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

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Reduced apoptosis in human intestinal cells cured of persistent poliovirus infection

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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.

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

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

61 and incubated overnight at 37 °C. Cells were then mock-infected or infected with a type 3 PV
62 strain: S3, L2-2 or H442-11, at a multiplicity of infection (moi) of 10^{-2} infectious doses 50
63 (ID_{50}) per cell. The percentage of cells surviving infection was determined 1, 2 and 3 days pi
64 by staining cells with trypan blue. The resistance of the clones to lysis was highly significant
65 3 days pi: with all type 3 PV strains tested, more than 80% of parental Caco-2 cells were
66 lysed whereas 60-100% of cl6 and 40-80% of b13 cells survived infection (Fig. 1a-1c).
67 Similar results were obtained with infections with Sabin 1 and Sabin 2 PV strains (not
68 shown), indicating that the resistance of cl6 and b13 cells to PV infection was not type-
69 specific. As S3 multiplication was slightly delayed in the clones (Fig. 1d), the resistance of
70 the clones was tested at a higher moi. At an moi of 10 ID_{50} per cell, S3 multiplication in cl6
71 was delayed by 1-2 h during the exponential phase. However, 24 h pi, viral yields were
72 similar for the three cell types and cl6 cells were much more resistant than Caco-2 cells to S3-
73 induced lysis (Fig. 1e and 1f). At this moi, b13 was more susceptible to infection than cl6, in
74 agreement with data at low moi.

75 Low levels of CD155 expression may contribute to resistance to virus-induced cell
76 lysis (8). We quantified the expression of CD155 at the surface of Caco-2, cl6 and b13 cells,
77 over a period of 6 days between two cell passages. We used flow cytometry after indirect
78 immunofluorescence labeling with the anti-CD155 monoclonal antibody 404 (16), as
79 described (13, 15). More than 90% of cells of each cell type were brightly stained and the
80 level of CD155 expression was slightly higher on the cured cells than on Caco-2 cells (Fig.
81 2a). This was confirmed normalizing the results for ICAM-1, detected with the control
82 antibody 8.4A6 (Sigma) (Fig. 2b). Thus, the delay before the cytopathic effects in the clones
83 was not due to a low level of CD155 expression.

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

86 mutated CD155 (19). We determined the sequence of CD155 mRNA from these cells and
87 from Caco-2 cells, in the region corresponding to the domain of CD155 that includes the PV-
88 binding site (14, 18), as described (20, 24). Nucleotide positions that were mutated in CD155
89 mRNA from persistently PV-infected IMR-32 cells were not mutated in Caco-2, cl6 or b13
90 cells: they were Gly_{39}, Ala_{67} and Arg_{104}. Also, no other mutation was detected, suggesting that
91 the partial resistance of cl6 and b13 cells to PV-induced lysis was not due to the expression of
92 CD155 forms mutated in the PV-binding domain. To exclude the role of other mutations in
93 CD155, cells were infected with coxsackievirus B3 (CVB3), an enterovirus that uses the
94 coxsackievirus and adenovirus receptor (3). Results were similar to those obtained with PV
95 (Fig. 2c), confirming that the resistance of clones to virus-induced lysis did not depend on
96 CD155.

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

101 PV infection induces apoptosis in several cell types, including Caco-2 cells (1, 2, 12,
102 21, 26). We therefore tested whether the resistance of clones to PV-induced lysis correlated
103 with partial resistance to PV-induced apoptosis, by comparing the percentage of the three cell
104 types with DNA fragmentation 3 days after infection with S3 at a moi of 10^{-2} ID_{50}/cell:
105 terminal deoxynucleotidyltransferase-mediated dUTP-rhodamine nick end labeling (TUNEL)
106 was used as described (12). In mock-infected cultures, less than 1 % of cells were TUNEL-
107 positive (not shown). In infected cultures, viral antigens were detected by indirect
108 immunofluorescence with a rabbit anti-PV type 3 antiserum (5). The percentages of cells that
109 were viral antigen-positive did not differ significantly between the cell types (Fig. 3a).
110 Interestingly, there were 3-fold fewer viral antigen- and TUNEL-double positive cells in

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

113 To test whether cells cured of persistent infection were also partially resistant to
114 apoptosis induced by non-viral inducers, we treated confluent cultures of each cell type with
115 either actinomycin D (AMD) (8 µM for 22 h) or staurosporine (2 µM for 18 h). Like PV, both
116 drugs activate the mitochondrial pathway of apoptosis, but staurosporine has a much more
117 specific effect than AMD. DNA fragmentation was evaluated with a Cell Death Detection
118 ELISA^{plus} kit (Roche). Cells of cl6 and b13 appeared to be about 2- and 3-fold more resistant
119 to AMD- and staurosporine-induced apoptosis, respectively, than Caco-2 cells (Fig 3b and
120 3c). These results indicate that the partial resistance of the cured clones to apoptosis is not
121 exclusively virus-specific.

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

125

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221

222 **Legend to Figure 1**.

223 Partial resistance of Caco-2-cell-derived clones cl6 and b13 to PV-induced cell lysis
224 after *de novo* infection. PV type 3 strains Sabin 3 (S3), L2-2 and H442-11 were used at an
225 moi of 10^{-2} (a-d), or 10 ID_{50}/cell (e, f). Caco-2 (dark gray), cl6 (light gray) and b13 (white)
226 cells were infected with the indicated virus strains and the number of surviving cells was
227 determined by trypan blue exclusion, 1, 2 and 3 days pi at the moi of 10^{-2} (a-c) and 1 day pi at
228 the moi of 10 (e). The results were obtained by calculating surviving cells in infected cultures
229 as a percentage of those in mock-infected homologous cultures, for the same day. S3 growth
230 curves in Caco-2 (circles), cl6 (squares) and b13 (triangles) cells infected at moi of 10^{-2} (d)
231 and 10 ID_{50}/cell (f) are shown. The results are means ± SEM from at least four cultures from
232 two independent experiments.

233

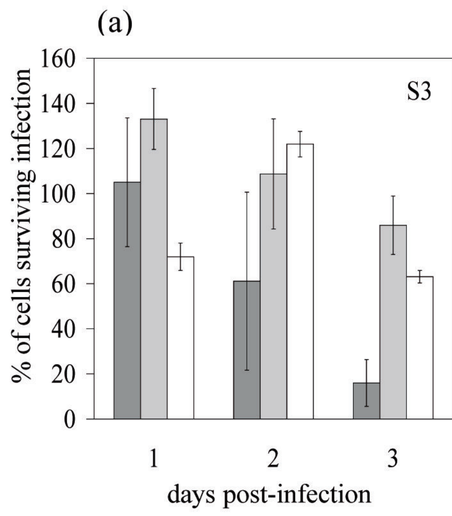
234 **Legend to Figure 2**.

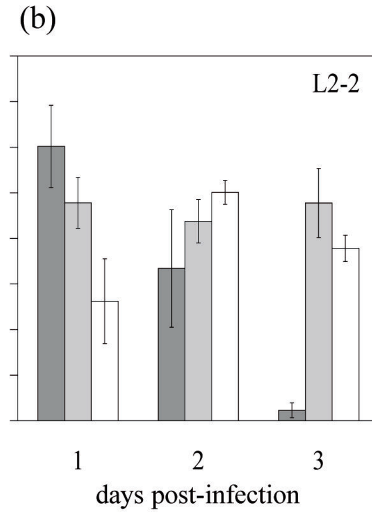
235 The resistance of clones to virus-induced lysis did not depend on CD155 expression.
236 The level of CD155 expression at the surface of Caco-2 (circles), cl6 (squares) and b13
237 (triangles) cells was determined by indirect immunofluorescence and the mean fluorescence
238 per positive cell was quantified by flow cytometry in arbitrary units, 1 to 6 days after cell
239 passage by trypsinization (a). The ratio of CD155 to ICAM-1 expression, used as a reference,
240 is shown for the three cell types (b). Partial resistance of Caco-2-cell-derived clones cl6 and
241 b13 to cell lysis after infection with CVB3 (c). Caco-2 (dark gray), cl6 (light gray) and b13
242 (white) cells were infected at an moi of 10^{-2} ID_{50}/cell, and the number of surviving cells was
243 determined by trypan blue exclusion 2 days pi. The results are means ± SEM from at least
244 four cultures from two independent experiments.

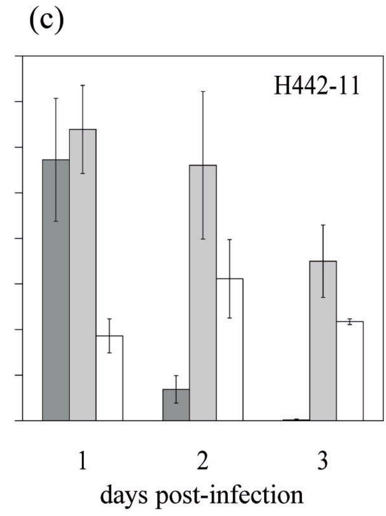
245

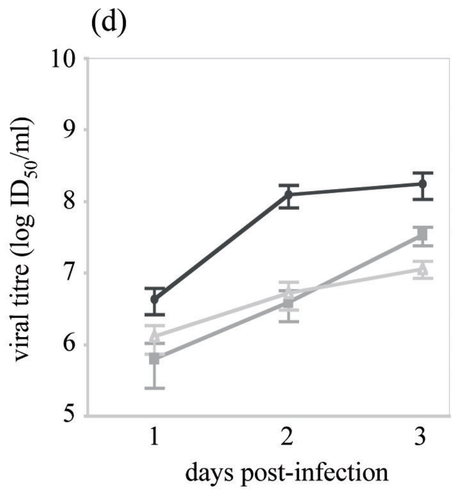
246 **Legend to Figure 3**.

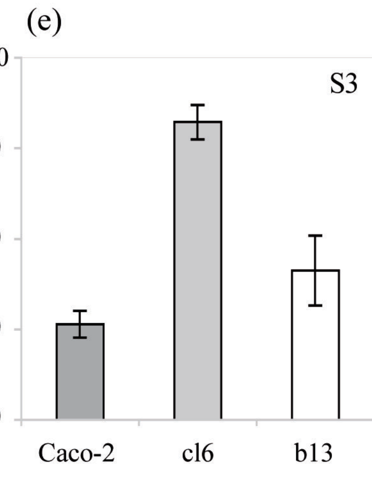
247 Cell clones cured of persistent PV infection were partially resistant to apoptosis.
248 Percentage of viral antigen- and TUNEL-positive cells in Caco-2, cl6 and b13 cultures 3 days
249 pi by PV Sabin 3 (moi of 10^{-2} ID_{50}/cell); about 300-400 cells were analyzed under the
250 microscope for each culture (a). Specific enrichment in mono- and oligo-nucleosomes
251 released into the cytoplasm of confluent Caco-2, cl6 and b13 cultures following treatment
252 with actinomycin D (AMD, 8 µM for 22 h) (b) or staurosporine (ST, 2 µM for 18 h) (c). The
253 enrichment factor was determined with a Cell Death Detection ELISA^{plus} kit (Roche). The
254 results are means ± SEM from at least two independent experiments.
255

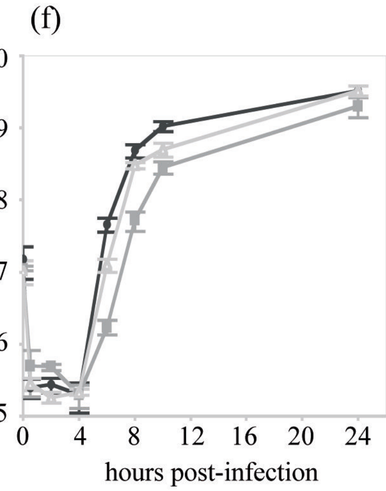

(a)
% of cells surviving infection
S3
days post-infection

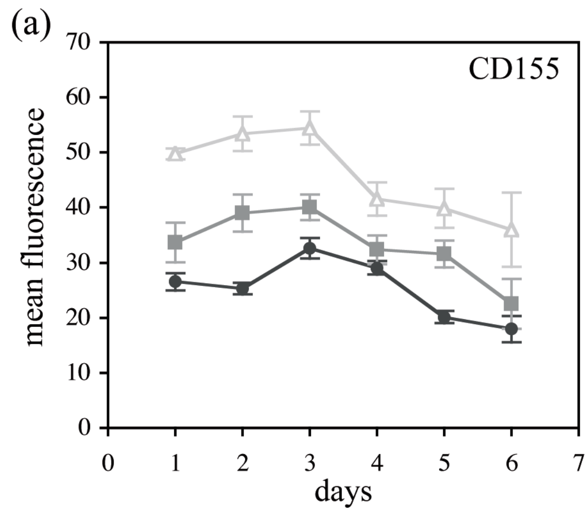

(b)
L2-2
days post-infection

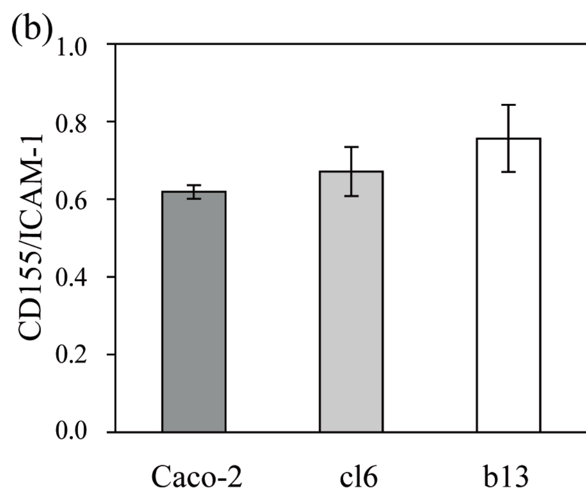

(c)
H442-11
days post-infection

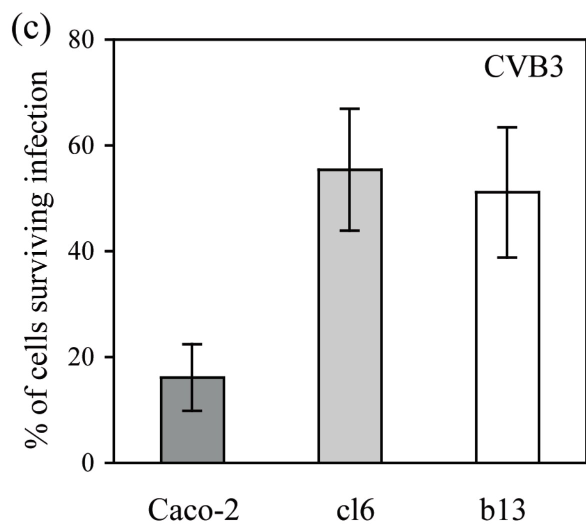

(d)
viral titre (log ID50/ml)
days post-infection


(e)
% of cells surviving infection
S3
Caco-2
cl6
b13


(f)
viral titre (log ID50/ml)
hours post-infection

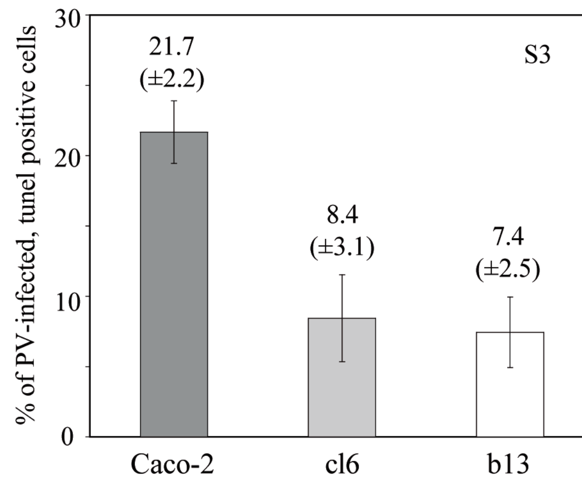

(a)
CD155
mean fluorescence
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
days


(b)
CD155/ICAM-1
1.0
0.8
0.6
0.4
0.2
0.0
Caco-2
cl6
b13

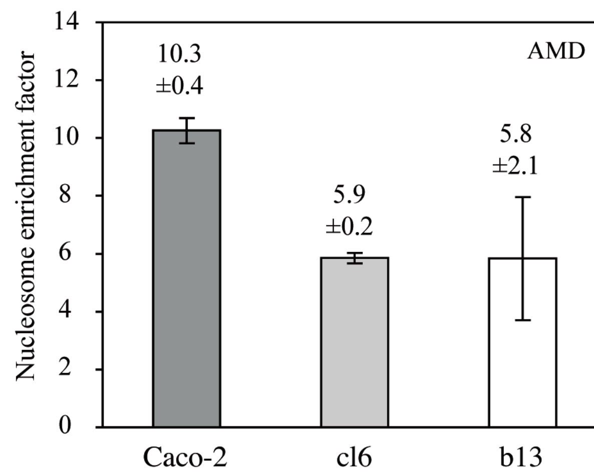

(c)
CVB3
% of cells surviving infection
80
60
40
20
0
Caco-2
cl6
b13

(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)



(b)



(c)