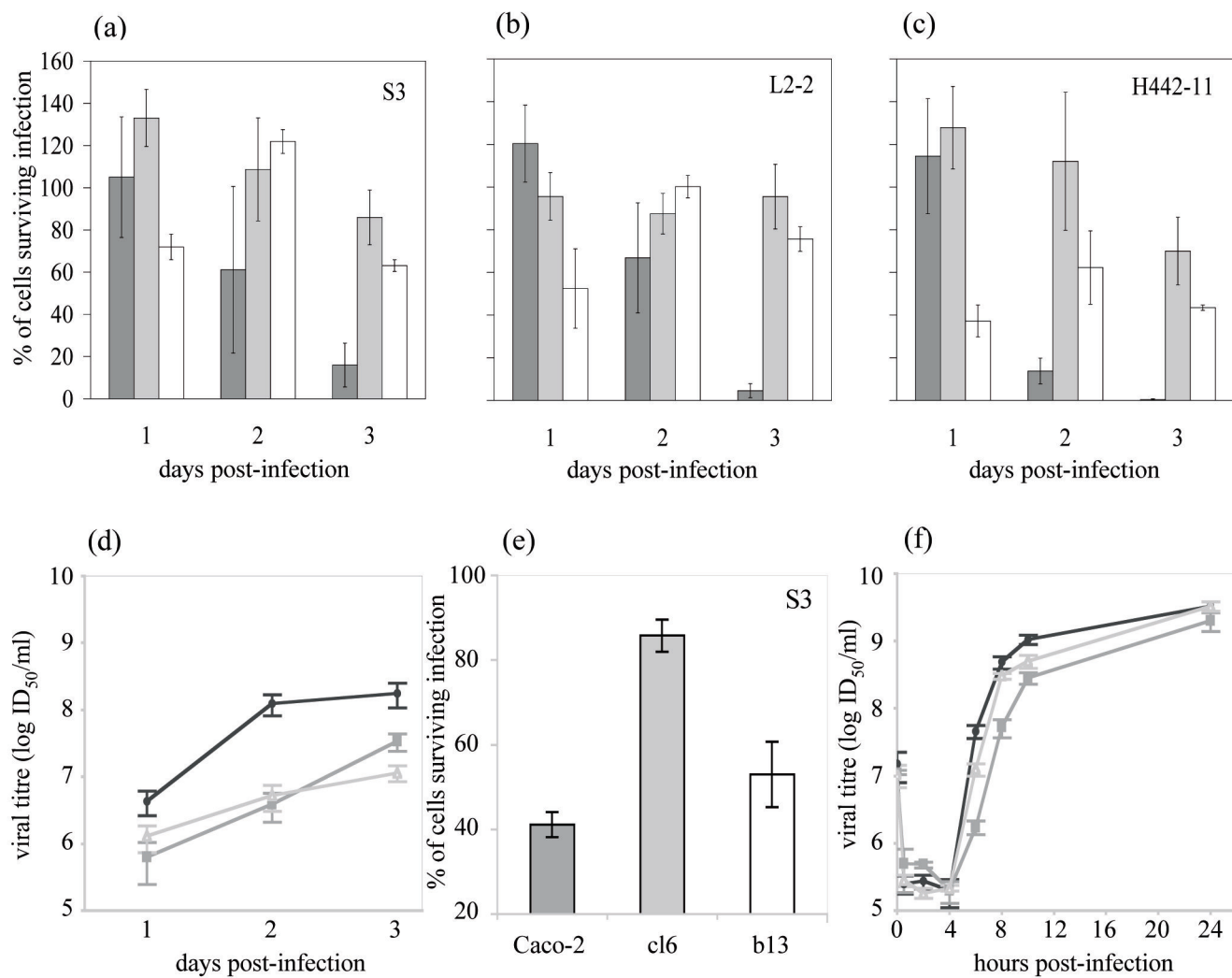
Partial resistance of Caco-2-cell-derived clones cl6 and b13 to PV-induced cell lysis after *de novo* infection. PV type 3 strains Sabin 3 (S3), L2-2 and H442-11 were used at an moi of 10^{-2} (a-d), or 10 ID₅₀/cell (e, f). Caco-2 (dark gray), cl6 (light gray) and b13 (white) cells were infected with the indicated virus strains and the number of surviving cells was determined by trypan blue exclusion, 1, 2 and 3 days pi at the moi of 10^{-2} (a-c) and 1 day pi at the moi of 10 (e). The results were obtained by calculating surviving cells in infected cultures as a percentage of those in mock-infected homologous cultures, for the same day. S3 growth curves in Caco-2 (circles), cl6 (squares) and b13 (triangles) cells infected at moi of 10^{-2} (d) and 10 ID₅₀/cell (f) are shown. The results are means $\pm$ SEM from at least four cultures from two independent experiments.

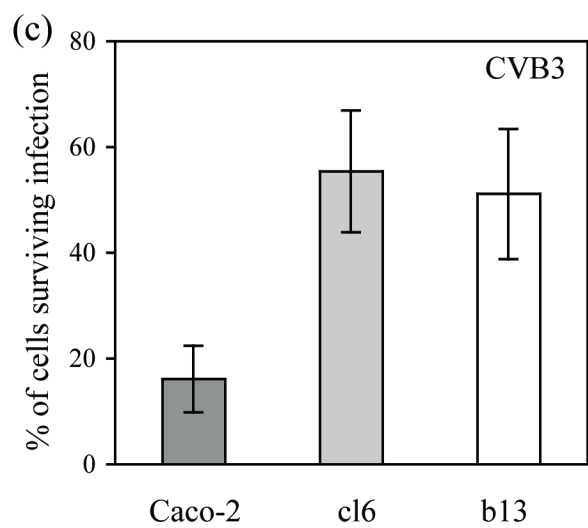
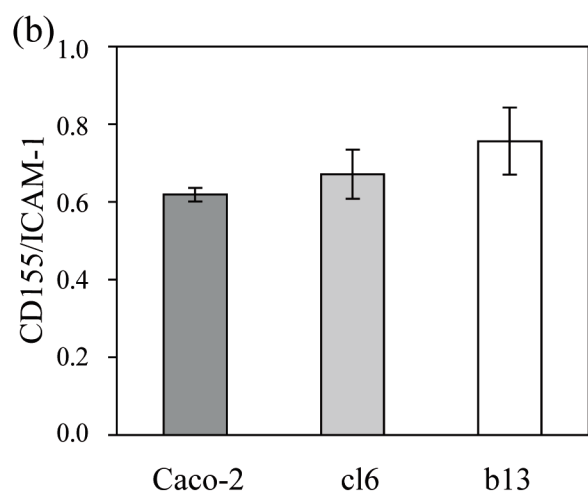
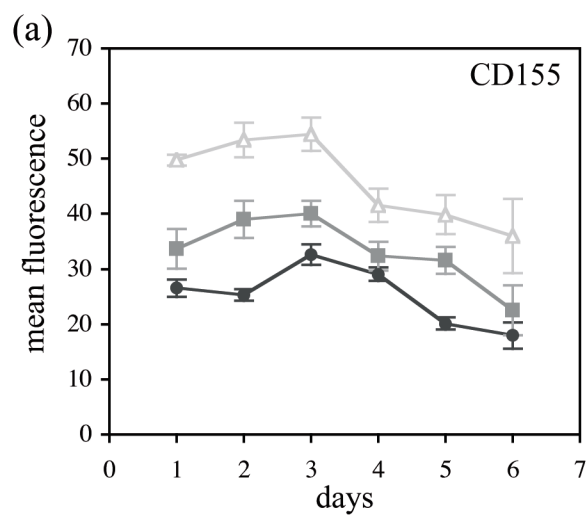
Legend to Figure 2.

The resistance of clones to virus-induced lysis did not depend on CD155 expression. The level of CD155 expression at the surface of Caco-2 (circles), cl6 (squares) and b13 (triangles) cells was determined by indirect immunofluorescence and the mean fluorescence per positive cell was quantified by flow cytometry in arbitrary units, 1 to 6 days after cell passage by trypsinization (a). The ratio of CD155 to ICAM-1 expression, used as a reference, is shown for the three cell types (b). Partial resistance of Caco-2-cell-derived clones cl6 and b13 to cell lysis after infection with CVB3 (c). Caco-2 (dark gray), cl6 (light gray) and b13 (white) cells were infected at an moi of 10^{-2} ID₅₀/cell, and the number of surviving cells was determined by trypan blue exclusion 2 days pi. The results are means $\pm$ SEM from at least four cultures from two independent experiments.

Legend to Figure 3.

Cell clones cured of persistent PV infection were partially resistant to apoptosis. Percentage of viral antigen- and TUNEL-positive cells in Caco-2, cl6 and b13 cultures 3 days pi by PV Sabin 3 (moi of 10^{-2} ID₅₀/cell); about 300-400 cells were analyzed under the microscope for each culture (a). Specific enrichment in mono- and oligo-nucleosomes released into the cytoplasm of confluent Caco-2, cl6 and b13 cultures following treatment with actinomycin D (AMD, 8 μ M for 22 h) (b) or staurosporine (ST, 2 μ M for 18 h) (c). The enrichment factor was determined with a Cell Death Detection ELISA^{plus} kit (Roche). The results are means $\pm$ SEM from at least two independent experiments.





(a)

	% of positive cells		
	Caco-2	cl6	b13
PV antigens	74.9 (± 9.4)	69.0 (± 12.0)	67.0 (± 10.8)
Tunel	23.7 (± 2.6)	9.3 (± 3.8)	8.8 (± 3.2)

