

Poliovirus mutants excreted by a chronically infected hypogammaglobulinemic patient establish persistent infections in human intestinal cells.

Karine Labadie, Isabelle Pelletier, Aure Saulnier, Javier Martin, Florence Colbère-Garapin

▶ **To cite this version:**

Karine Labadie, Isabelle Pelletier, Aure Saulnier, Javier Martin, Florence Colbère-Garapin. Poliovirus mutants excreted by a chronically infected hypogammaglobulinemic patient establish persistent infections in human intestinal cells.. Virology, 2004, 318 (1), pp.66-78. 10.1016/j.virol.2003.09.005 . pasteur-00146530

HAL Id: pasteur-00146530
https://pasteur.hal.science/pasteur-00146530

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.

Poliovirus mutants excreted by a chronically infected hypogammaglobulinemic patient establish persistent infections in human intestinal cells

Karine Labadie[1], Isabelle Pelletier[1], Aure Saulnier[1], Javier Martin[2] & Florence Colbère-Garapin[1]*

Unité de Neurovirologie et Régénération du Système Nerveux, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris cedex 15, France,[1] Division of Virology, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, United Kingdom[2].

Running title: Persistent PV infection in intestinal cells

*Correspondence Footnote:
Tel: (33) 1 45 68 87 64
Fax: (33) 1 45 68 87 80
e-mail: fcolbere@pasteur.fr

ABSTRACT

Immunodeficient patients whose gut is chronically infected by vaccine-derived poliovirus (VDPV) may excrete large amounts of virus for years. To investigate how poliovirus (PV) establishes chronic infections in the gut, we tested whether it is possible to establish persistent VDPV infections in human intestinal Caco-2 cells. Four type 3 VDPV mutants, representative of the viral evolution in the gut of a hypogammaglobulinemic patient over almost two years (J. Martin *et al.*, J. Virol., 2000, 74, 3001-10), were used to infect both undifferentiated, dividing cells, and differentiated, polarized enterocytes. A VDPV mutant excreted 36 days post-vaccination by the patient was lytic in both types of intestinal cell cultures, like the parental Sabin 3 strain. In contrast, three VDPVs, excreted 136, 442 and 637 days post-vaccination, established persistent infections both in undifferentiated cells and in enterocytes. Thus, viral determinants selected between day 36 and 136 conferred on VDPV mutants the capacity to infect intestinal cells persistently. The percentage of persistently VDPV-infected cultures was higher in enterocytes than in undifferentiated cells, implicating cellular determinants involved in the differentiation of enterocytes in persistent VDPV infections. The establishment of persistent infections in enterocytes was not due to poor replication of VDPVs in these cells, but was associated with reduced viral adsorption to the cell surface.

Key words: poliovirus, vaccine, enterovirus, picornavirus, persistent infection, enterocyte, intestine, Caco-2 cells, CD155.

INTRODUCTION

Poliovirus (PV) is an enterovirus that causes paralytic poliomyelitis (Landsteiner and Popper, 1909; Levaditi, 1913). The PV genome, a single-stranded RNA molecule of positive polarity, is protected by an icosahedral capsid composed of 60 copies of polypeptides VP1 to VP4 (Rossmann, 2002). The cellular receptor common to all three PV serotypes is CD155, a glycoprotein member of the immunoglobulin superfamily (Koike et al., 1990; Mendelsohn, Wimmer, and Racaniello, 1989). At the surface of susceptible cells, CD155 binds the virus and induces conformational transitions of the capsid that lead to viral uncoating at physiological temperature (Hogle and Racaniello, 2002). During these transitions, virions sedimenting at 160S externalize VP4 and the N-terminal part of VP1, thereby forming A particles sedimenting at 135S. Some A particles are released from cells at 37°C, while others remain cell-associated. The production of empty capsids sedimenting at 80S is believed to result from viral uncoating. CD155 is a major determinant of PV tissue tropism and it is present at the surface of human neurons (Gromeier and Nomoto, 2002; Pavio, Buc-Caron, and Colbère-Garapin, 1996). It is also present at the surface of human polarized enterocytes and M (microfold) cells, both on the apical face and more abundantly on the basolateral face (Iwasaki et al., 2002).

Poliomyelitis is efficiently prevented by either of two vaccines: the inactivated poliovaccine and the attenuated oral poliovaccine, OPV (Minor and Almond, 2002). The three Sabin strains of the OPV multiply to high titers in the human intestine, but normally do not invade the central nervous system. This vaccine is very safe. However, in immunodeficient persons with hereditary antibody deficiencies, Sabin PV strains of each of the three serotypes can continue replicating for long periods of times, more than 10 years, sometimes causing chronic poliomyelitis (Bellmunt et al., 1999; Buttinelli et al., 2003; Kew et al., 1998; Minor, 2001; Yoneyama et al., 2001). Prolonged virus multiplication in the intestine of patients causes long-term excretion of neurovirulent vaccine-derived PV (VDPV) in the stools. Recently, Martin et al. characterized Sabin 3-derived PV isolates, which were excreted by a hypogammaglobulinemic patient over a period of 637 days post-vaccination (Martin et al., 2000). Virus excretion appeared to cease spontaneously after this period. The isolates followed a main lineage of evolution, with a rate (2.78% per year) of nucleotide substitution at synonymous third-base codon positions that was very similar to that estimated for wild-type PV during person-to-person transmission (Kew et al., 1995). Some mutations in the

isolates are in regions of the capsid possibly involved in adaptation for growth in the human gut and virus persistence (Martin et al., 2000).

PV persistence has never been studied in intestinal cells, although it has been studied in various cell culture models, including neuronal and epidermoid cells. The molecular mechanisms of PV persistence vary greatly between different cell types, but modification of either CD155 expression or of PV-CD155 interactions is common to many persistent PV infections (Duncan and Colbère-Garapin, 1999; Duncan, Pelletier, and Colbère-Garapin, 1998; Pelletier, Duncan, and Colbère-Garapin, 1998). CD155 expression is reduced in cervix carcinoma HeLa cells (Kaplan and Racaniello, 1991) and in some clones of larynx carcinoma HEp-2c cells (Calvez et al., 1995) following persistent PV infection. In addition, PV mutants (PVpi) selected during persistent infection of neuroblastoma IMR-32 cells harbor capsid mutations in regions interacting with CD155 (Pelletier et al., 1998). The kinetics of adsorption of PVpi onto CD155-expressing cells and of elution, and the conformational modification of the capsid induced by CD155 at 37°C are often different from those of the parental lytic PV strains (Duncan and Colbère-Garapin, 1999). We have recently shown that 147S forms are produced upon interaction of particular PV mutants with either CD155-expressing cells or the soluble receptor PVR-IgG2a, at 0°C (Duncan, Pelletier, and Colbère-Garapin, 1998; Pelletier et al., 2003). These 147S forms have the same composition as the virion and may be a conformational intermediate between 160S virions and 135S A particles (Pelletier et al., 2003).

To develop a cell culture model relevant to chronic PV infection of the human intestine, we studied PV persistence in Caco-2 cells, derived from a human colon adenocarcinoma. Caco-2 cells grown on permeable filters express genes that are specifically expressed in villus absorptive enterocytes of the small intestine (Hidalgo, Raut, and Borchsardt, 1989; Pinto et al., 1983; Van Beers et al., 1995). Therefore, despite their colonic origin, Caco-2 cells differentiate into small intestine enterocyte-like cells (Costa de Beauregard et al., 1995; Pinto et al., 1983). We show here that several of the PV mutants excreted by an immunodeficient patient can establish persistent infection in human intestinal cells, and that there is a correlation between this capacity and reduced adsorption kinetics onto cells.

RESULTS

Characterization of the intestinal cell culture models.

To study the capacity of VDPV mutants to establish persistent infections in undifferentiated dividing intestinal cells and in polarized enterocyte-like cells, we first characterized our intestinal cell culture models. Caco-2 cells were seeded onto Transwell filters as described in Materials and Methods. The interactions of PV with CD155 are generally involved in persistent PV infections, and therefore we tested for CD155 expression at the cell surface, by indirect immunofluorescence with a monoclonal antibody specific for CD155 and secondary antibodies labeled with fluorescein isothiocyanate (FITC). As previously shown (Tucker et al., 1993a), CD155 was present on both faces of the Caco-2 cells, and more abundant on the basal face of both 3 day- and 14 day-old cultures (Fig. 1). Two to 4 day-old Caco-2 cells were used to study VDPV-CD155 interactions (see below). We verified that the average expression of CD155 at the cell surface was constant over this period of time by immunofluorescence and analysis with a fluorescence activated cell sorter (not shown). The structural protein F-actin, that is associated with the cytoskeleton and present in the microvilli of the brush border of polarized enterocytes, was detected by its specific binding to phalloïdin-alexa. F-actin was principally expressed at the basal face of 3-day old Caco-2 cells (Fig. 1, M). In contrast, it was principally expressed on the apical face of 14-day old cells, consistent with the presence of F-actin in brush border microvilli of 14-day old Caco-2 cells (Fig. 1, N). The presence of the brush border on the apical face of 14-day old Caco-2 cells was confirmed by labeling terminal fucose residues of the brush border glycocalyx with the lectin UEA1 conjugated to FITC, and by transmission electron microscopy (data not shown), as previously shown for enterocyte-like cells obtained by differentiation of a clone of Caco-2 cells (Ouzilou et al., 2002). The trans-epithelial resistance increased from about 400 to 750 ohms.cm^2 between days 3 and 14 of culture, indicating that 14 day-old cells formed tight monolayers, due to the presence of tight junctions. Thus, 14 day-old Caco-2 cells acquired characters of polarized enterocytes, in particular cell polarity, a brush border and tight junctions.

Persistent poliovirus infection in Caco-2 cells.

Seven isolates of type 3 VDPVs obtained from a hypogammaglobulinemic patient, chronically infected by Sabin 3-derived PV mutants over almost two years until the patient spontaneously stopped excreting virus, were characterized previously (Martin et al., 2000).

The number in the name of isolates (H36, H136, H307, H391, H442, H480 and H637) indicates the day post-vaccination when the virus was collected. The virus excreted by the patient on any one day could have been a mixture, and thus clones were obtained by plaque-purification, twice, and named H36-11 to H637-11. The genomic region encoding capsid proteins, which harbors all the determinants required to confer to PV a persistent phenotype in HEp-2c cells (Calvez et al., 1993; Duncan, Pelletier, and Colbère-Garapin, 1998), was sequenced for each of these viral clones. This genomic region in four of the clones (H36-11, H136-11, H442-11 and H637-11) contained no missense mutations with reference to the consensus sequence of the corresponding isolates. It thus seems probable that these viral clones correspond to the major virus populations in the respective isolates. These 4 clones correspond to the first and last isolates, and to two intermediate isolates, and therefore cover the entire period of the evolution of the VDPVs. The 3 other clones had either a supplementary mutation ($VP1_{30}$ Ala>Val in H307-11, and $VP3_{79}$ Ser>Met in H480-11) or lacked one missense mutation (at residue $VP1_{34}$ in H391-11). Other clones of the same isolates also differed from the respective consensus sequences. These clones may be quasi-species. Therefore only VDPV clones H36-11, H136-11, H442-11 and H637-11 were included in all experiments.

The capacity of the four viral clones H36-11, H136-11, H442-11 and H637-11 to establish persistent infections was tested in undifferentiated, 4 to 5 day-old Caco-2 cells, using as controls the parental Sabin 3 strain, that is lytic in HEp-2c cells, and a type 3 PVpi, L2-2, that establishes persistent infections in 100 % of HEp-2c cell cultures (Duncan, Pelletier, and Colbère-Garapin, 1998). During the first three weeks post-infection, cytopathic effects were detected in all infected Caco-2 cultures, and one month post-infection, a few growing cell colonies appeared in cultures infected with either PVpi L2-2 or one of VDPV H136-11, H442-11 or H637-11. Caco-2 cells persistently infected by VDPV mutants could be grown for at least 6 months. However, the percentage of persistently infected cultures was determined two months post-infection (Fig. 2). PVpi L2-2 established persistent infections in $47\pm12\%$ of Caco-2 cell cultures. The Sabin 3 strain and viral clone H36-11 - the first isolate - were entirely lytic (Fig. 2). H136-11, H442-11 and H637-11 established persistent infections in 8 to 17% of cultures (Fig. 2). Thus, only 3 of 4 VDPVs were persistent, implicating viral determinants in the establishment of persistent infections in Caco-2 cells. These determinants were acquired (or became dominant) in the patient after day 36 and were present in VDPVs from day 136 to day 637 post-vaccination.

In HEp-2c cells, H36-11 was lytic, and H136-11, H442-11 and H637-11 mutants established persistent infections in 61±16, 17±10 and 69±16% of HEp-2c cell cultures, respectively. Therefore, each VDPV mutant was either lytic or persistent in both HEp-2c and Caco-2 cells.

Interestingly, 20 to 80% of Caco-2 cell cultures persistently infected by the VDPV mutants stopped excreting infectious virus between 1 month and 1 year post-infection.

Persistent poliovirus infection in polarized enterocyte-like cells.

We investigated whether differentiation of Caco-2 cells affects the establishment of persistent infections. Caco-2 cell cultures were grown for two weeks on microporous membranes until they were differentiated into polarized enterocyte-like cells, and then they were infected with H36-11, H136-11, H442-11 and H637-11, the parental S3 strain and PVpi L2-2. Although the level of CD155 expression is higher on the basolateral than on the apical face of cells, the level on the apical face is sufficient to mediate efficient infection (Tucker et al., 1993a), and therefore cells were infected either by the apical or by the basolateral face (Fig. 3). Five days post-infection, apical and basolateral supernatants were collected and the virus titered: all cultures excreted virus, and the titers were above 10^5 infectious doses 50 per ml (ID_{50}/ml) in the apical chamber. Cytopathic effects were detected in all infected cultures. The percentage of persistently infected cultures was determined 4 weeks post-infection, because the filters were not appropriate to grow enterocyte-like cells for longer periods of time. The parental S3 strain and H36-11 were lytic, as in undifferentiated Caco-2 cells. PVpi L2-2 established persistent infections in more than 65% of infected cultures, and the clones H136-11, H442-11 and H637-11 in more than 75% of infected cultures, independently of the face of cells used for virus inoculation (Fig. 3). The percentage of persistently VDPV-infected cultures was higher in enterocyte-like cells than in 4-5 day-old Caco-2 cell cultures, one and two months post-infection, suggesting that cellular determinants involved in the differentiation of enterocytes also contribute to the establishment of persistent infections by the VDPV mutants. No spontaneous cure was observed in VDPV-infected enterocyte-like cells, even when these cells could be maintained for periods of time longer than one month.

One-step virus growth cycles in polarized enterocytes.

We tested whether multiplication of the persistent VDPV mutants (H136-11, H442-11, H637-11) was impaired in polarized enterocyte-like cells. One-step growth experiments were performed at a m.o.i. of 10 ID_{50} per cell with two of the persistent mutants (H136-11 and

H442-11) and with the parental lytic S3 strain. No significant difference was observed between the multiplication of the persistent VDPVs and that of the S3 strain: the exponential growth and the intracellular yields were comparable for the 3 viruses. Intracellular yields were high (about 10^9 ID_{50} per ml and per culture of 1.5 x 10^6 cells) (Fig. 4, upper panel). Virus liberation was also similar for all three viruses, and about 1000-fold more efficient from the apical face than from the basolateral face of cells, 48 h post-infection (Fig. 4, lower panel), in agreement with the results previously published for the Mahoney PV strain (Tucker et al., 1993b).

Reduced adsorption of the persistent VDPVs onto Caco-2 cells.

There are numerous mutations in the capsid proteins of the persistent VDPVs: some in the canyon ($VP2_{139}$, $VP2_{140}$), in or near antigenic site 3a ($VP1_{288}$), site 4 ($VP3_{79}$, $VP3_{116}$) or at the interface of protomers ($VP3_{91}$) (Martin et al., 2000). These residues may affect binding of VDPVs to CD155 and the plasticity of the viral capsid, respectively. Therefore, the early steps of the viral cycle were studied in Caco-2 cells for the three persistent VDPVs, the lytic VDPV H36-11 and the lytic S3 strain. The adsorption of H36-11 was more efficient than that of S3 (Fig. 5A), and similar to that of the wild-type strain T7/Leon used as an internal control (not shown). In contrast, the three persistent VDPVs had a lower adsorption than S3 onto Caco-2 cells (Fig. 5A). VDPVs H442-11 and H637-11, excreted late, had the lowest binding efficiency, about half that of S3. Thus, reduced adsorption correlated with the persistent phenotype of mutants H136-11, H442-11 and H637-11.

Virus elution and conformational transitions of the capsid

Increased elution or modified conformational transitions may contribute to the mechanism of the persistent infection, as previously shown for PVpi in HEp-2c cells (Duncan, Pelletier, and Colbère-Garapin, 1998; Pelletier, Duncan, and Colbère-Garapin, 1998). Therefore, these steps were studied and to compare the proportion of eluted particles regardless of the number of attached virions, values are expressed as a percentage of the total cell-associated radioactivity (Fig. 5B). The H442-11 and H637-11 viruses eluted from cells at 37°C faster and more efficiently than the lytic VDPV H36-11 and the S3 strain. The enhanced elution of H442-11 and H637-11 may thus contribute to the persistent phenotype of these viruses. However, the kinetics of elution of VDPV H136-11 was similar, and even slightly slower than that of S3. The VDPV H36-11 had the lowest elution rate (Fig. 5B) and its profile of elution was similar to that of the wild-type T7/Leon strain (not shown).

The uncoating transitions from 160S virions to 135S A particles were then analyzed by ultracentrifugation in sucrose gradients. Sedimentation profiles of cell-associated particles are shown for a 0 or 20 min incubation period at 37°C (Fig. 6). Like S3, all of the mutants sedimented at 160S after binding to Caco-2 cells for 3 h at 0°C, and the corresponding peak decreased after incubation at 37°C, leading to the formation of 135S A particles. The alteration ratio (i.e. the ratio of the area under the 135S peak to the area under the 160S peak) was between 0.3 and 0.4 for VDPVs H36-11, H136-11 and H637-11, and 0.5 for the control strain, S3 (Fig. 6). VDPV H442-11 had an alteration ratio almost twice as high as that of S3, and after 20 min the uncoating process of this VDPV appeared more complete than that of the other viruses tested. Thus, the extent of the uncoating transitions in 20 min did not correlate with the capacity of the VDPVs to establish persistent infections.

DISCUSSION

Immunodeficient patients whose gut is chronically infected by VDPVs can excrete large amounts of virus for years. Unfortunately, there is no convenient small animal model to investigate how PV establishes chronic infections in the gut. We therefore tested whether it is possible to establish persistent PV infections *in vitro* in human intestinal cells. The expression of CD155 was first verified at the surface of Caco-2 cell-derived enterocytes, and the differentiation of cells was monitored by examining the distribution of F-actin in the cells. In addition, the formation of a typical brush border was observed by transmission electron microscopy, and the presence of fucose residues in the glycocalyx was verified.

We have previously shown that the P1 region of PV type 3, the region that encodes the capsid proteins, harbors the main determinants involved in the phenotype persistence in epithelial-like HEp-2c cells (Duncan, Pelletier, and Colbère-Garapin, 1998). In this study, four sequential VDPV clones excreted by an immunodeficient individual and covering a 637-day period (Martin et al., 2000) were studied in terms of their ability to establish persistent infections in intestinal cell cultures. H36-11, the first isolate from the patient was, like the parental S3 strain, lytic in dividing 4 to 5 day-old Caco-2 cultures. Interestingly, H136-11 and two other mutants isolated later (H442-11 and H637-11) established persistent infections in 8 to 17% of Caco-2 cell cultures. The same VDPVs established persistent infections in HEp-2c cells. Thus, viral mutations acquired or selected between 36 and 136 days post-vaccination were responsible for the phenotype of persistence. Seven capsid residues in VP1 and VP2 differentiate H136-11, which is persistent, from H36-11 which is lytic (Table 1). They include

mutations at residues $VP2_{14}$ and $VP1_{288}$ close to residues $VP2_{13}$ and $VP1_{290}$ respectively, involved in the capacity of a type 3 PVpi to establish persistent infections in HEp-2c cells (Duncan, Pelletier, and Colbère-Garapin, 1998). Therefore, residues $VP2_{14}$ and $VP1_{288}$ may contribute to the capacity of H136-11, H442-11 and H637-11 to establish persistent infections in Caco-2 (and HEp-2c) cells. However, this remains to be confirmed, and the involvement of the other mutations cannot be excluded.

Viral determinants play a major role in the establishment of persistent infections both in Caco-2 and in HEp-2c cells. Spontaneous cures occurred in persistently VDPV-infected Caco-2 cells, as previously observed in persistently PVpi-infected HEp-2c cells (Calvez et al., 1995). Therefore, persistent infections in Caco-2 and HEp-2c cells seem to have several common features, that differ from those in cultured neuronal cells, in which viral determinants do not play the major role, and in which no spontaneous cure has ever been observed (Colbère-Garapin, Pelletier, and Ouzilou, 2002).

VDPV mutants H136-11, H442-11 and H637-11 established persistent infections in enterocyte-like cells as they did in young Caco-2 cell cultures, but with a higher frequency, i.e. in more than 75% of cultures. This suggests that the same viral determinants play a role in the establishment of persistent infections in both types of intestinal cell cultures, but that cellular determinants involved in the differentiation of enterocytes also contributed to persistence. No spontaneous cure was observed in VDPV-infected enterocytes, possibly because these cultures, unlike Caco-2 cells, could not be maintained for several months. Alternatively, the absence of cure in VDPV-infected enterocytes suggests either that cell division plays a role in the spontaneous cure of Caco-2 cells, or that the expression of cellular genes involved in enterocyte differentiation is not favorable to the cure of cells. If the absence of spontaneous cure in enterocyte-like cells is relevant to infection of fully differentiated enterocytes in patients, it is probable that these cells are normally eliminated *in vivo* when they are replaced by newly differentiated enterocytes.

Presumably several mechanisms are involved in persistence. The restriction of PV replication sometimes contributes to the mechanism of PV persistence (Borzakian et al., 1992; Calvez et al., 1995; Girard et al., 2002). However, the establishment of persistent infections in enterocyte-like cells was not due to poor replication of VDPVs in these cells: one-step growth cycles were similar for the lytic S3 strain and the persistent VDPVs H136-11 and H442-11, with high intracellular yields and efficient, polarized virus liberation by the apical face. The early steps of PVpi multiplication are generally modified in HEp-2c cells (Duncan, Pelletier, and Colbère-Garapin, 1998; Pelletier, Duncan, and Colbère-Garapin, 1998), and consequently

we studied adsorption, elution and uncoating transitions of VDPVs. The mutant H36-11, like T7/Leon (not shown), had an adsorption rate significantly higher than that of S3 (Fig. 5A). This may be related either to the reversion in H36-11 of an attenuating determinant, at residue $VP3_{91}$, toward the T7/Leon genotype, or to H36-11 residues $VP2_{186}$ Met and $VP1_{275}$ Thr (Table 1), the latter residue being close to the footprint of CD155 on the PV3 capsid (He et al., 2003). Interestingly, the adsorption rates of persistent VDPVs (H136-11, H442-11 and H637-11) were lower than those of the lytic strains (S3 and H36-11). The high elution rate of H442-11 and H637-11 may contribute to the phenotype of persistence of these viruses, as previously reported for a type 1 PVpi (Pelletier, Duncan, and Colbère-Garapin, 1998). However, the elution rate of H136-11 was slightly slower than that of S3, and yet H136-11 established persistent infections efficiently in enterocyte-like cells. As in the case of elution, there was no strict correlation between the efficiency of uncoating transitions and the capacity of VDPVs to establish persistent infections. No 147S particle was detected following adsorption of any of the VDPV mutants onto Caco-2 cells, confirming that there is no strict correlation between the formation or accumulation of these particles and PV persistence (Duncan and Colbère-Garapin, 1999; Pelletier et al., 1998).

Therefore, adsorption was the only early step potentially involved in the mechanism of persistence of the three persistent VDPVs in intestinal cell cultures. Although this reduced adsorption did not prevent a high level of viral replication, as mentioned above, it is possible that reduced interactions between persistent VDPVs and CD155 correlates with a low level of apoptosis in infected cells. Indeed, PV induces apoptosis in Caco-2 cells (Ammendolia et al., 1999) and we have recently shown that the frequency of PV-induced apoptosis is reduced when PV-CD155 interactions are affected by a mutation in the N-terminal domain of CD155, which interacts with PV (Gosselin et al., 2003). The establishment of a persistent VDPV infection may also depend on several viral factors. A mutation in the 3A coding region of a PV is associated with reduced Vero cell lysis following viral multiplication (Lama, Sanz, and Carrasco, 1998). Moreover, our results strongly suggest that cellular factors involved in the differentiation of enterocytes may contribute to the establishment of persistent infections in human intestinal cells. It would be particularly interesting to identify these factors and discover their role in persistent VDPV infection.

MATERIALS AND METHODS

Cells and viruses

The human colon adenocarcinoma cell line Caco-2 (passages 10 to 40) was grown at 37°C in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS). Enterocyte-like cells were obtained by seeding Caco-2 cells in the upper chamber of polyester Transwell-Clear filters (Corning Costar) with pores of 0.4 μm or 3 μm diameter, in DMEM supplemented with glucose (1 g/l) and 1% non-essential aminoacids (NEAA) in a humidified 9% CO_2 incubator.

We used the vaccinal attenuated P3/Leon $12a_1b$ Sabin strain (Sabin 3 or S3) and a PV mutant (PVpi), L2-2, derived from wild type P3/Leon/37 after 2.5 months of persistent infection in human neuroblastoma cell line IMR-32, and persistent in HEp-2c cells (Duncan, Pelletier, and Colbère-Garapin, 1998). The seven sequential Sabin 3-derived viruses (H36 to H637) were isolated from a hypogammaglobulinemic patient who had excreted virus for 637 days (Martin et al., 2000). We cloned each VDPV isolate twice by plaque purification on HEp-2c cells under an agarose (0.8%) overlay. All viral stocks were prepared on HEp-2c cells and virus infectivity was titered on these cells by an endpoint micromethod. The infectious titers are expressed per milliliter as the $\log_{10}$ of the highest dilution for which cytopathic effects and cell lysis were observed in 50% of wells 7 days after infection at 37°C. Titers varied between 0.05 and 5 x 10^8 ID_{50}/ml. The capsid protein-encoding region in each viral clone was sequenced.

Viral RNA sequencing

cDNA was synthesized exactly as previously described (Oprisan et al., 2002) using 4 antisense primers (C1484, C2474, C2658, C3586; the number indicating primer position in viral genome). The synthesized cDNA was then amplified with 10 pmol genomic primers (G614, G1913, G2402) and 2.6 U Enzyme mix high fidelity (Roche). Amplification involved 29 cycles of denaturation at 94°C for 15 sec, annealing at 45°C for 30 sec and elongation at 68°C for 2 min, followed by a final cycle of denaturation at 94°C for 15 sec, annealing at 45°C for 30 sec and elongation at 68°C for 7 min. PCR products were analyzed by electrophoresis in ethidium bromide-stained 0.8% agarose gels and then purified using the QIAquick Spin Purification (Qiagen), according to the manufacturer's instructions. The amplified DNA fragments were sequenced using the Big-Dye Terminator Cycle Sequencing

Ready Reaction Kit (Perkin Elmer Applied Biosystems) on the ABI Prism DNA 377 Sequencer (Perkin Elmer Applied Biosystems). Nucleotide sequences were aligned with CLUSTAL W (Thompson, Higgins, and Gibson, 1994).

Indirect immunofluorescence

Caco-2 cells seeded on Transwell filters for 3 or 14 days were fixed for 15 min at 20°C in 4% paraformaldehyde and then treated for 10 min with NH_4Cl (50 mM). Cells were incubated either with the anti-CD155 monoclonal antibody 404 (14 µg/ml) (Lopez et al., 1999), or with a control immunoglobulin G1 for 60 min at 20°C, washed twice with phosphate-buffered saline (PBS) -Ca^{2+} -Mg^{2+} containing 2% filtered FCS and then incubated for 60 min with anti-mouse immunoglobulin conjugated to FITC (15 µg/ml). Cells were washed again, then permeabilized for 2 min with Triton X100 (0.5%), and were incubated with phalloïdin-alexa (0.4 U/ml) for 30 min to stain F-actin. Filters were washed again and mounted in Vectashield and cells were viewed under a Leica confocal microscope with a X63 objective. Fluorescence (green and red channels) from basolateral to apical face was quantified with MetaMorph Offline *(Universal Imaging Corporation, version 4.6 r0)*.

Establishment of persistent infection in Caco-2 cells

Confluent 4 or 5-day-old Caco-2 cells, cultivated in 24-well plates, were washed with DMEM and then infected at a multiplicity of infection (m.o.i.) of about 10^{-4} ID_{50}/cell. Infected cells were grown at 37°C in a humidified 4% CO_2 incubator, in DMEM-2% FCS for the first week and then in DMEM-10% FCS for a minimum of 8 weeks, changing the medium once per week.

To induce Caco-2 cell differentiation into polarized enterocyte-like cells, Caco-2 cells were seeded at a density of 4.10^4 cells per cm^2 on tissue culture-treated polyester Transwell filters. Apical and basal media were replaced every 3 or 4 days for 14 days with DMEM-10% FCS until cells reached confluency and then with DMEM-2% FCS. Two weeks after seeding, cells were infected either from the apical or basolateral side as described above except that cells were grown in a humidified 9% CO_2 incubator for at least one month.

Single-cycle growth experiments

Enterocyte-like Caco-2 cells were infected, 2 weeks after seeding on filters by the apical face, at a m.o.i. of 10 ID_{50}/cell in 200 µl of DMEM for 30 min at 37°C. Cells were

washed three times and then incubated for 2, 4, 6, 12, 24 or 48 h at 37°C in 9% CO_2 atmosphere. For each time point, both apical and basolateral supernatants were collected. Intracellular virus was recovered by freezing and thawing, and all samples were clarified before titering virus on Caco-2 cells.

Preparation of radiolabeled virions

Confluent HEp-2c cells were infected at a m.o.i. of about 10 ID_{50}/cell and agitated for 30 min at 37°C. Medium supplemented with 2% FCS was then added, and cells were incubated for 2 h at 37°C. The medium was then changed to methionine- and cysteine-free medium for 30 min and 600 µCi of ^{35}S-methionine and ^{35}S-cysteine (TRAN^{35}S-LABEL; ICN) were added. Cells were lysed 24 to 48 h post-infection by freezing and thawing. Virus was clarified and purified from cytoplasmic extracts by CsCl density gradient centrifugation as previously described (Blondel et al., 1983; Pelletier et al., 1991). Purified virus was desalted by gel filtration on a PD-10 column (Pharmacia Biotech) and then stored at -80°C. As the specific activities of S3, PVpi L2-2 and VDPV mutants varied between 0.7 and 18 x 10^{-4} cpm/ID_{50}, the same m.o.i. (140 or 200 ID_{50}/cell) was used in experiments with radiolabeled viruses. The results of virus adsorption and elution are expressed as percentages of cell-associated radioactivity.

Virus adsorption onto Caco-2 cells

Virus adsorption was studied, as previously described (Duncan, Pelletier, and Colbère-Garapin, 1998; Pelletier, Duncan, and Colbère-Garapin, 1998), using 2 to 4-day-old Caco-2 cells in suspension on ice. Cells (0.75 x 10^6) were infected with radiolabeled virions at a m.o.i. of 140 ID_{50}/cell in 150 µl of DMEM-2%FCS. Infected cells were agitated on ice for 30, 60, 90, 120, 150, 180 and 210 min after which cells were washed twice with DMEM-2%FCS and once with PBS. Cell-associated radioactivity was determined by scintillation counting.

Particle elution from Caco-2 cells at 37°C

Undifferentiated Caco-2 cells (0.75 x 10^6) in suspension were infected, as previously described (Duncan, Pelletier, and Colbère-Garapin, 1998; Pelletier, Duncan, and Colbère-Garapin, 1998), with radiolabeled virions at a m.o.i. of 140 ID_{50}/cell in 150 µl of ice-cold DMEM-2%FCS. After 2.5 h of adsorption, infected cells were washed twice and incubated for various times (0, 15, 30, 60, 90, 120 and 150 min) in 3 ml of DMEM-2%FCS preheated to

37°C. Cells were then washed once with ice-cold PBS and cell-associated radioactivity was determined by scintillation counting.

Capsid conformational transitions

Undifferentiated Caco-2 cells (0.5×10^7) in suspension were infected with radiolabeled virions at a m.o.i. of 200 ID_{50}/cell for 3h at 0°C and then treated exactly as previously described (Pelletier, Duncan, and Colbère-Garapin, 1998). Briefly, Caco-2 cells were incubated at 37°C for either 0 or 20 min and lysed. The 20 min time point was chosen because at this time, about 50% of the capsids of the reference S3 strain were transformed into uncoating intermediates. The lysates were layered onto 15 to 30% (w/v) sucrose gradients that were centrifuged in a Kontron TST 41.14 rotor for 2h at 40,000 rpm at 4°C.

Acknowledgements. We would like to thank Dr. Marc Lopez for providing the anti-CD155 monoclonal antibody 404. We are grateful to B. Blondel and B. Baron for critical reading of the manuscript. We thank E. Perret (Centre d'Imagerie Dynamique) for help with confocal microscopy and C. Bronnert (Plate-Forme de Microscopie électronique) for help with electron microscopy. This work was supported by grants from the Pasteur Institute.

REFERENCES

Ammendolia, M. G., Tinari, A., Calcabrini, A., and Superti, F. (1999). Poliovirus infection induces apoptosis in CaCo-2 cells. *Journal of Medical Virology* **59,** 122-129.

Bellmunt, A., May, G., Zell, R., Pring-Akerblom, P., Verhagen, W., and Heim, A. (1999). Evolution of poliovirus type I during 5.5 years of prolonged enteral replication in an immunodeficient patient. *Virology* **265,** 178-184.

Blondel, B., Akacem, O., Crainic, R., Couillin, P., and Horodniceanu, F. (1983). Detection by monoclonal antibodies of an antigenic determinant critical for poliovirus neutralization present on VP1 and on heat-inactivated virions. *Virology* **126,** 707-710.

Borzakian, S., Couderc, T., Barbier, Y., Attal, G., Pelletier, I., and Colbère-Garapin, F. (1992). Persistent poliovirus infection: establishment and maintenance involve distinct mechanisms. *Virology* **186,** 398-408.

Buttinelli, G., Donati, V., Fiore, S., Marturano, J., Plebani, A., Balestri, P., Soresina, A. R., Vivarelli, R., Delpeyroux, F., Martin, J., and Fiore, L. (2003). Nucleotide variation in Sabin type 2 poliovirus from an immunodeficient patient with poliomyelitis. *J. Gen. Virol.* **84,** 1215-1221.

Calvez, V., Pelletier, I., Borzakian, S., and Colbère-Garapin, F. (1993). Identification of a region of the poliovirus genome involved in persistent infection of HEp-2 cells. *J. Virol.* **67,** 4432-4435.

Calvez, V., Pelletier, I., Couderc, T., Pavio-Guedo, N., Blondel, B., and Colbère-Garapin, F. (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.

Colbère-Garapin, F., Pelletier, I., and Ouzilou, L. (2002). Persistent infections by picornaviruses. *In* "Molecular Biology of Picornaviruses" (B. L. S. E. Wimmer, Ed.), pp. 437-450. ASM Press, Washington.

Costa de Beauregard, M. A., Pringault, E., Robine, S., and Louvard, D. (1995). Suppression of villin expression by antisense RNA impairs brush border assembly in polarized epithelial intestinal cells. *EMBO Journal* **14,** 409-421.

Duncan, G., and Colbère-Garapin, F. (1999). Two determinants in the capsid of a persistent type 3 poliovirus exert different effects on mutant virus uncoating. *Journal of General Virology* **80,** 2601-2605.

Duncan, G., Pelletier, I., and Colbère-Garapin, F. (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.

Girard, S., Gosselin, A. S., Pelletier, I., Colbère-Garapin, F., Couderc, T., and Blondel, B. (2002). Restriction of poliovirus RNA replication in persistently infected nerve cells. *Journal of General Virology* **83,** 1087-1093.

Gosselin, A. S., Simonin, Y., Guivel-Benhassine, F., Rincheval, V., Vayssiere, J. L., Mignotte, B., Colbère-Garapin, F., Couderc, T., and Blondel, B. (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.

Gromeier, M., and Nomoto, A. (2002). Determinants of poliovirus pathogenesis. *In* "Molecular Biology of Picornaviruses" (B. L. S. E. Wimmer, Ed.), pp. 367-380. ASM Press, Washington.

He, Y., Mueller, S., Chipman, P. R., Bator, C. M., Peng, X., Bowman, V. D., Mukhopadhyay, S., Wimmer, E., Kuhn, R. J., and Rossmann, M. G. (2003). Complexes of poliovirus serotypes with their common cellular receptor, CD155. *Journal of Virology* **77,** 4827-4835.

Hidalgo, I. J., Raut, T. J., and Borchsardt, R. T. (1989). Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* **96,** 736-749.

Hogle, J. M., and Racaniello, V. R. (2002). Poliovirus receptors and cell entry. *In* "Molecular Biology of Picornaviruses" (B. L. S. E. Wimmer, Ed.), pp. 71-84. ASM Press, Washington.

Iwasaki, A., Welker, R., Mueller, S., Linehan, M., Nomoto, A., and Wimmer, E. (2002). Immunofluorescence analysis of poliovirus receptor expression in Peyer's patches of humans, primates, and CD155 transgenic mice: implications for poliovirus infection. *Journal of Infectious Diseases*. **186,** 585-592.

Kaplan, G., and Racaniello, V. R. (1991). Down regulation of poliovirus receptor RNA in HeLa cells resistant to poliovirus infection. *J. Virol*. **65,** 1829-1835.

Kew, O. M., Mulders, M. N., Lipskaya, G. Y., da Silva, E. E., and Pallansch, M. A. (1995). Molecular epidemiology of polioviruses. *Semin. Virol*. **6,** 401-414.

Kew, O. M., Sutter, R. W., Nottay, B. K., McDonough, M. J., Prevots, D. R., Quick, L., and Pallansch, M. A. (1998). Prolonged replication of a type 1 vaccine-derived poliovirus in an immunodeficient patient. *Journal of Clinical Microbiology* **36,** 2893-2899.

Koike, S., Horie, H., Ise, I., Okitsu, A., Yoshida, M., Iizuka, N., Takeuchi, K., Takegami, T., and Nomoto, A. (1990). The poliovirus receptor protein is produced both as membrane-bound and secreted forms. *EMBO J.* **9,** 3217-3224.

Lama, J., Sanz, M. A., and Carrasco, L. (1998). Genetic analysis of poliovirus protein 3A: characterization of a non-cytopathic mutant virus defective in killing Vero cells. *Journal of General Virology* **79,** 1911-1921.

Landsteiner, K., and Popper, E. (1909). Ubertragung der Poliomyelitis acuta auf Affen. *Z. Immunitätsforsch.* **2,** 377-390.

Levaditi, C. (1913). Virus de la poliomyélite et culture des cellules in vitro. *Compt. Rend. Soc. Biol. Paris* **75,** 202-205.

Lopez, M., Jordier, F., Bardin, F., Coulombel, L., Chabannon, C., and Dubreuil, P. (1999). Identification of a new class of Ig superfamily antigens expressed in hemopoiesis. *In* "Leucocyte typing VI, White Cell Differentiation Antigens." (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.), pp. 1081. Garland.

Martin, J., Dunn, G., Hull, R., Patel, V., and Minor, P. D. (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.

Mendelsohn, C. L., Wimmer, E., and Racaniello, V. R. (1989). Cellular receptor for poliovirus : molecular cloning, nucleotide sequence and expression of a new member of the immunoglobulin superfamily. *Cell* **56,** 855-865.

Minor, P. (2001). Characteristics of poliovirus strains from long-term excretors with primary immunodeficiencies. *Dev Biol (Basel)* **105,** 75-80.

Minor, P. D., and Almond, J. (2002). Poliovirus vaccines: molecular biology and immune response. *In* "Molecular Biology of Picornaviruses" (B. L. S. E. Wimmer, Ed.), pp. 381-390. ASM Press, Washington.

Oprisan, G., Combiescu, M., Guillot, S., Caro, V., Combiescu, A., Delpeyroux, F., and Crainic, R. (2002). Natural genetic recombination between co-circulating heterotypic enteroviruses. *Journal of General Virology* **83,** 2193-2200.

Ouzilou, L., Caliot, E., Pelletier, I., Prevost, M. C., Pringault, E., and Colbère-Garapin, F. (2002). Poliovirus transcytosis through M-like cells. *Journal of General Virology* **83,** 2177-2182.

Pavio, N., Buc-Caron, M. H., and Colbère-Garapin, F. (1996). Persistent poliovirus infection of human fetal brain cells. *J. Virol.* **70,** 6395-6401.

Pelletier, I., Couderc, T., Borzakian, S., Wyckoff, E., Crainic, R., Ehrenfeld, E., and Colbère-Garapin, F. (1991). Characterization of persistent poliovirus mutants selected in human neuroblastoma cells. *Virology* **180,** 729-737.

Pelletier, I., Duncan, G., and Colbère-Garapin, F. (1998). One amino acid change on the capsid surface of poliovirus Sabin 1 allows the establishment of persistent infections in HEp-2c cell cultures. *Virology* **241,** 1-13.

Pelletier, I., Duncan, G., Pavio, N., and Colbère-Garapin, F. (1998). Molecular mechanisms of poliovirus persistence: key role of capsid determinants during the establishment phase. *Cellular & Molecular Life Sciences*. **54,** 1385-1402.

Pelletier, I., Ouzilou, L., Arita, M., Nomoto, A., and Colbère-Garapin, F. (2003). Characterization of the poliovirus 147S particle: new insights into poliovirus uncoating. *Virology* **305,** 55-65.

Pinto, M., Robine-Leon, S., Appay, M., Kedinger, M., Triadou, N., Dussaulx, E., Lacroix, B., Simon-Assmann, P., Haffen, K., Fogh, J., and Zweibaum, A. (1983). Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biology of the Cell* **47,** 323-330.

Rossmann, M. G. (2002). Picornavirus structure overview. *In* "Molecular Biology of Picornaviruses" (B. L. S. E. Wimmer, Ed.), pp. 27-38. ASM Press, Washington.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22,** 4673-4680.

Tucker, S. P., Thornton, C. L., Wimmer, E., and Compans, R. W. (1993a). Bidirectional entry of poliovirus into polarized epithelial cells. *J. Virol.* **67,** 29-38.

Tucker, S. P., Thornton, C. L., Wimmer, E., and Compans, R. W. (1993b). Vectorial release of poliovirus from polarized human intestinal epithelial cells. *J. Virol*. **67,** 4274-4282.

Van Beers, E. H., Al, R. H., Rings, E. H., Einerhand, A. W., Dekker, J., and Bueller, H. A. (1995). Lactase and sucrase-isomaltase gene expression during Caco-2 cell differentiation. *Biochemical Journal* **308,** 769-775.

Yoneyama, T., Yoshida, H., Shimizu, H., Yoshii, K., Nagata, N., Kew, O., and Miyamura, T. (2001). Neurovirulence of Sabin 1-derived polioviruses isolated from an immunodeficient patient with prolonged viral excretion. *Dev Biol (Basel)* **105**, 93-98**.**

TABLE 1

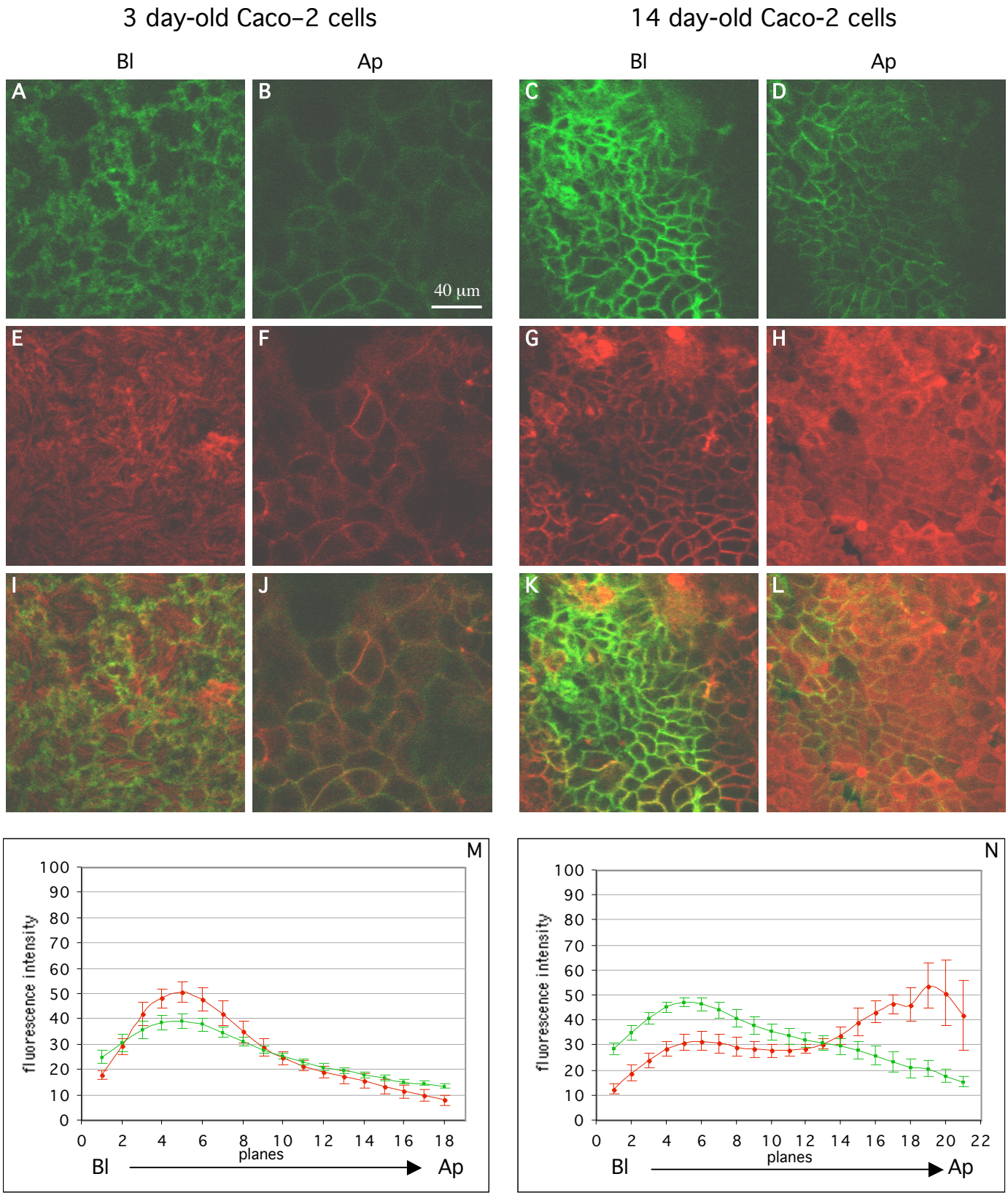
Nucleotide position and amino acid differences between the lytic and persistent strains of VDPV in the capsid region.

Genome region	Nucleotide position	Amino acid position	Amino acid in lytic strain		Amino acid in persistent strain		
			S3	H36-11	H136-11	H442-11	H637-11
VP2	989	14	Leu	-	Met	Met	Met
	1176	76	Lys	-	Ile	-	-
	1366	139	Gln	-	-	His	His
	1368	140	Arg	-	Met	Met	Met
	1413	155	Lys	-	-	Ile	-
	1451	168	Ser	-	Pro	-	-
	1458	170	Lys	-	-	-	Arg
	1505	186	Leu	Met	Met	Met	Met
VP3	1997	79	Ser	-	-	Thr	Thr
	2034	91	Phe	Ser	Ser	Ser	Ser
	2108	116	Phe	-	-	Leu	Leu
	2387	209	Ser	-	-	-	Cys
VP1	2510	12	Gly	-	-	Ser	-
	2577	34	Ala	-	Val	Val	Val
	3242	256	Val	-	-	-	Ile
	3299	275	Ala	Thr	Ser	Thr	Thr
	3338	288	Asn	-	Asp	Asp	Asp

Amino acid residues that differentiate lytic and persistent VDPV strains, and are common to the three persistent VDPV strains, are underlined.

Figure 1.

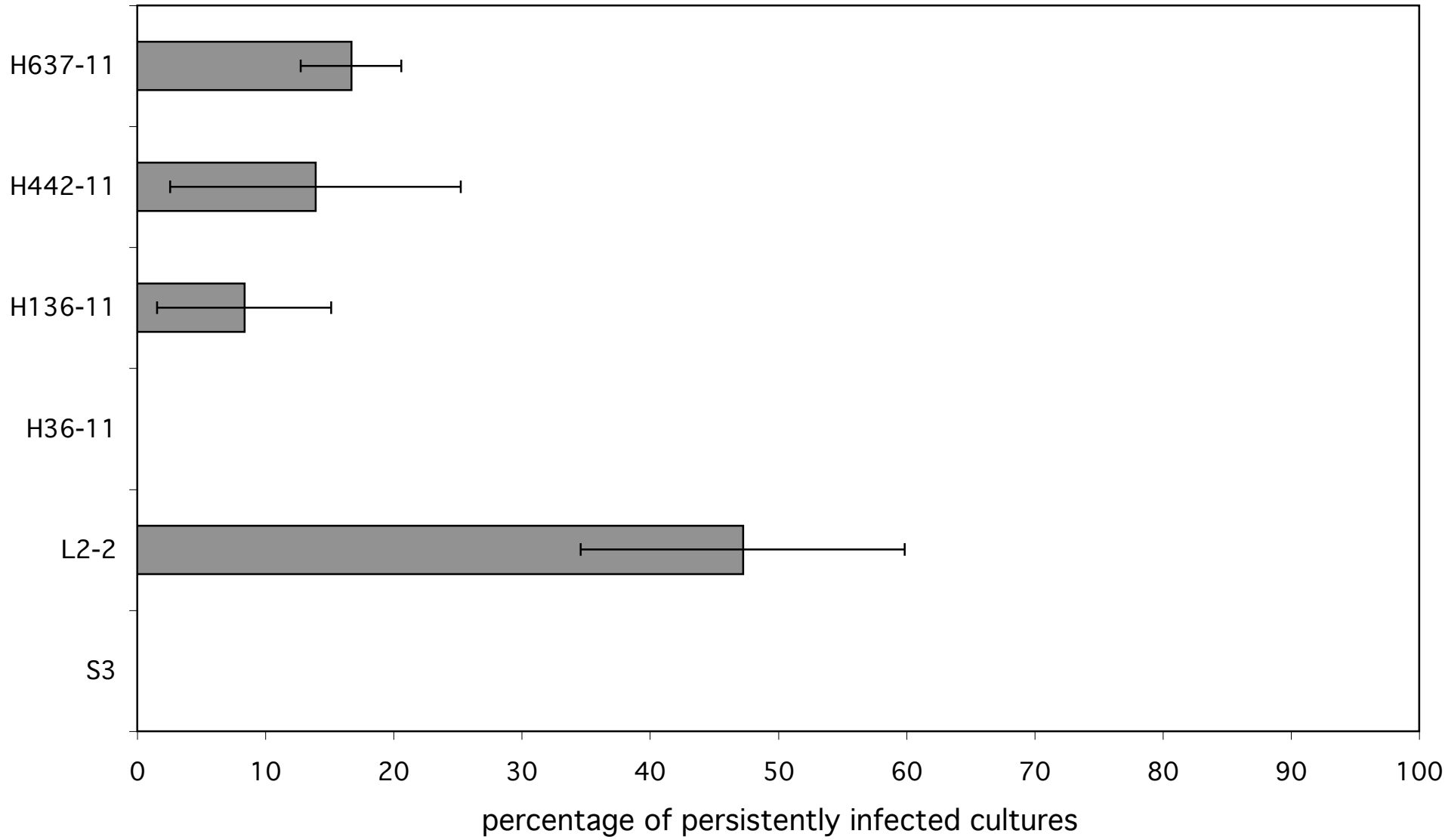
Immunofluorescence analysis of CD155 expression in 3-day-old Caco-2 cells and polarized enterocytes. Caco-2 cells were seeded and cultured on Transwell filters for 3 days (left-hand panels) or 14 days (right-hand panels). Fluorescence is shown both on basolateral (Bl) and apical (Ap) planes of Caco-2 cells. The Bl and Ap planes of 3-day-old Caco-2 cells are designated by reference to differentiated enterocytes (the basal face is adjacent to the filter). The PV receptor (PVR or CD155) was detected with the monoclonal antibody 404 (Lopez et al., 1999) and anti-mouse immunoglobulin conjugated to FITC (A-D). To visualize Caco-2 cell differentiation, F-actin was stained with phalloïdin-alexa (E-H). Simultaneous detection of CD155 and F-actin is presented (I-L). Images were captured by confocal microscopy through a 63X objective lens. Panels M and N show fluorescence intensity profiles (in arbitrary units) for green (FITC) and red (alexa) channels, from Bl to Ap planes of 3- and 14-day-old Caco-2 cells, respectively. Each value in M and N represents the average fluorescence intensity for 3 separate fields in the same plane. Vertical bars indicate the standard errors of the means (SEM) for 18 and 21 planes of 3- and 14-day old cultures, respectively. The distance between two planes is 1 μm and the thickness of each slide is 0.3 μm. Fluorescence was quantified with MetaMorph Offline (Universal Imaging Corporation, version 4.6 r0).



K. Labadie *et al.*, Figure 1

Figure 2.

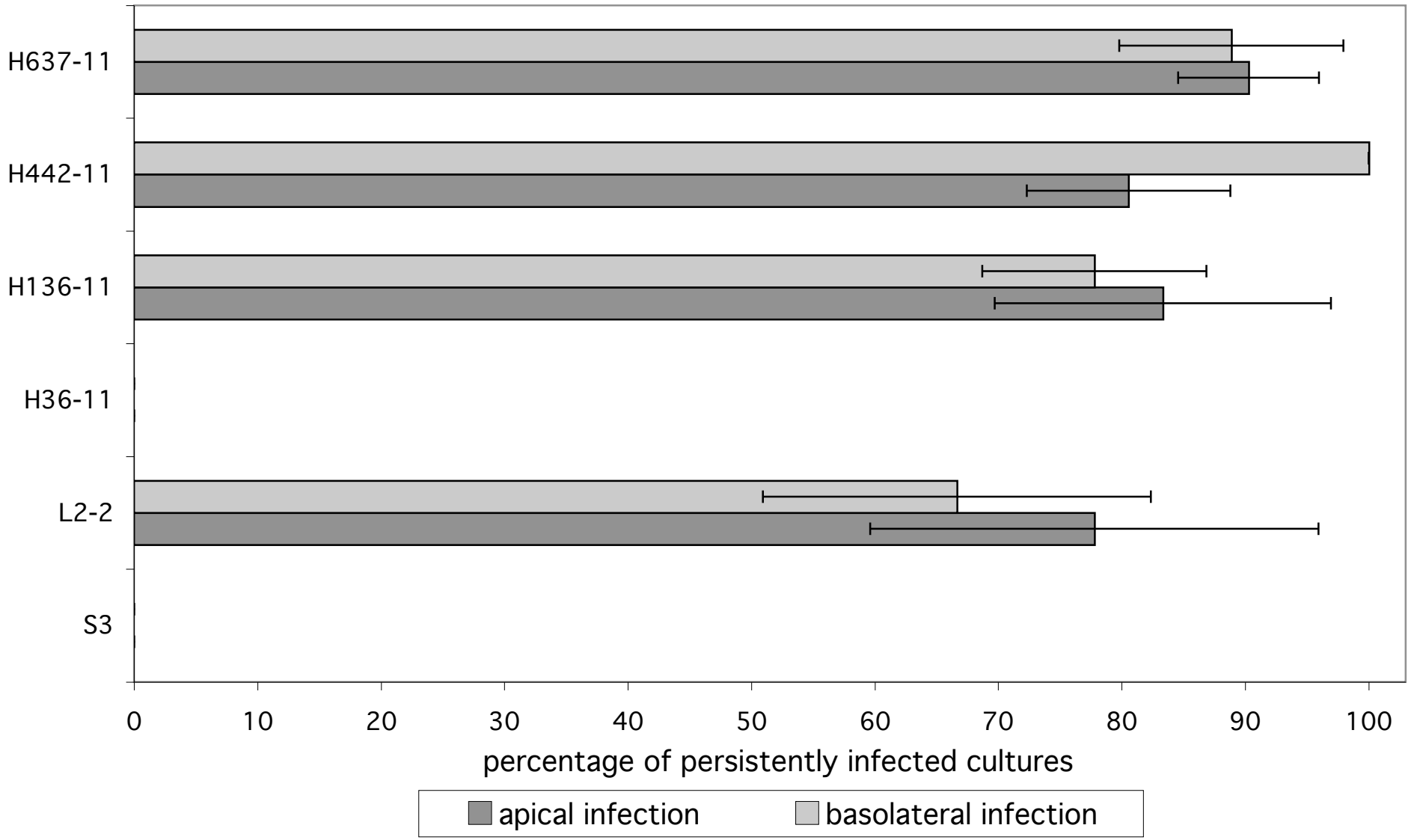
Persistent poliovirus infection in Caco-2 cell cultures. Confluent 4- or 5-day-old Caco-2 cells were infected at a m.o.i. of 10^{-4} ID_{50} per cell with the Sabin 3 strain (S3), the mutant PVpi L2-2 and the 4 VDPV clones H36-11 to H637-11. The results are expressed as the percentage of persistently infected cultures 2 months post-infection. The mean of 3 experiments is presented, and the horizontal error bars represent the SEM.



K. Labadie *et al.*, Figure 2

Figure 3.

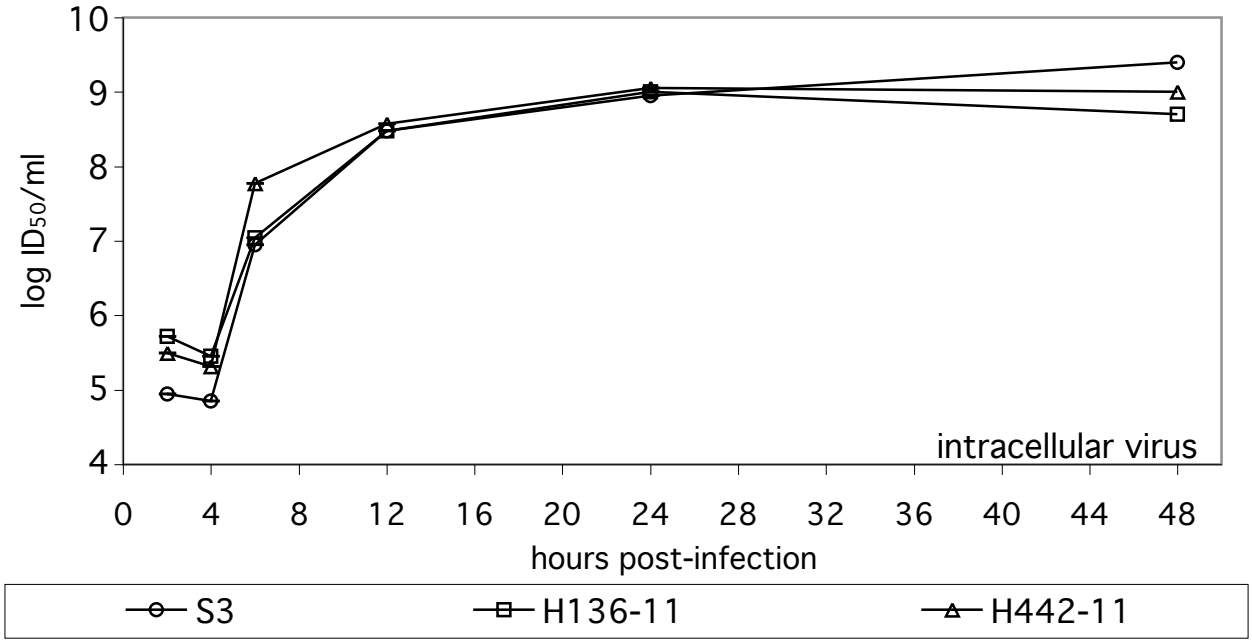
Persistent poliovirus infection in polarized enterocyte-like cells. Caco-2 cells seeded on Transwell filters were grown for 14 days to induce the differentiation of Caco-2 cells into enterocyte-like cells. Cells were then infected at a m.o.i. of 10^{-4} ID_{50}/cell with the Sabin 3 strain (S3), the PVpi L2-2 or the 4 VDPV clones H36-11 to H637-11, either from the apical face (dark gray) or from the basolateral face (light gray). At least 3 independent experiments were performed with each virus, and the percentage of persistently infected cultures one month post-infection is presented with SEM (horizontal error bars).

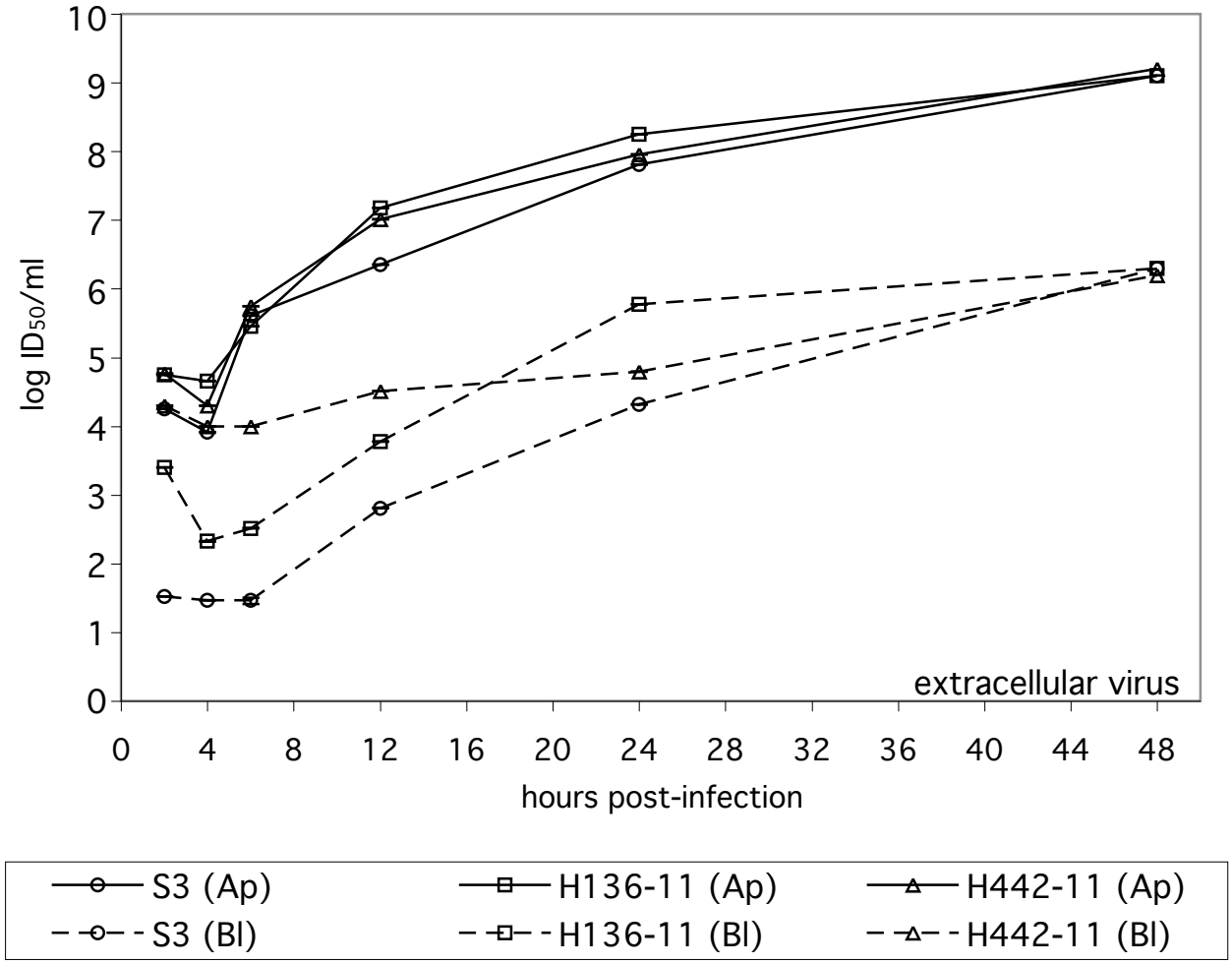


K. Labadie *et al.*, Figure 3

Figure 4.

One-step virus growth cycles in polarized enterocytes. Caco-2 cells were seeded on Transwell filters and were infected 2 weeks later from the apical side at a m.o.i. of 10 ID_{50}/cell with the lytic strain S3, and the persistent VDPV clones H136-11 and H442-11. Intracellular virus (upper panel) and extracellular apical or basolateral virus (lower panel) were titered on Caco-2 cells. Averages of 4 cultures from 2 independent experiments are presented and the SEM are indicated by vertical bars (they are barely visible because of the log scale).

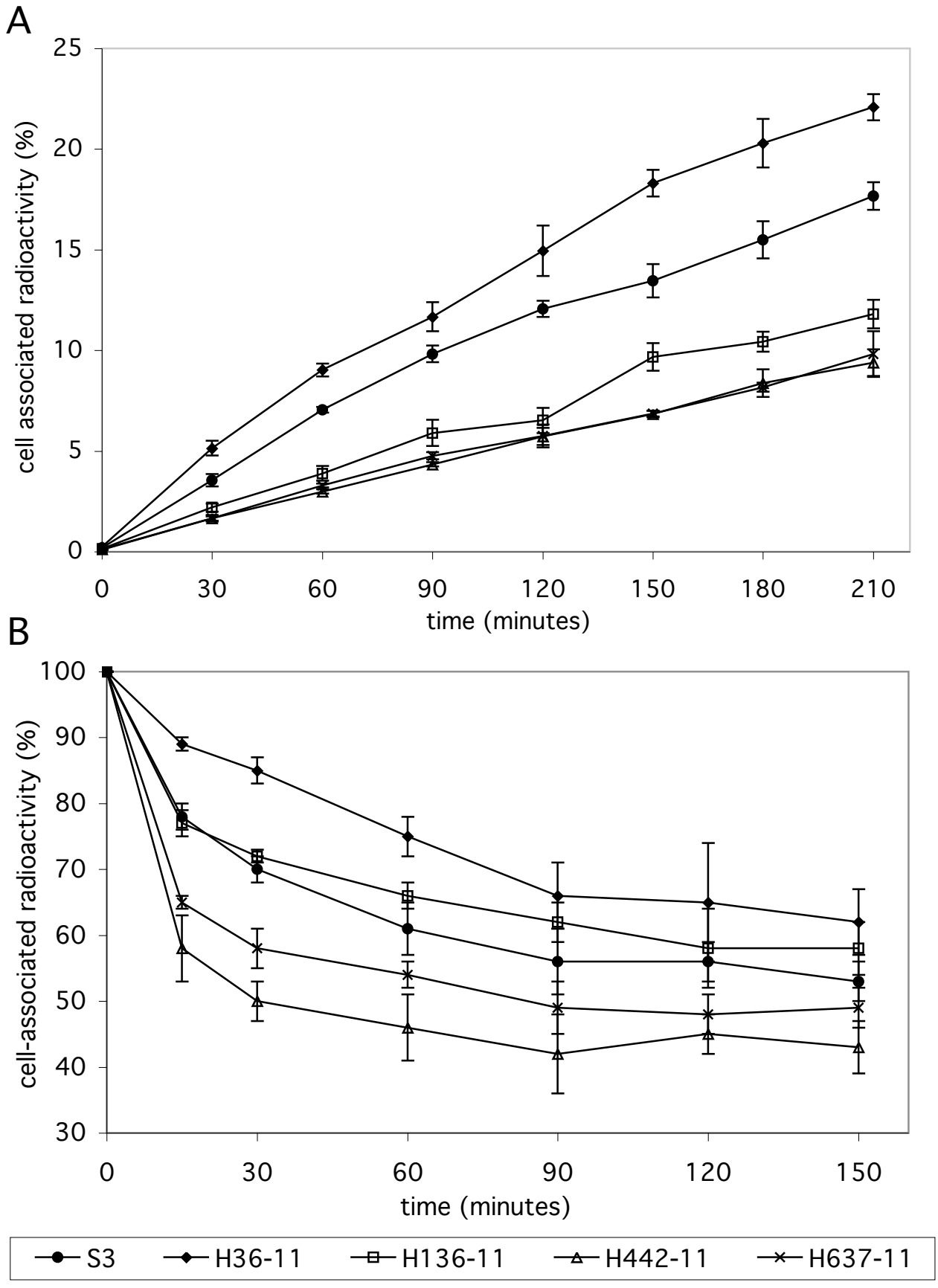




K. Labadie *et al.*, Figure 4

Figure 5.

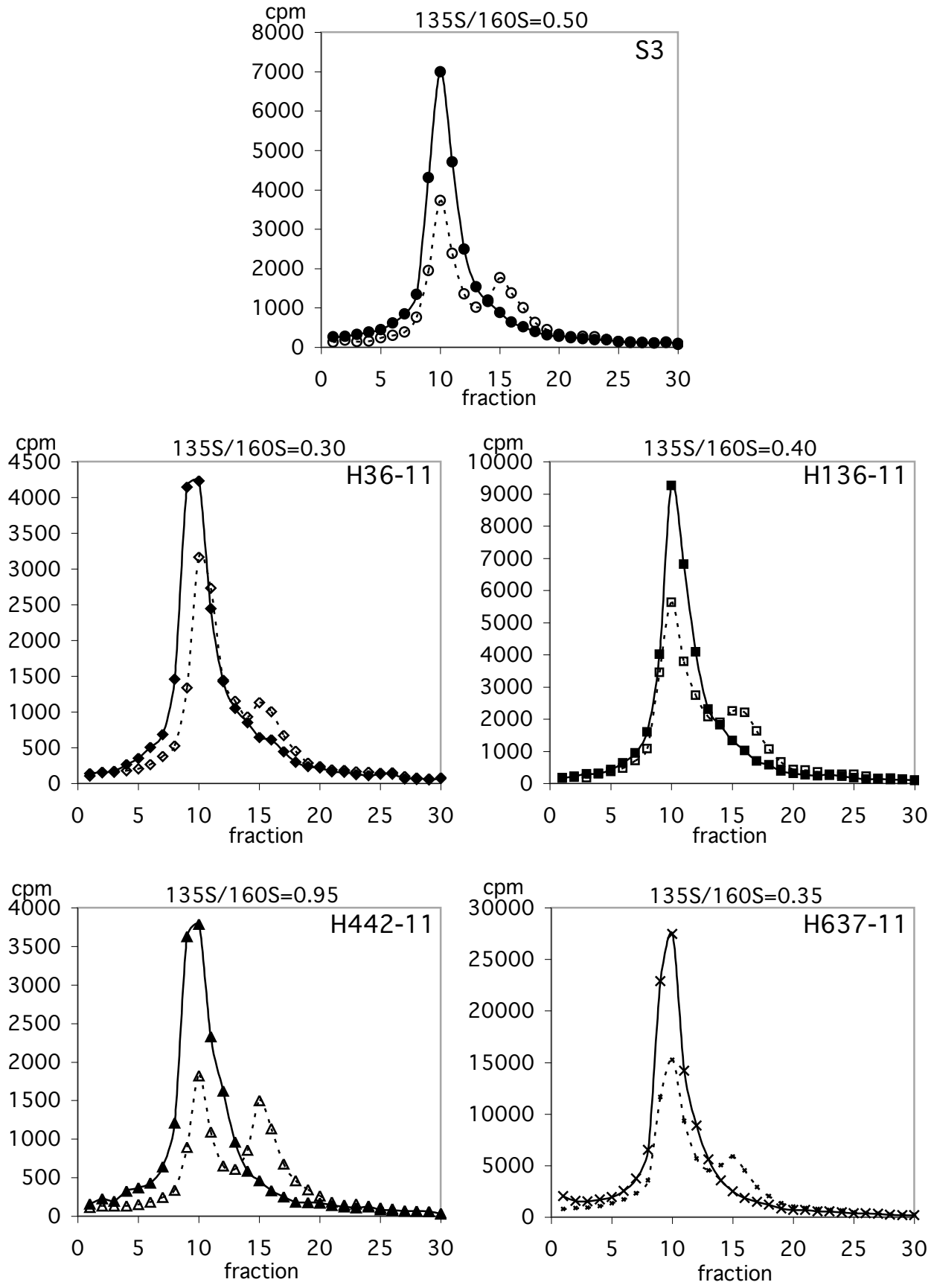
Analysis of virus adsorption (panel A) and particle elution (panel B) at the surface of Caco-2 cells for the lytic strain S3, the lytic VDPV clone H36-11 and the 3 persistent VDPV clones H136-11, H442-11 and H637-11. (A) Caco-2 cells in suspension were infected with radiolabeled virions at 0°C for the times indicated. The cell-associated radioactivity was counted and is expressed as the percentage of the radioactivity present in the inoculums. (B) Caco-2 cells were infected in suspension. After 2.5 h of adsorption, unattached virus was removed and cells were incubated at 37°C for the times indicated. The cell-associated radioactivity was measured and is expressed as a percentage of the radioactivity present prior to the 37°C incubation period. For both the adsorption and elution experiments, each point represents the mean value of 3 independent experiments and the SEM are indicated by vertical bars.



K. Labadie *et al.*, Figure 5

Figure 6.

Capsid conformational transitions from 160S to 135S particles. Caco-2 cells in suspension were infected with radiolabeled virions for 3 h at 0°C. Cells were washed and incubated for either 0 (continuous line) or 20 min (broken line) at 37°C to permit CD155-induced conformational transitions in the viral capsid. Cells were then lysed and viral particles were separated by sucrose gradient (15-30%) centrifugation. The gradients were fractionated from the bottom, and fractions were counted for radioactivity. The 135S/160S alteration ratio is presented for each virus.



K. Labadie *et al.*, Figure 6