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Review

Molecular aspects of poliovirus biology with a special focus on the interactions with nerve cells

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Poliovirus (PV), the pathogenic agent of paralytic poliomyelitis, is the prototype of the picornavirus family. Although paralytic poliomyelitis has been nearly totally eradicated in most industrialized countries, PV continues to be an important public health problem in many developing countries. Moreover, in industrialized countries, two current concerns are the occurence, albeit at a very low frequency, of vaccine-associated paralytic poliomyelitis, due to the genetic instability of the attenuated oral PV strains in vaccinees, and the emergence of a neuro-muscular pathology in many survivors of the acute disease, called the post-polio syndrome. PV has been targeted by the World Health Organization for world-wide eradication in the coming decade and continues to be the subject of intensive research. The advances made in the molecular biology of PV, taken together with the development of new animal and cell models, have permitted a new look at a key step in the pathogenesis of poliomyelitis, i.e. the interactions between PV and nerve cells. These aspects of PV biology are developed in this review according to three themes: (i) the PV host range; (ii) the molecular determinants of PV neurovirulence and attenuation; and (iii) the persistence of PV in nerve cells, which has proven to be an interesting new domain in the field of PV research.

Keywords: poliovirus; neurovirulence; persistence; host range

Introduction

Poliovirus (PV) was discovered to be the etiological agent of poliomyelitis by Landsteiner and Popper in 1908 (Landsteiner and Popper, 1908). Since then, PV has been identified as a member of the picornavirus family which includes many important human and animal pathogens. This family is divided into five generas: the enteroviruses, the cardioviruses, the rhinoviruses, the aphtoviruses and the hepatoviruses (Minor, 1991). PV, the prototype virus of the enteroviruses, can be further classified into three serotypes: PV-1, PV-2, and PV-3. The emergence of poliomyelitis as an epidemic disease in Europe and in the United States at the turn of the century made PV the focus of considerable attention. In 1949, Enders *et al*. (1949) demonstrated that PV could be grown in cultured cells, a discovery which proved to be a turning point in PV research, leading to the elaboration of a polio vaccine. In fact, both a killed and an oral live attenuated vaccine were developed, independently, in the 1950s by Salk (1955) and Sabin (Sabin and Boulger, 1973), respectively. Intensive vaccination, starting in the early 1960s, has allowed for nearly total eradication of PV in most industrialized countries and significant progress has been made towards the global eradication of poliomyelitis (Hull *et al*, 1997). However, PV continues to be an important public health concern in many developing countries, and in particular, in Africa and Asia. Outbreaks of poliomyelitis still occur even in countries in which poliomyelitis has been controlled for many years such as the Netherlands in 1992 (Mulders *et al*, 1995) and more recently, Albania in 1996 (WHO, 1996). These outbreaks involve unvaccinated or inadequately vaccinated subgroups within highly immunized communities. Another problem in industrialized countries is related to the wide use of the oral PV vaccine (OPV) and concerns the occurence, albeit at a very low frequency, of vaccine-associated paraly-

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tic poliomyelitis (VAPP), which is due to the genetic instability of the attenuated OPV strains in vaccinees. Finally, many survivors of the acute disease, after decades of clinical stability, develop a new neuro-muscular pathology called the post-polio syndrome (PPS).

During the past 15 years, the research into the molecular biology of PV, which is presented briefly in the first part of this review, has advanced considerably. The progress made in this field, together with the development of new animal and cell models, have permitted researchers to address a key step in the pathogenesis of poliomyelitis at the molecular level: the interactions between PV and nerve cells. These aspects will be developed here according to three themes: (i) the PV host range; (ii) the molecular determinants of PV neurovirulence and attenuation; (iii) the persistence of PV in nerve cells.

Structure of the virion

The poliovirion is composed of a single-stranded RNA molecule of positive polarity in a non-enveloped icosahedral protein capsid. The three-dimensional structure of the virion has been determined by X-ray crystallography for the three serotypes of poliovirus (Filman *et al*, 1989; Hogle *et al*, 1985; Lentz *et al*, 1997). The capsid consists of 60 copies of each of the four viral structural proteins VP1, VP2, VP3, and VP4 (Figure 1). Five molecules of VP1 surround the fivefold axis of symmetry, whereas VP2 and VP3 alternate around the threefold axis of symmetry; VP4 is exclusively internal. VP1, VP2 and VP3 share a common 'core' structure composed of a wedge-shaped eight-stranded anti-parallel beta-barrel with two flanking alpha helices connected together by dissimilar loops. The C-termini of these three proteins as well as most of the connecting loops are exposed on the outer surface of the protein shell and contain the three major antigenic sites (van der Werf, 1994). Their N-termini are located on the inside and form an elaborate network together with VP4 which is myristoylated at its N-terminus. A deep surface depression, called the 'canyon', surrounds a star-shaped protrusion at the center of each fivefold axis of symmetry (Figure 1). This depression was first proposed to contain the site for cell receptor binding by analogy with rhinovirus 14 and 16 (Colonno *et al*, 1988; Olson *et al*, 1993; Rossmann *et al*, 1985). Further evidence that the receptor binding site for PV is located in the canyon comes from the study of two types of viral mutants: soluble receptor resistant mutants and viruses adapted to mutant PV receptors (Colston and Racaniello, 1994, 1995; Racaniello, 1996).

The PV genome is a single-stranded molecule of infectious RNA, 7441 nucleotides long for PV-1, which is poly-adenylated at its 3′-terminus and

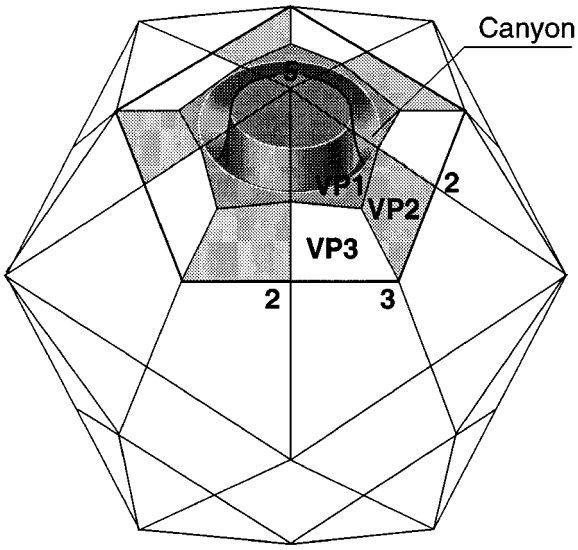

Figure 1 Schematic representation of the poliovirus capsid. The two, three and fivefold axes of symmetry and the positions of capsid proteins VP1, VP2 and VP3 are indicated for one protomer. The depression surrounding the fivefold axis is called the canyon (Adapted from (Hogle *et al*, 1985)).

covalently linked to a small viral protein, VPg, at its 5′-terminus (Figure 2) (Lee *et al*, 1977). The complete nucleotide sequences of the virulent strains (Kitamura *et al*, 1981; La Monica *et al*, 1986; Racaniello and Baltimore, 1981; Stanway *et al*, 1983) as well as those of the attenuated Sabin strains (Nomoto *et al*, 1982; Toyoda *et al*, 1984) have been determined for each of the three serotypes. The PV genome can be divided into three regions: a long open-reading frame flanked by two non-coding regions (NCR) of 742 nucleotides at the 5′ end and 72 nucleotides at the 3′ end. A significant amount of complex secondary structure has been predicted for the 5′NCR (Agol, 1991; Pilipenko *et al*, 1989; Skinner *et al*, 1989). The first 100 nucleotides form a clover-leaf structure involved in the initiation of positive-strand replication (Andino *et al*, 1990), and further downstream, secondary structures form the internal ribosomal entry site (IRES) which is necessary for the initiation of translation (Figure 3) (Pelletier *et al*, 1988; Rohll *et al*, 1994; Sonenberg, 1987; Trono *et al*, 1988). Elements of the IRES have also recently been shown to be involved in the replication of the viral genome (Borman *et al*, 1994; Shiroki *et al*, 1995). The 5′ NCR is known to be involved in the attenuation of all three serotypes (Macadam *et al*, 1994a). The 3′NCR includes the poly-A tail and is involved in the initiation of negative-strand replication. Its biological function probably depends on either a predicted pseudoknot structure (Jacobson *et al*, 1993) or interactions of a novel type, involving a 'kissing' interaction between two hairpin loops in this region (Pilipenko *et al*, 1996).

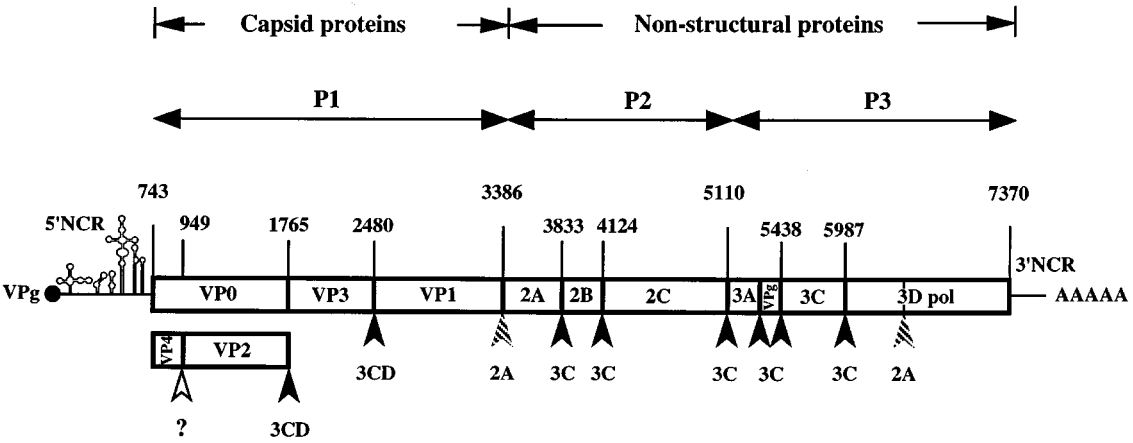

Figure 2 Genetic organization of PV-1/Mahoney. The 5′ and 3′ noncoding regions indicated as 5′NCR and 3′NCR respectively, flank the single open-reading frame, encoding the polyprotein which is shown as an elongated rectangle. The protein precursors P1, P2 and P3, are designated by arrows above the genome, and the viral proteins are indicated in the rectangles. The small viral protein VPg is covalently linked to the 5′ end of the RNA genome. The capsid proteins VP4, VP2, VP3, VP1 and VPg are also known as 1A, 1B, 1C, 1D and 3B according to the L434 nomenclature (Rueckert and Wimmer, 1984). Proteolytic cleavages occur between the amino acid pairs Asn-Ser, Gln-Gly and Tyr-Gly, as indicated by empty, solid, and cross-hatched arrowheads respectively. The cleavage sites of proteases 2A, 3CD and 3C are shown. The mechanism of cleavage of the precursor VP0 giving VP4 and VP2 is not known (Adapted from (Kitamura *et al*, 1981)).

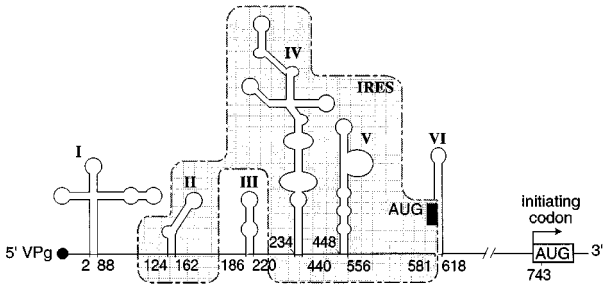

Figure 3 Diagram of the computer-predicted secondary structure of the 5′ noncoding region of PV-1/Mahoney RNA. The six stem-loop structures are labeled I through VI. The boundaries of the IRES are indicated by a dotted line. The cryptic AUG at position 586, which plays an essential role in the internal initiation of translation (Pilipenko *et al*, 1992), is represented by a black rectangle. The initiating codon AUG of the viral polyprotein is represented by an open rectangle (Adapted from Andino *et al*, 1990; Jackson *et al*, 1994; Pilipenko *et al*, 1989; Wimmer *et al*, 1993).

Viral cycle

Receptor-binding, cell entry and uncoating

The viral cycle of PV in cultured cells occurs entirely in the cytoplasm of the host cell (Rueckert, 1996). It is among the shortest known of the viral cycles, lasting approximately 8 h at 37°C. The initial event is attachment of the virion to the receptor which is found only on the surface of primate cells. The human poliovirus receptor (hPVR) has been identified as a member of the immunoglobulin superfamily, whose cellular role is not yet known (Koike *et al*, 1990; Mendelsohn *et al*, 1989; Racaniello, 1996; Wimmer *et al*, 1994). The hPVR, a highly glycosylated protein with an apparent molecular weight of 80 kDa, has three extracellular immunoglobulin-like domains, a transmembrane domain, and a cytoplasmic tail. The virion interacts with the first of the extracellular domains (Bernhardt *et al*, 1994; Koike *et al*, 1991a; Morrison *et al*, 1994; Selinka *et al*, 1991, 1992). Simian (Koike *et al*, 1992) and murine (Morrison and Racaniello, 1992) homologs of the hPVR gene have been isolated but only the corresponding monkey protein (mPVR) is a functional receptor for PV. After transfection of murine L cells, both hPVR and mPVR are sufficient to render these normally resistant cells susceptible to PV (Koike *et al*, 1992; Mendelsohn *et al*, 1989). Similarly, transgenic (Tg) mice expressing the hPVR (hPVR-Tg mice) become susceptible to infection by all three PV serotypes (Koike *et al*, 1991b; Ren *et al*, 1990). Like many members of the immunoglobulin superfamily, the hPVR and its homologs may play a role in cell adhesion and recognition. Some recent data suggest a physiological function of hPVR proteins in conjunction with early embryonic development of the spinal cord and its cellular components (Gromeier *et al*, 1997).

The hPVR performs a dual function in PV infection: it is responsible for both the attachment of the virus to the cell surface and for the destabilization of the virion which leads to the conformational transitions of the viral capsid necessary for uncoating (Racaniello, 1996; Wimmer *et al*, 1994). These conformational transitions include the loss of the internal capsid protein VP4 and the extrusion of the internal N-terminus of VP1

(Everaert *et al*, 1989; Flore *et al*, 1990; Fricks and Hogle, 1990; Gomez Yafal *et al*, 1993; Kaplan *et al*, 1990; Lonberg-Holm *et al*, 1975; Wien *et al*, 1996). The particles thus altered have a reduced sedimentation coefficient (135S *versus* 160S for native virions), are protease sensitive and are considerably more hydrophobic than the native virion (Everaert *et al*, 1989; Fricks and Hogle, 1990; Lonberg-Holm *et al*, 1975, 1976). The 135S particles are capable of infecting cells in a receptor-independent fashion when supplied at sufficiently high concentrations (Curry *et al*, 1996) and are thought to be a necessary intermediate in the cell entry pathway (Fricks and Hogle, 1990). However it has been recently shown that cold-adapted mutants of PV have gained the ability to infect cells at 25°C without alteration to 135S particles (Dove and Racaniello, 1997). A hydrocarbon binding pocket containing sphingosine, and lying in VP1 below the canyon floor, is believed to regulate the ability of the capsid to undergo receptor-mediated structural transitions (Colston and Racaniello, 1994; Filman *et al*, 1989). The viral particles penetrate into the cell, either directly through the plasma membrane after binding to the PV receptor (Dunnebacke *et al*, 1969) or by receptor-mediated endocytosis (Zeichardt *et al*, 1985). However, it has been demonstrated that a low pH step is not required for poliovirus uncoating (Gromeier and Wetz, 1990; Kaplan *et al*, 1990; Perez and Carrasco, 1993). The amino terminus of VP1 and the myristoylated N-terminus of VP4 could form a channel through the cytoplasmic or endosomal membranes, permitting the viral RNA to enter into the cytoplasm (Fricks and Hogle, 1990; Mosser *et al*, 1994; Tosteson and Chow, 1997).

Translation of viral RNA

Immediately after the viral RNA is released into the cytoplasm, a cellular protease cleaves the VPg from the 5′ end of the genome (Ambros *et al*, 1978), and translation is performed by host cell ribosomes (Haller and Semler, 1995; Jackson *et al*, 1995). The initiation of translation of PV mRNA does not require a 5′ m^7G cap structure and host cell ribosomal 40S subunit binding occurs at an internal sequence, called the IRES (Figure 3), which is located about 100 nucleotides upstream of the AUG start codon (Pelletier and Sonenberg, 1989; Pilipenko *et al*, 1989). Both a cryptic (non-initiating) AUG at position 586 and an oligopyrimidine tract situated 22 nucleotides upstream of it (oligopyrimidine/AUG tandem) are involved in the efficient functioning of this element (Pilipenko *et al*, 1992). Several host cell proteins which bind to the 5′ NCR appear to be necessary to ensure proper initiation and stimulation of viral translation (Belsham and Sonenberg, 1996). Some of these proteins have been identified, such as: the human La autoantigen (Meerovitch *et al*, 1993), the polypyrimidine tract-binding protein (PTB) (Hellen *et al*, 1993), the cellular translation initiation factor eIF-2α, (del Angel *et al*, 1989) and the poly (rC) binding protein 2 (PCBP2) (Blyn *et al*, 1996).

The long open-reading frame is translated to produce a 247 kDa polyprotein which is then cleaved co-translationally by viral proteases to yield the viral proteins (Figure 2). The first third, called the P1 region, encodes for the capsid proteins, while the last two-thirds, divided into the P2 and P3 regions, encodes for the non-structural proteins (Wimmer *et al*, 1993). Proteins 2A, 3CD and 3C are viral proteases involved in polyprotein processing (Hämmerle *et al*, 1991; Hellen *et al*, 1992; Kean *et al*, 1991). The protein 2A also plays a role in RNA synthesis (Molla *et al*, 1993; Yu *et al*, 1995) and acts in the shutoff of host cell protein synthesis (Belsham and Sonenberg, 1996; Jackson *et al*, 1995). Recent studies indicate that the protein 2A also acts as a transactivator for the translation of viral mRNA (Borman *et al*, 1997; Hambidge and Sarnow, 1992). The protein 3D is the viral RNA-dependent RNA polymerase ($3D^{pol}$) which also has an unwinding activity (Cho *et al*, 1993), terminal adenylyl transferase activity (Neufeld *et al*, 1994), and could be involved in the uridylylation of VPg (3B) during the initiation of RNA replication (Toyoda *et al*, 1987). Both the protein 2B and the 2C are considered to play important roles in RNA synthesis (Barton *et al*, 1995; Bienz *et al*, 1990; Johnson and Sarnow, 1991). It has been demonstrated that the protein 2C binds RNA and has ATPase and GTPase activity (Mirzayan and Wimmer, 1994; Rodriguez and Carrasco, 1993; Teterina *et al*, 1992). The protein 3AB has a dual function in PV genome replication, acting as both a precursor for VPg and as a co-factor for $3D^{pol}$ (Lama *et al*, 1994, 1995; Plotch and Palant, 1995). Furthermore, the protein 3AB associates tightly with newly synthesized cytoplasmic membranes in a manner that would allow it to serve as a lipophilic anchor for the assembly of the PV RNA replication complex (Towner *et al*, 1996).

Replication of viral RNA

Replication of viral RNA occurs on the surface of membranous vesicles that bud from several host cell organelles including the endoplasmic reticulum and the Golgi (Bienz *et al*, 1983; Caliguiri and Tamm, 1970; Schlegel *et al*, 1996). The protein 2BC has been proposed to play a role in the induction of these membranous vesicles (Bienz *et al*, 1990; Cho *et al*, 1994). Despite years of intensive research, the process of PV RNA replication is not yet completely understood. Replication starts by the formation of a complementary negative-stranded RNA molecule which serves as the template for the synthesis of progeny positive-stranded viral RNAs. Biochemical and genetic evidence have identified poliovirus non-structural proteins 2A, 2BC, 2B, 2C, 3AB, 3B (VPg), 3CD and $3D^{pol}$ as participants in the process

of viral RNA replication (Johnson and Sarnow, 1995; Wimmer *et al*, 1993). Cellular factors have also been proposed to be involved in viral RNA synthesis (Andino *et al*, 1993; Andrews *et al*, 1985; Dasgupta *et al*, 1980; McBride *et al*, 1996; Roehl and Semler, 1995). The different steps of genome replication, as well as the roles of the proteins involved, are discussed in reviews by Wimmer *et al* (1993) and Johnson and Sarnow (1995).

Virion assembly and release

The formation of viral particles seems to be coupled to RNA synthesis (Ansardi *et al*, 1996; Hellen and Wimmer, 1995), both events occuring on the surface of virus-induced membranous vesicles found in the cytoplasm of infected cells (Pfister *et al*, 1992). The protein 2C could facilitate virion assembly (Li and Baltimore, 1990). In the first step, the myristoylated P1 polyprotein, the precursor for all of the capsid proteins, is cleaved by the 3CD protease (Jore *et al*, 1988; Ypma-Wong *et al*, 1988) to give VP0, VP1 and VP3 which then aggregate to form a protomer (Bruneau *et al*, 1983; Rueckert, 1996). Five of these molecules combine to form a pentamer (Phillips and Fennel, 1973; Watanabe *et al*, 1965), and then 12 of these pentamers join together to form a procapsid (Jacobson and Baltimore, 1968). Two controversial models exist concerning the formation of the provirion, the next intermediate in PV assembly: either the viral RNA is inserted into the procapsid giving the provirion (Jacobson and Baltimore, 1968), or the pentamers condense around the viral RNA forming the provirion (Ghendon *et al*, 1972; Nugent and Kirkegaard, 1995). During the last step of virus assembly, VP0 is cleaved to give VP2 and VP4, transforming the provirion into a mature viral particle (Basavappa *et al*, 1994; Holland and Kiehn, 1968; Jacobson and Baltimore, 1970). The mechanism of cleavage remains unknown, but it could be autocatalytic (Basavappa *et al*, 1994; Hogle *et al*, 1985). The myristoylation of VP0, and therefore VP4, is essential for morphogenesis and the stability of the capsid (Kräusslich *et al*, 1990; Marc *et al*, 1989). Once assembled, the virions accumulate in the cytoplasm of infected cells in the form of crystalline inclusions which are eventually liberated by the bursting of vacuoles at the cell surface (Bienz *et al*, 1973; Dunnebacke *et al*, 1969) or by active release (Tucker *et al*, 1993). Cell lysis is accompanied by the massive release of new progeny virions. It has been suggested that the PV-induced lysis may be due to the virus-receptor interactions which occur right at the beginning of the virus cycle (Morrison *et al*, 1994).

Effect of PV infection on the host cell

As PV infection progresses *in vitro*, the host cell undergoes dramatic metabolic and morphological changes including rounding and detachment of infected cells from the substrate. Collectively, these changes are commonly referred to as cytopathic effects (Haller and Semler, 1995; Schlegel and Kirkegaard, 1995).

Infection of cells by PV results in rapid inhibition of host cell protein synthesis, mainly by way of the viral protein 2A, which induces, either directly or indirectly, the cleavage of the cellular eukaryotic initiation factor eIF4G (formerly called p220 or eIF-4γ) (Kräusslich *et al*, 1987; Novoa *et al*, 1994; Sommergruber *et al*, 1994; Wyckoff *et al*, 1990, 1992). Other modifications of initiation factors such as the phosphorylation of eIF2α may also contribute to the inhibition of host cell protein synthesis (O'Neill and Racaniello, 1989). In addition, it has recently been shown that PV activates the translational suppressor 4E-BP1, which inhibits cap-dependent translation by binding to the cap-binding subunit eIF4E (Gingras *et al*, 1996).

Infection with PV also leads to the inhibition of host cell transcription catalyzed by all three classes of host cell RNA polymerases (Contreras *et al*, 1973; Zimmerman *et al*, 1963), possibly due to the 3C-induced alteration of cellular transcription factors (Clark and Dasgupta, 1990; Clark *et al*, 1991, 1993; Kliewer and Dasgupta, 1988; Kliewer *et al*, 1990; Rubinstein and Dasgupta, 1989; Yalamanchili *et al*, 1996, 1997).

In contrast to RNA and protein syntheses, lipid synthesis is stimulated by PV infection, and much of the newly synthesized lipid is found in membranous vesicles that accumulate in the cytoplasm of infected cells (Mosser *et al*, 1972). Furthermore, during the course of PV infection, intracellular calcium concentrations increase greatly (Aldabe *et al*, 1997; Irurzun *et al*, 1995) and the plasma membrane shows increased permeability to both monovalent cations and translation inhibitors which are normally non-permeate (Lopez-Rivas *et al*, 1987; Munoz and Carrasco, 1983). It has been recently proposed that PV non-structural proteins 2B (Doedens and Kirkegaard, 1995) and 3AB (Lama and Carrasco, 1996) might have the capacity to modify plasma membrane permeability. In addition, PV proteins 2BC, 2B and 3A might inhibit the host cell protein secretory pathway in PV infected cells (Barco and Carrasco, 1995; Doedens and Kirkegaard, 1995).

Morphological and cytoskeletal rearrangements were described in PV infected cells many years ago (Caliguiri and Tamm, 1969; Dales *et al*, 1965; Lenk and Penman, 1979). More recently, a cytoskeletal protein, microtubule-associated protein 4 (MAP-4), was shown to be cleaved in PV infected cells (Joachims and Etchison, 1992). This cleavage seems to be mediated by the 3C protease (Joachims *et al*, 1995). Cytoskeletal rearrangement induced by infection may be important for virus propagation by increasing cell lysis and therefore virus release (Doedens *et al*, 1994).

Finally, evidence showing that PV encodes separate functions which trigger and suppress the development of apoptosis in infected cells, has been described (Tolskaya *et al*, 1995). The molecular mechanisms underlying these phenomena remain to be determined.

Poliomyelitis pathogenesis

The term poliomyelitis is derived from the Greek words 'polios', meaning gray, and 'myelos', meaning marrow, referring to the fact that the disease results from the destruction of neurons located in the gray matter of the anterior horn of the spinal cord. However, PV is primarily an enteric pathogen that causes, most often, a silent or abortive infection and, only in rare cases (about 1%), central nervous system (CNS) lesions and poliomyelitis.

The major sequence of events in the multiplication and spread of PV was revealed by studies in both the chimpanzee and man, as well as in cell cultures (Minor, 1997; Racaniello and Ren, 1996). The incubation period is usually between 7 and 14 days. PV infection is initiated by ingestion of virus followed by its primary multiplication in the oropharynx and intestine. Usually, the virus is present in oropharyngeal secretions for 1 to 2 weeks and is excreted in the stool for several weeks or months. Bodian's observations revealed that extensive viral multiplication takes place in the tonsils and the Peyer's patches of the small intestine (Bodian and Howe, 1955). The specific cell type carrying out the primary round of PV replication has not yet been identified. However, in the gut, there is evidence that PV selectively binds to specialized microfold cells (M cells) which overlie the Peyer's patches, suggesting that PV can be transcytosed across the epithelial layer by M cells and thus delivered to the cells of lymphoid origin found in the Peyer's patches (Sicinski *et al*, 1990). Further, the ability of PV to replicate in monocytes (Eberle *et al*, 1995; Freistadt *et al*, 1993) suggests that resident mononuclear phagocytic cells in the Peyer's patches may be the site of the initial rounds of PV replication. It is not yet known how the virus returns from the lymphoid tissue to the gut where it is found in high titer. *In vitro* studies with polarized epithelial cells (Caco-2 cells) showed that PV release occurs almost exclusively from the apical cell surface, suggesting that the dissemination of progeny virus into the gut may be mediated by the infection of intestinal wall epithelial cells via the basolateral surface, followed by the vectorial release of virus from these cells into the luminal environment (Tucker *et al*, 1993).

From the lymphoid tissues of the oropharynx and intestine, the virus moves into the regional lymph nodes (deep cervical and mesenteric nodes), and then into the blood, causing a transient and clinically silent viremia. Among Sabin vaccine strains, only PV-2 causes viremia (Horstmann *et al*, 1964). In a minority of cases, virus in the blood disseminates to other susceptible tissues, such as the systemic lymph nodes and the brown fat (suprasternal, upper axillary and paravertebral), in addition to the regional lymph nodes (Bodian and Horstmann, 1965). In these extra-neural sites, the virus replicates, and is continually fed back into the blood stream to maintain the viremia. Monocytes in the blood may also become infected and shed additional virus. Neutralizing antibodies are usually present in the serum by the time that paralysis appears.

Persistent viremia is required for viral spread to the CNS and the major pathway of penetration of PV into the CNS is probably through capillary walls (Bodian and Horstmann, 1965). The precise mechanism by which the virus breaches the blood-brain barrier remains unknown. If blood mononuclear phagocytes actually permit viral replication, they may act as carriers of PV into the CNS. Moreover, viral transmission along nerve fibers cannot be excluded and may provide an additional route for entry into the CNS. In fact, viral spreading to the CNS via nerve fibers, following intramuscular inoculation, has been demonstrated under experimental conditions (Ren and Racaniello, 1992b; Wenner and Kamitsuka, 1957; Wyatt, 1990). Evidence for virus spreading along nerve fibers from the alimentary tract to the CNS, however, has not been conclusive (Bodian and Horstmann, 1965).

Whereas virus replication in the intestine is not associated with any specific histologic lesions, viral infection of the CNS is associated with lesions characteristic of poliomyelitis infections (Bodian, 1959). Actually, histopathological observations of the CNS from both human cases of poliomyelitis and from experimentally infected primates, revealed a characteristic pattern of virus multiplication and lesion distribution in the CNS. PV has a predilection for the motor neurons of the anterior horn of the cervical and lumbar regions of the spinal cord. In severe cases, lesions can be observed in the intermediate and even the posterior gray columns and in the sensory spinal ganglia. It is particularly striking that the thoracic cord which separates the cervical cord from the lumbar cord is spared. Similarly, in the brain, while most of the brain stem centers are involved, other areas are entirely unaffected. In the brain stem, the reticular formation and most of the nuclei of cranial nerves, including the motor nuclei (facial, hypoglossal, nucleus ambiguus) and the sensory nuclei (vestibular and trigeminal) are involved. Neurons of the cerebellar vermis and the deep cerebellar nuclei may also be involved. In the forebrain, neuronal lesions are usually mild and restricted to the precentral gyrus (motor cortex), the thalamus and the globus pallidus. The highly selective nature of

the damage could be due to two principal factors: the inherent variation in susceptibility of the nervous centers to infection and the restricted movement of the virus along certain fiber pathways (Bodian and Horstmann, 1965; Brown *et al*, 1987; Jubelt *et al*, 1980).

Nerve cell lesions are the result of lytic viral replication (Bodian, 1959; Couderc *et al*, 1989). Cytological changes consist of chromatolysis with disappearance of the Nissl bodies, nuclear abnormalities, and shrunken cytoplasm. The necrotic neurons may be removed by inflammatory cells (neuronophagia). Glial, endothelial and inflammatory cells are spared (Hashimoto *et al*, 1984). The changes in the nerve cells are accompanied by a secondary inflammatory reaction involving meningeal, perivascular and parenchymal infiltrates with lymphocytes, macrophages and some polymorphonuclear cells (Bodian, 1959).

The localization and degree of paralysis depend on the site and the severity of neuronal lesions. Clinically, paralytic poliomyelitis can be divided into three types: spinal, bulbar and encephalitic (Bodian, 1959). Spinal poliomyelitis is the most frequent and is characterized by flaccid paralysis of muscles innervated by the motor neurons of the spinal cord. Bulbar poliomyelitis involves the destruction of neurons of the reticular formation and of the nuclei of cranial nerves in the brain stem. In the most severe cases, a combination of the two forms occurs. Encephalitic poliomyelitis, which involves neurons of the brain, is rare. Spinal and bulbar poliomyelitis can be fatal due to respiratory or cardiac failure resulting from damage of neurons in the spinal cord that innervate the diaphragm, the intercostal and abdominal muscles and/or neurons of the respiratory and vasomotor nuclei in the brain stem. The non-fatal cases are associated with highly variable patterns of residual paralysis. Many years following paralytic poliomyelitis, these survivors may develop a new disease, the post-polio syndrome, which will be discussed later in this review.

PV infection is characterized by a restricted tissue tropism despite the presence of virus in many organs during the viremic phase of infection (Bodian and Howe, 1955; Sabin, 1956). Although the basis for this restriction has not been determined, the presence of the hPVR at the cell surface is believed to be a major determinant of PV tissue tropism (Holland, 1961). However, studies revealing the presence of hPVR mRNA in human and hPVR-Tg mouse tissues, which are refractory to PV infection, suggest that susceptibility to PV may not be determined simply by the expression of hPVR (Freistadt, 1994; Freistadt *et al*, 1990; Mendelsohn *et al*, 1989; Ren and Racaniello, 1992a; Ren *et al*, 1990). Further studies, overcoming the current difficulties encountered when studying the expression of functional hPVR, will be needed in order to determine the precise tissue distribution of the hPVR protein (Freistadt, 1994; Freistadt *et al*, 1990; Macadam *et al*, 1989; Mendelsohn *et al*, 1989). In their absence, the assessment of the respective roles of both hPVR and other cellular factors, in mediating poliomyelitis, remains difficult (Freistadt, 1994).

Prophylaxis of poliomyelitis and vaccine-associated paralytic poliomyelitis

The demonstration that serum immunoglobulin can protect against the paralytic disease was one of the crucial results which favoured the development of antipoliomyelitis vaccines (Modlin, 1995). Since the 1960's, poliomyelitis has been effectively controlled by the use of both inactivated and live attenuated vaccines. The inactivated vaccine (IPV) was elaborated using wild pathogenic strains rendered non-infectious by formaldehyde treatment (Salk, 1955). IPV is safe and induces a protective immune response in vaccinees which needs to be boosted regularly. Unfortunately however, IPV induces poor local immunity and is thus not an effective tool for controlling virus transmission in the human population. In contrast, the Sabin live oral polio vaccine (OPV), which is composed of attenuated strains selected by numerous passages of wild-type viruses in monkey tissues *in vivo* and *in vitro* (Sabin and Boulger, 1973), induces a strong and long-lasting immune response including local intestinal immunity (Sabin, 1985). OPV strains are thus recommended tools for the eradication of poliomyelitis. OPV strains of all three serotypes replicate in the human gut, and have a good safety record. However, in a very small number of cases, vaccination is associated with paralytic poliomyelitis (VAPP), which affects either individuals recently vaccinated with the OPV strains, or non-vaccinated individuals who live in direct contact with healthy vaccinees (Strebel *et al*, 1994). VAPP is probably caused by the genetic variability of the Sabin strains, due to point mutations and genomic recombination. Indeed, neurovirulent vaccine-derived strains are found in the gut of healthy vaccinees, and in the CNS of VAPP patients. PV-2/Sabin and PV-3/Sabin-derived strains are more frequently isolated from VAPP cases (about 88% of cases) than PV-1/Sabin-derived strains. The pathogenic character of these strains is in general associated with mutations, or even reversions to the wild-type genotype, at the positions corresponding to the determinants of attenuation/neurovirulence. It is currently estimated that there is one case of VAPP per 2.5 million doses of OPV administered, although this number may reach as many as one case per 200 000 doses in certain circumstances. As reported recently (Strebel *et al*, 1995), multiple intramuscular injections of antibiotics, within 30 days of immunization with OPV, have provoked a

disproportionately large number of cases of VAPP in vaccine recipients in Romania, where muscular injections are a popular means of administering medications to young children. One of the mechanisms proposed to explain this phenomenon known as 'provocation' poliomyelitis is that injury at the site of an intramuscular injection may allow PV to gain direct access to peripheral nerve endings and then to travel by retroaxonal transport to the anterior horn (Strebel *et al*, 1995).

Host range

Although humans are the only natural hosts for PV, paralytic poliomyelitis clinically and histologically similar to the human disease, can be reproduced experimentally by intracerebral inoculation in the monkey, and exceptionally, in the mouse (Bodian and Howe, 1955; Jubelt *et al*, 1980; Koike *et al*, 1991b; Ren *et al*, 1990; Sabin 1956). *In vitro*, however, PV multiplies exclusively in primate cell lines of either human or monkey origin. This host range restriction in cultured cells is determined by a blockage at the level of the host cell receptor, as when a variety of non-primate cells are transfected with purified viral RNA, one replicative cycle occurs and infectious virus is released (Holland and McLaren, 1959; Holland *et al*, 1959a, 1959b). Furthermore, expression of the hPVR in mouse L cells renders these normally resistant cells susceptible to multi-cycle viral infection (Koike *et al*, 1990; Mendelsohn *et al*, 1989). *In vivo*, in the monkey as well as in hPVR-Tg mice, the three wild-type serotypes of PV are neurovirulent. In contrast, in the normal mouse, excepting certain strains such as PV-2/Lansing (Armstrong, 1939) and PV-1/LS-a (Li and Schaeffer, 1953) which have been found to be capable of infecting the murine CNS, most strains, and notably PV-1/Mahoney, are avirulent.

The construction of PV-2/Lansing-PV-1/Mahoney recombinant viruses revealed that the molecular determinants allowing PV-2/Lansing to infect the murine CNS are localized in the region of the genome encoding for the capsid proteins (La Monica *et al*, 1986). Further studies using monoclonal antibodies showed that the loop connecting β-strands B and C (BC loop) of VP1 (amino acids 94–102) plays a major role in the neurovirulence of PV-2/Lansing in the mouse (La Monica *et al*, 1987b). The critical role of this region was confirmed by the construction of a chimeric virus in which the BC loop of PV-1/Mahoney was replaced by the equivalent region of PV-2/Lansing, yielding a virus neurovirulent in the mouse (Martin *et al*, 1988; Murray *et al*, 1988). Moreover, it seems that the structural conformation of the BC loop plays a primordial role in this phenotype (Couderc *et al*, 1991; Martin *et al*, 1991; Yeates *et al*, 1991).

Additional molecular determinants of mouse-adaptation were identified by the characterization of other viruses neurovirulent in the mouse selected by several different approaches: a single passage in the murine CNS of a chimeric mouse-avirulent PV-2/Lansing carrying the BC loop from PV-1/Mahoney (Moss and Racaniello, 1991) or a single passage in the murine CNS of PV-1/Mahoney (Couderc *et al*, 1993); neuroblastoma cells persistently infected with PV-1/Sabin or PV-1/Mahoney (Couderc *et al*, 1994); mutant hPVR-expressing cells infected with PV-1/Mahoney (Colston and Racaniello, 1995). These determinants can be classified into two groups: (i) those which are exposed, like the BC loop, on the surface of the capsid i.e. the residues at positions 160 of VP1, 142 of VP2 and 60 of VP3 (Colston and Racaniello, 1995; Couderc *et al*, 1994), and (ii) those located inside the viral capsid, i.e., the residues at positions 22, 40, 43 and 54 of VP1, 31 of VP2 and 62 of VP4 (Couderc *et al*, 1993, 1994; Moss and Racaniello, 1991). Each of these determinants are sufficient, when present independently, to confer a mouse-adapted phenotype to PV-1/Mahoney. In contrast, the mouse adaptation of PV-1/LS-a is determined by multiple mutations found in regions encoding both VP1 and the proteinase 2A (Lu *et al*, 1994).

Since hPVR-Tg mice are susceptible to infection by all three serotypes of PV (Koike *et al*, 1991b; Ren *et al*, 1990), it seems likely that PV strains which are avirulent in the normal mouse are blocked at one or more of the early steps of the viral cycle: adsorption, penetration and uncoating. In the simplest model, mouse-virulent mutants could have acquired mutations which allow them to attach to receptors in the mouse CNS. Direct assessment of this model has been hampered by the inability to demonstrate receptor binding of either primate-specific or mouse-adapted strains to mouse brain homogenates (Holland, 1961). However, a more recent study showed that binding sites for even primate-specific PV strains may be present on mouse L cells in culture (Barnert *et al*, 1992). These results suggest an alternative model in which PV-1/Mahoney is able to attach to the as of yet unidentified murine receptor with a weak affinity, but is unable to undergo conformational changes required for subsequent steps (Couderc *et al*, 1991, 1993). The superficial determinants localized in or around the region of the putative attachment site for the hPVR could therefore increase the affinity of PV for this mouse receptor. On the contrary, the mechanism by which the internal determinants overcome the blockage of the early steps is less evident. However, their localization in the three-dimensional structure of the capsid has revealed that they are situated in contact with VP4 (Couderc *et al*, 1993, 1994; Moss and Racaniello, 1991) and our results show that these areas are implicated in the receptor-mediated conformational changes necessary for uncoating in

human cells (Couderc *et al*, 1996). These determinants could therefore act by lowering the energy barrier of transition from the 160S to the 135S particle in order to compensate for the lower free energy of binding to the suboptimal receptor expressed by the murine nerve cell.

As described above, the PV host range is primarily dependent on the presence of a cell surface molecule that functions as the PV receptor. However several data indicate that other host factors could play a role in the host range specificity of PV. First, it has been shown that PV-1/Mahoney plus-strand RNA synthesis is restricted at an initiation step at 40°C in mouse TgSVA cells (kidney cell line established from the PV-sensitive hPVR-Tg mice), but not in human HeLa cells (Shiroki *et al*, 1993). Genetic analysis of temperature-resistant mutants identified a point mutation within the stem-loop II of the IRES (Shiroki *et al*, 1995) thereby revealing a new cis-element for PV RNA replication, located in the IRES. More recently it has been shown that the viral cycle in the TgSVA cells could also be specifically restricted in an IRES-dependent initiation step of translation by mutations in the stem-loop II of the IRES (Shiroki *et al*, 1997). Neurovirulence tests of the corresponding mutants using monkeys and hPVR-Tg mice revealed that these mutants were strongly attenuated only in the case of the Tg mouse model, suggesting that interactions between the IRES and host factor(s) could potentially be an additional host range determinant (Shiroki *et al*, 1997). It is interesting to note that the proteinase 2A which contributes to the mouse-neurovirulent phenotype of the mouse-adapted strain PV-1/LS-a (Lu *et al*, 1994), is also known to enhance the efficiency of PV translation initiation (Hambidge and Sarnow, 1992; Macadam *et al*, 1994b).

Determinants of PV neurovirulence

The neurovirulence of the PV strains refers to the capacity of the strains (i) to replicate in the neuronal target cells and (ii) to kill enough of these cells to induce the paralytic disease. The neurovirulence phenotype is usually determined in monkeys (rhesus or cynomolgus) by monitoring paralysis and the development of histological lesions in the CNS after intrathalamic or intraspinal injection (WHO, 1990). Recently, hPVR-Tg mice have proven to be suitable replacements for monkeys for the PV neurovirulence test (Abe *et al*, 1995; Koike *et al*, 1991b; Ren *et al*, 1990). The hPVR-Tg mice develop paralysis after inoculation of PV by the intracerebral, intraspinal, intraperitoneal, intravenous, and intramuscular routes (Gromeier *et al*, 1995; Koike *et al*, 1991b, 1994; Racaniello and Baltimore, 1981; Ren and Racaniello, 1992a, b; Ren *et al*, 1990). However, they are poorly sensitive to oral PV inoculation (Koike *et al*, 1994) and it has been shown that the inability of PV to replicate in the alimentary tract of hPVR-Tg mice is not solely due to the low level of hPVR expression in the small intestine (Zhang and Racaniello, 1997). Studies have also been carried out in non-Tg mice with virus strains that have been adapted for growth in these mice although these virus strains are not infectious when administered orally (La Monica *et al*, 1987a; Martin *et al*, 1988, 1991; Moss *et al*, 1989; Ren *et al*, 1991; Tardy-Panit *et al*, 1993). However, at present, no convenient animal model is available for studying the diffusion of PV from the portal of entry of the virus to the CNS. Moreover, when using the existing animal models, it must be taken into account that the measurement of neurovirulence for a given strain is influenced by both the choice of the host animal and the route of inoculation.

PV neurovirulence has been studied mainly through the characterization of attenuated viral mutants and in particular the live oral PV Sabin vaccine strains. Attempts have been made to find *in vitro* phenotypic markers which could correlate with the attenuation/neurovirulence phenotype (Nakano *et al*, 1978). Among the numerous markers which differentiate the original wild-type from the attenuated vaccine strains, only the temperature-sensitive (ts) phenotype of the attenuated strains correlates rather well with the attenuated phenotype (Christodoulou *et al*, 1990; Georgescu *et al*, 1994; Lwoff, 1959, Omatu *et al*, 1986).

The first approach used to attempt to identify the molecular basis of the attenuation/neurovirulence of PV was the comparison of the RNA nucleotide sequences of virulent and attenuated PV strains. However, this approach did not lead directly to the identification of the genomic segment(s) responsible for the attenuated or neurovirulent phenotype of PV strains, due to the great number of mutations involved, especially in the case of PV-1. In order to better address this question, it was necessary to study the neurovirulence of genomic recombinants made between the attenuated Sabin strains and closely related neurovirulent strains. Further analysis of site-directed mutants also contributed to the identification of attenuation/neurovirulence determinants (Figure 4) (Agol, 1993; Koike *et al*, 1994; Minor, 1992, 1996; Racaniello and Ren, 1996).

The attenuated PV-3/Sabin strain (P3/Leon $12a_1b$) genome differs from that of the virulent wild-type progenitor PV-3/Leon (P3/Leon/USA/1937) by 11 mutations (Stanway *et al*, 1984) but only two are strong determinants of attenuation/neurovirulence: a nucleotidic change in the 5′NCR at position 472 and an amino acid substitution in the capsid protein VP3 at position 91 (Figure 4) (Westrop *et al*, 1989). A third amino acid change, at position 6 of VP1, has been shown to have a weak attenuating effect (Tatem *et al*, 1992). The mutation at position 472 confers a slight ts effect to the PV-3/Sabin strain

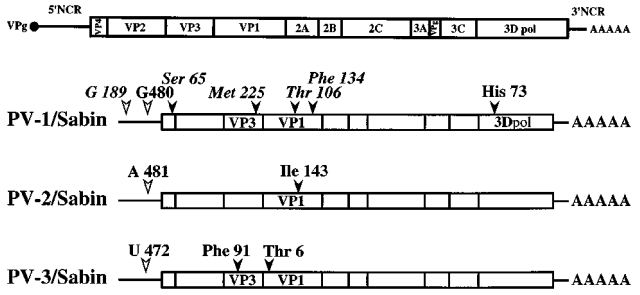

Figure 4 Localization of attenuating determinants in the PV Sabin strains of all three serotypes. The nucleotides (empty triangles) and amino acids (solid triangles) involved in the attenuation of neurovirulence are indicated in the 5′ noncoding region (5′NCR) and in the coding region respectively, of the Sabin strain genomes. These determinants have been identified using different animal models; in most cases both the monkey and either common or transgenic mice carrying the hPVR gene were used, however, certain determinants, indicated in italics, were identified using only the transgenic mouse.

(Macadam *et al*, 1992) and it could affect the stability of the RNA secondary structure in domain V of the 5′NCR (Pilipenko *et al*, 1989; Skinner *et al*, 1989). The VP3 residue, located at the interface between protomers of the viral capsid, is mainly responsible for the ts phenotype of the strain, affecting the assembly of 14S capsid pentamers, and that of virions at supraoptimal temperature (Macadam *et al*, 1991a). The residue at position 6 of VP1 is located inside the capsid in an area involved both in the assembly and in the receptor-mediated conformational changes required for uncoating (Fricks and Hogle, 1990; Kirkegaard, 1990). This residue does not appear to be involved in the ts phenotype (Tatem *et al*, 1992).

Reversion towards the wild-type genotype at position 472 of the 5′NCR is rapidly selected in the gut of vaccinees (Dunn *et al*, 1990; Minor and Dunn, 1988), and is found in all isolates from VAPP patients (Evans *et al*, 1985; Georgescu *et al*, 1994; Minor, 1992). This reversion is closely associated with an increase in the neurovirulence, although it does not, on its own, give rise to a fully neurovirulent phenotype. The Sabin-type residue at position 91 of VP3 is found to be reverted in only a few cases (Macadam *et al*, 1989), nevertheless, second site mutations in the capsid proteins could suppress its attenuating effect (Filman *et al*, 1989). The presence of the reversion at position 6 of VP1 has not been systematically investigated, but has been found in some vaccine lots and in some viruses isolated from inoculated monkeys (Lu *et al*, 1996; Rezapkin *et al*, 1995).

As the PV-2/Sabin strain was derived from the strain P2/712/56 a naturally attenuated isolate that is no longer available, different approaches were taken to investigate the attenuating determinants of this strain. Analysis of recombinant strains between P2/P712, an attenuated strain closely related to PV-2/Sabin, and the mouse-adapted neurovirulent PV-2/Lansing strain, in both normal and hPVR-Tg mice, showed that only two nucleotides, at position 481 in the domain V of the 5′NCR and at residue 143 of VP1, are major determinants of attenuation/neurovirulence (Figure 4) (Moss *et al*, 1989; Ren *et al*, 1991). The study of intratypic recombinants of PV-2/Sabin and P2/117, a neurovirulent strain isolated from a VAPP case, revealed that nucleotide 481 and residue VP1-143 of the P2/117 strain both confer a highly neurovirulent phenotype to PV-2/Sabin in monkeys (Macadam *et al*, 1993). Moreover, the suppression of the ts phenotype of PV-2/Sabin correlated with a mutation at nucleotide 481, although other changes were also involved (Macadam *et al*, 1991b). The residue 143 of VP1 is exposed on the external surface of the virion in the loop connecting β-strands D and E (DE loop) of VP1 (Hogle *et al*, 1985). The DE loop interacts closely with the BC loop of VP1 (Filman *et al*, 1989; Yeates *et al*, 1991) to make up the discontinuous antigenic neutralization site 1 (Wiegers *et al*, 1989) and, as mentioned earlier, the BC loop is believed to influence PV host range through receptor-mediated events early in infection (Martin *et al*, 1988; Moss and Racaniello, 1991; Murray *et al*, 1988). Thus, it is possible that the attenuating effect of the residue VP1-143 affects early receptor-mediated events (Macadam *et al*, 1993; Ren *et al*, 1991). In vaccinees, both nucleotide 481 and residue 143 of VP1 are unstable attenuation determinants, and most of the neurovirulent PV-2/Sabin strains isolated from VAPP patients carry mutations at these positions (Equestre *et al*, 1991; Georgescu *et al*, 1994; Macadam *et al*, 1991a, 1993).

The genome of the PV-1/Sabin strain, LS-c, 2ab, differs from that of the virulent wild-type progenitor PV-1/Mahoney by 54 point mutations (Nomoto *et al*, 1982). As with PV-2 and PV-3, neurovirulence determinants of PV-1 have been studied in monkeys and hPVR-Tg mice, but also in normal mice with PV-1 strains adapted to the mouse due to modifications in the BC loop of VP1 (Agol, 1993; Koike *et al*, 1994; Racaniello and Ren, 1996). PV-1 attenuation/neurovirulence determinants were found scattered throughout the viral genome, rendering the analysis more complex than for the other serotypes (Agol *et al*, 1985; Omata *et al*, 1986). One well-characterized determinant has been identified in the domain V of the 5′NCR at position 480 (Figure 4) (Kawamura *et al*, 1989; Macadam *et al*, 1989; Martin *et al*, 1991; McGoldrick *et al*, 1995; Tardy-Panit *et al*, 1993). This position is only moderately implicated in neurovirulence when compared to the important contribution of nucleotide 472 in PV-3 and probably of nucleotide 481 in PV-2 (Horie *et al*, 1994; McGoldrick *et al*, 1995). Nevertheless, a second determinant located in domain III of the 5′NCR of

PV-1, at position 189, could also play a role in neurovirulence (Horie *et al*, 1994). Four additional attenuating determinants have been located in the capsid protein encoding region (Figure 4) (Bouchard *et al*, 1995; Christodoulou *et al*, 1990). Two of them are located inside the viral capsid: residue 65 of VP4 is near the seven-stranded β-sheet that acts to stabilize the association of pentamers (Filman *et al*, 1989), and residue 134 of VP1 is within a hydrophobic pocket below the canyon floor. The two others are exposed on the capsid surface: residue 106 of VP1 is near the VP1 BC loop region on the north rim of the hPVR foot-print and residue 225 of VP3 is at the interface between protomers. Additional weak determinants of attenuation/neurovirulence may be located in the 3′-terminal half of the PV-1 genome (Agol *et al*, 1985; Bouchard *et al*, 1995; Christodoulou *et al*, 1990; Horie *et al*, 1994; Omata *et al*, 1986), and in particular, in codon 73 of $3D^{pol}$ (McGoldrick *et al*, 1995; Tardy-Panit *et al*, 1993). Several of the determinants contributing to attenuation, including nucleotide 480 and residues 65 of VP4, 225 of VP3, and 73 of $3D^{pol}$, are involved in the ts phenotype of the PV-1/Sabin strain. The mutation at position 480 has been found in the genomes of PV-1/Sabin strains isolated from both healthy vaccinees and from the rare cases of VAPP associated with this strain (Li *et al*, 1996; Otelea *et al*, 1993). However, this mutation was found to appear less rapidly than that at position 472 of PV-3/Sabin (Dunn *et al*, 1990). Mutations in codons 106 of VP1, 225 of VP3 and 73 of $3D^{pol}$ are also found in some strains isolated from VAPP cases (Figure 4) (Furione *et al*, 1993; Georgescu *et al*, 1995; Li *et al*, 1996; Otelea *et al*, 1993).

Remarkably, all three Sabin vaccine strains contain strong attenuation determinants in domain V of the 5′NCR of the viral genome. Free-energy calculations showed that these determinants disrupt a highly conserved base-paired stem and this structural perturbation was correlated with the temperature sensitivity of the attenuated strains (Macadam *et al*, 1992). This domain is an essential part of the IRES element, involved in the initiation of translation. It has been shown that nucleotide 472 of PV-3/Sabin affects the level of translation of the viral genome in either a cell-free translation system using Krebs-2 cell extracts (Svitkin *et al*, 1990), or in neuroblastoma cells but not in HeLa cells (La Monica and Racaniello, 1989). In addition, in rabbit reticulocyte lysates the relatively poor efficiency of translation of PV wild-type strains can be restored by the addition of either HeLa or neuroblastoma cell extracts, whereas only HeLa cell extracts can restore the efficiency of translation for PV carrying mutations in domain V (Haller *et al*, 1996). Thus, the attenuated phenotype observed in neural cells could be due, at least in part, to a decrease in the translation of the attenuated viral RNA (La Monica and Racaniello, 1989). It has been recently described that the substitution of the native PV-1/Mahoney IRES by the human rhinovirus 2 IRES drastically reduced neurovirulence (Gromeier *et al*, 1996). These experiments underline the importance of the IRES in the determination of the neurovirulent phenotype. Attenuating mutations which destabilize the RNA secondary-structure of the domain V of the IRES may interfere with the binding of essential cellular translation initiation factors. If such factors are less numerous or slightly different in neural cells, their interaction with the 5′NCR of attenuated viruses might be altered, resulting in reduced translation and therefore diminished multiplication of attenuated viruses. Alternatively, the altered nucleotide sequences of the attenuated stains could generate a binding site for negatively-acting factor found only in neural cells. Two lines of evidence support the first hypothesis. First, an initiation correcting factor (ICF), which restores the efficiency of PV translation in rabbit reticulocyte lysates, has been isolated from Krebs-2 cell extracts (Svitkin *et al*, 1988). This ICF appears to be a complex formed of initiation factors eIF-2 and eIF-2B (Svitkin *et al*, 1988). Interestingly, this ICF appears to act less effectively on attenuated PV-1 and PV-3 RNA templates than on RNAs from their neurovirulent counterparts. Secondly, it was shown that the mutation at position 472 of PV-3/Sabin reduces the cross-linking between the PV 5′NCR and polypyrimidine tract-binding protein, PTB, in neuroblastoma cells but not in HeLa cells (Gutierrez *et al*, 1997). Recently it was also shown that attenuated PV strains could be obtained by modifying the cryptic AUG (at position 586) which, in tandem with an appropriately spaced oligopyrimidine tract, is involved in translation initiation (Slobodskaya *et al*, 1996). In fact, the presence of an initiating rather than a cryptic AUG at this position in a pathogenic mouse-adapted PV strain, strongly suppresses PV growth in the murine CNS.

Although the situation is undoubtedly much more complicated *in vivo*, two different mechanisms could explain how attenuation determinants could confer the attenuated phenotype to the Sabin strains in terms of PV pathogenesis in humans. The first mechanism involves the nature of the target cells in the human body. Indeed, a correlation has been observed between the attenuated phenotype and limited viral replication, not only in human neuroblastoma cell lines (Agol *et al*, 1989; La Monica and Racaniello, 1989) as mentioned above, and in human fetal brain cells (Pavio *et al*, 1996), but also in peripheral blood mononuclear cells (PBMC) (Freistadt and Eberle, 1996). If PV replication occurs in PBMC during a natural infection, the reduced multiplication of PV in PBMC may be therefore responsible for a reduced viral load, and thus the capacity of PV to reach the CNS would also be affected. Whether or not neurons and PBMC share common cellular factors responsible for the

reduction in the replication of attenuated PV strains remains to be investigated. The second mechanism of PV attenuation in humans involves a non-specific property of attenuated viruses, their thermosensitivity, which limits their replication at physiological temperature. However, the distinction between specific and non-specific mechanisms of attenuation may be complicated, since certain PV mutations confer a ts phenotype dependent on the cell type used for the assay. Therefore, the attenuated phenotype seems to act at several stages of viral pathogenesis, leading to limited viral growth in humans and leaving enough time for the immune system to develop an effective defense to prevent invasion of the CNS, contrary to the efficiently and rapidly multiplying neurovirulent viruses.

The fact that the attenuated phenotype concerns not only neural cells, but also extra-neural target cells could explain the somewhat surprising counter-selection of attenuating mutations during PV replication in the intestine of vaccinees. The simplest explanation would be that the physiological temperature of both the intestine and the CNS (37°C) is the principal agent responsible for the counter-selection of attenuating mutations, as most of them are also ts mutations. However, since ts, as well as non-ts, attenuating mutations can equally well be counter-selected in some types of cell cultures at a rather low temperature (34°C) (Rezapkin *et al*, 1994; Taffs *et al*, 1995) it would seem that cellular factors in addition to or aside from temperature are probably involved. It is tempting to hypothesize that the multiplication of the attenuated strains is affected by cellular factors common to both neurons and intestinal cells, and therefore that these common factors may contribute to the counter-selection of attenuating mutations in the intestine.

Post-polio syndrome

Ten to fifty percent of patients having recovered from acute poliomyelitis develop, after 30 to 40 years of clinical stability, new clinical signs known as the post-polio syndrome (PPS) (Dalakas 1986; 1995; Dalakas *et al*, 1984). These symptoms range from fatigue and muscle pains, to slowly progressive muscle weakness and atrophy (Agre *et al*, 1989; Diard *et al*, 1994). Both muscles originally affected and those having apparently escaped the effects of the acute infection can be affected. Muscle biopsies indicate both recent and ancient denervation. Several non-exclusive hypotheses have been proposed to explain this new pathological phenomenon including: the gradual attrition of the remaining, healthy motor neurons which have been forced to compensate for the neurons lost during the acute infection, an immunopathological mechanism and the reactivation of PV, i.e. a persistent PV infection (Dalakas, 1986). Analyses of the spinal fluid of PPS patients have revealed the presence of anti-PV IgM antibodies and, at the same time, high levels of IL-2 and the soluble IL-2 receptor, both of which have been found to be produced locally during viral infections of the CNS (Sharief *et al*, 1991). Further, fragmentary genetic sequences of PV RNA or PV-related RNA have been found in spinal fluid taken from PPS patients (Leon-Monzon and Dalakas, 1995: Leparc-Goffart *et al*, 1996; Muir *et al*, 1995). Although the link between PV persistence and the PPS remains controversial, all of these results suggest that PV is present in the CNS of PPS patients.

Persistence of poliovirus

Persistence in vitro

In order to test the capacity of PV to establish persistent infections, several *in vitro* models have been developed. In fact, although it is commonly believed that PV is an exclusively lytic virus in the cell cultures used for its growth, carrier-state types of infections in which a minority of cells in each generation are permissive to the virus, have been successfully established. Indeed, HeLa cell cultures could be persistently infected by supplying anti-PV antisera (Ackermann and Kurtz, 1955), by washing cell monolayers extensively (Pasca, 1961) or by co-transfection of PV RNA with a PV subgenomic RNA (Kaplan *et al*, 1989). Carrier-states have also been established in human lymphoid cell cultures (Carp, 1981; Fagraeus *et al*, 1981; Wallace, 1969). It was recently shown that steady-state types of infection, in which the majority of cells in the culture produce virus in the absence of cell lysis, could be established in human erythroblastoid K562 cells (Lloyd an Bovee, 1993).

Given that PV replicates in nerve cells *in vivo*, we were interested in studying PV-host cell interactions in cell lines more closely related to cells of the neuronal lineage, such as the human neuroblastoma cell lines SK-N-MC and IMR-32. Interestingly, when we infected these two cell lines, we found that the three PV serotypes were not fully lytic in these cultures. Indeed, in the first few days following cell infection, a large proportion of the cells died, while the surviving cells started to grow. Cytopathic effects were observed for the first few weeks following infection, then disappeared, and cells reached confluency, despite the fact that virus continued to be released into the cell medium at high titers ($10^6 - 10^7$ ID50/ml) (Colbère-Garapin *et al,* 1989). This showed that PV was capable of establishing a persistent infection in cultured cells of neuronal origin.

Neuroblastoma cell cultures persistently infected with PV are resistant to superinfection by other strains of PV, but sensitive to another enterovirus, coxsackievirus B3, suggesting that cells more

resistant to PV than the parental IMR-32 cells were selected during persistent infection (Colbère-Garapin *et al*, 1989). Although the establishment of a persistent infection may involve strong cell selection, the neuronal character of persistently infected cells was maintained as confirmed by the detection of neurofilaments in the majority of cells having survived the crisis period (Colbère-Garapin *et al*, 1989). After six months of persistent infection, between 5 and 25% of the cells, depending on the cell line and the PV strain, were found to be still releasing virions, as determined by infectious center assaying (Colbère-Garapin *et al*, 1989). The majority of the cells expressed viral antigen as shown by immunofluorescence. However, only 5 to 7% of the cells were strongly immuno-labeled and this percentage is in agreement with that of cells releasing virus (Colbère-Garapin *et al*, 1989). Viral RNA having the expected size of 7.5 kilobases was detected in cell cultures by Northern blotting, which, when taken together with results based on interference tests, suggests that defective interfering particles were not generated during persistent infection (Pelletier *et al*, 1991).

The fact that persistently infected neuroblastoma cell cultures could be cured by several passages in the presence of neutralizing antibodies (Borzakian *et al*, 1992), is in agreement with the carrier-state model of persistent viral infection. However, it has been shown that the addition of antiviral serum to PV-infected neuroblastoma cells harboring up to 350 virions per cell prevented cell death and allowed the cells to continue to divide (Tolskaya *et al*, 1992). It was speculated that the curing of cells was due to the penetration of antibodies into the cells at a relatively late step of the infectious cycle (Tolskaya *et al*, 1992). The fact that we have shown that PV (or PV RNA) can remain intracellular for up to 10 days in the presence of neutralizing antibodies (Borzakian *et al*, 1992) is not in agreement with the carrier-state model. Therefore, it is probable that PV persistently infects human neuroblastoma cells by mechanisms different from those implicated in classic carrier-state persistent infections.

In an attempt to elucidate the mechanism of PV persistence in neuroblastoma cell cultures, we first analyzed the expression of the hPVR on the cell surface. Depending on the multiplicity of infection, PV killed up to 100% of IMR-32 cells in the majority of cultures, indicating that the hPVR is expressed on all IMR-32 cells. However, once IMR-32 cell cultures were persistently infected, the hPVR could be detected in only one third to one-half of the cells in the culture by immunofluorescence, and the average intensity of fluorescence per positive cell was slightly lower than that of uninfected IMR-32 cells. Thus, the reduced expression or the expression of a modified hPVR molecule in persistently infected IMR-32 cell cultures may be responsible for, or contribute to, the maintenance of the persistent infection (Borzakian *et al*, 1992).

Phenotypic and genotypic properties of mutant viruses (PVpi) selected during the persistent infection of neuroblastoma IMR-32 cells were then analyzed. Phenotypic analysis of PVpi revealed that they differ greatly from their corresponding parental strains. In particular, both the size of plaques and the titers of PVpi are higher on IMR-32 cells than on non-neural HEp-2 cells, in contrast to those of the parental viruses which are similar on the two cell types. Another remarkable property of PVpi is their capacity to establish secondary persistent infections in non-neural HEp-2 cell cultures, in contrast to the parental PV which are lytic in these cultures (Pelletier *et al*, 1991) (Figure 5).

The entire genome of PVpi S11, a PVpi derived from the attenuated PV-1/Sabin strain isolated 6 months after the establishment of a persistent

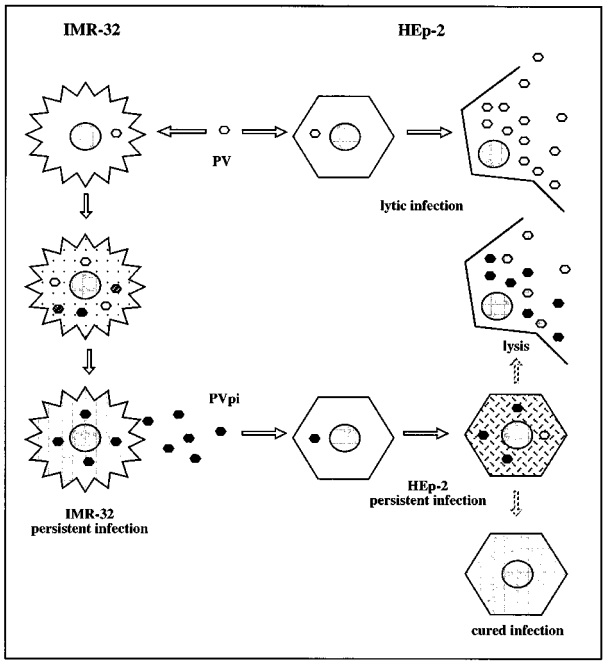

Figure 5 Simplified models of persistent PV infection in human neuroblastoma IMR-32 cells (star-shapes) and non-neural HEp-2 cells (large hexagons). The majority of PV strains (small white hexagons), which are grown on HEp-2 cells, are able to establish persistent infections in IMR-32 cells. Virus and cell coevolution occurs, leading to the selection of PVpi (small black hexagons). These PVpi, in contrast to the parental PV strains, are able to establish secondary persistent infections in non-neural HEp-2 cells. Coevolution of both cells and viruses also occurs in the HEp-2 cell system. However, the two types of persistent infections seem to differ, as the maintenance of the persistent infection in HEp-2 cells probably relies on an equilibrium between a lytic and an abortive infection (dotted arrows), whereas in IMR-32 cells, neither spontaneous curing nor lysis of persistently infected cultures has been observed.

infection in IMR-32 cells, was cloned and sequenced. Thirty-one mutations were found, scattered throughout the entire genome and 12 of them induce an amino acid change (Borzakian *et al*, 1993). Remarkably, some mutations were repeatedly selected in cell cultures, when independent cultures were infected by the same virus (Borzakian *et al*, 1993). All missense mutations in the PVpi S11 genome except one are clustered in the genes encoding for two of the capsid proteins, VP1 and VP2 (Figure 6). The capsid encoding region of a second PVpi, PVpi170, isolated 10 weeks after infection in IMR-32 cells with PV-1/Mahoney was also analyzed (Couderc *et al*, 1994). Sequencing revealed only five mutations inducing amino acid substitutions: two are located inside the capsid at positions 62 of VP4 and 43 of VP1 and three are located on the capsid surface at positions 95 of VP1, 142 of VP2 and 60 of VP3 (Figure 6). All of these mutations, except for the mutation affecting the position VP1-95, were capable of conferring the mouse-virulent phenotype to the normally mouse-avirulent PV-1/Mahoney (Couderc *et al*, 1994) indicating that these residues play a role in the early steps of the viral cycle. Residues VP4-62 and VP1-43 are located in an area known to be involved in receptor-mediated conformational transitions required for viral uncoating (Couderc *et al*, 1993, 1994, 1996; Moss and Racaniello, 1991). Residue VP2-142 is located in the canyon-foot-print of the hPVR and it is involved in receptor binding (Colston and Racaniello, 1995). Residue VP3-60 belongs to the antigenic neutralization site 3a in a loop near the south rim of the canyon and it could be involved in the interactions with the hPVR (Harber *et al*, 1995). Although residue VP1-95 did not confer the mouse-virulent phenotype, its location in the BC loop (antigenic site 1) and near the hPVR foot-print, suggests that it is involved in receptor binding (Colson and Racaniello, 1995; Harber *et al*., 1995). Remarkably, PVpi S11 also contains seven amino acid changes located in areas known to regulate receptor binding and receptor-mediated conformational transitions, and two of them, VP1-43 and VP2-142, are identical to those identified in PVpi170 (Borzakian *et al*, 1993). Furthermore, some of the amino acid substitutions of PVpi S11 and PVpi 170 were also selected in PV-1/Mahoney after growth in murine L cells expressing mutated hPVR molecules (Colston and Racaniello, 1995). Taken together, these studies suggest that interactions between PV and its receptor probably play a crucial role in persistent infection. In agreement with this hypothesis, it has been shown that L cells expressing mutated hPVR molecules permit virus replication in the absence of cytopathic effects (Morrison *et al*, 1994). Moreover, viral entry steps are also targeted by

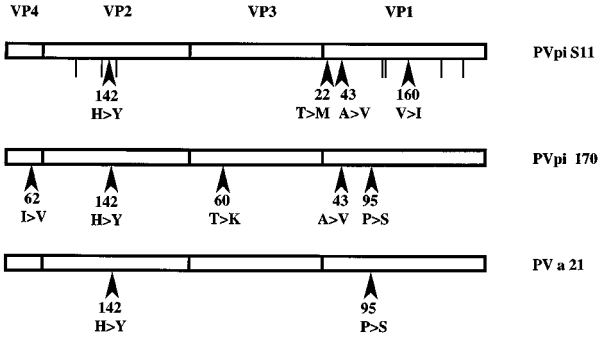

Figure 6 Amino acid substitutions in the P1 capsid protein-encoding region of PV mutants (PVpi) selected in persistently infected human neural cells. PVpi S11 was cloned 6 months after infection of neuroblastoma cells with PV-1/Sabin, PVpi 170 was cloned 2.5 months after infection of neuroblastoma cells with PV-1/Mahoney and PV a 21 was isolated 3 weeks after infection of anterior brain cells with PV-1/Mahoney. Amino acid substitutions are indicated either by vertical bars or by arrowheads. The arrowheads indicate the substitutions which may affect the early steps of the virus cycle; all of them were selected in PVpi isolated from at least two independent infections. With the exception of residue 95 of VP1, all substitutions indicated by arrowheads confer the capacity to infect murine motoneurons to PV-1/Mahoney.

mutations selected during persistent infections with reovirus (Dermody *et al*, 1993) and mouse hepatitis virus (Chen and Baric, 1996).

In human neuroblastoma cells the maintenance of persistent infection could depend on continuing virus-cell coevolution, as described for reovirus (Dermody *et al*, 1993), minute virus (Ron and Tal, 1985), foot-and-mouth disease virus (de la Torre *et al*, 1988) and mouse hepatitis virus (Chen and Baric, 1996), since both cells more resistant to PV than the parental IMR-32 cells, and PVpi phenotypically different from the parental PV, were selected during persistent infection.

It is interesting to note that mutations selected in neuroblastoma cells conferred the ability to infect murine neurons to PV-1. It would therefore be worthwhile to know if these mutations which play a role in the early steps of the viral cycle are selected by cellular factors similar in both human neuroblastoma and murine cells, and if these factors are specifically expressed by neuronal cells.

The model of PV persistence in IMR-32 cells has also permitted the development of a second model of PV persistence, in non-neural HEp-2 cells (Figure 5). As mentioned above, PVpi selected during the persistent infection of neuroblastoma IMR-32 cells are able to persistently infect HEp-2 cell cultures. In this cell system, there is also virus-cell coevolution (Borzakian *et al*, 1992). However, persistent infection in HEp-2 cells differs from that in neuroblastoma cells by at least two criteria: first, cytopathic effects are always observable in HEp-2 cell cultures, whereas

they are no longer detectable in infected IMR-32 cells after the crisis period, and second, either spontaneous curing or complete lysis of the cell cultures sometimes occur in HEp-2 cells while neither have ever been observed in infected IMR-32 cells (Calvez *et al*, 1995). The HEp-2 model has one great advantage over the IMR-32 cell system for studying the mechanisms of establishment and maintenance of persistent PV infection, as we possess both persistent PVpi mutants and their fully lytic parental strains for these cells. Indeed, the construction of recombinant viruses between the genomes of PV-1/Sabin and PVpi S11 permitted the localization of the determinants involved in the establishment of persistent infection in HEp-2 cell cultures to a region including the VP1 and VP2 capsid protein genes (Calvez *et al*, 1993). We have proposed that the maintenance of the persistent infection in these cultures relies on an equilibrium between a lytic and an abortive infection (Figure 5) (Borzakian *et al*, 1992).

Persistence ex vivo

In order to study a persistent infection closer to that which may occur *in vivo*, we have recently developed an *ex vivo* model using primary cultures of human fetal brain cells infected with a wild-type strain of PV (Pavio *et al*, 1996). These cells have the advantage of being neither transformed nor immortalized, and in addition they have the potential to differentiate into derivatives of both the neuronal and glial cell lineages (Buc-Caron, 1995). As in neuroblastoma cells, at the beginning of infection, cytopathic effects were observed, accompanied by an initial massive production of virus which then stabilized at about $10^7 - 10^8$ ID_{50}/ml. Some cells survived and the persistent infection could be maintained for up to 7 weeks, at which time these primary cells no longer adhered to the dish (Pavio *et al*, 1996).

Two missense mutations, corresponding to two amino acids on the surface of the viral capsid, VP2-142 and VP1-95, were repeatedly selected as early as 2 weeks post-infection (Figure 6). Remarkably these same two mutations had also been selected in neuroblastoma IMR-32 cells persistently infected by the same wild-type PV strain, suggesting that in primary cultures of human fetal brain cells, there is a similar strong selective pressure for capsid protein residues which affect virus-receptor interactions.

Early in infection, viral antigens were found in both neuronal and glial cells, indicating that both cell types were infected. The astrocytes then disappeared progressively from the infected cultures, and 2 weeks after infection, viral antigens were detected almost exclusively in cells of the neuronal lineage. Further identification of infected cells revealed that viral antigens were found predominantly in cells expressing a marker of early commitment to the neuronal lineage, although they were also found in cells expressing a neuroepithelium marker and in cells expressing a marker of post-mitotic neurons. The presence of viral antigens in post-mitotic neurons is particularly interesting, in that it suggests that PV is capable of persisting in the motoneurons of patients having survived paralytic poliomyelitis.

Persistence in vivo

As mentioned above, one hypothesis to explain the PPS is persistent PV infection in the spinal cord. However, up until now, no adequate animal model has been available to test whether or not PV can persist in the nerve cells of the spinal cord after the onset of paralysis. In fact, as previously described, poliomyelitis can be induced experimentally in mice by inoculation of PV directly into the CNS, however, most normal and hPVR-Tg mice which develop paralysis following PV inoculation, die (Jubelt and Meagher, 1984a; Koike *et al*, 1991b; Miller, 1981). Prolonged infections of the brain, lasting up to 4 months, have been described for normal mice which do not develop paralysis after inoculation with the mouse-adapted PV-2/Lansing strain, but PV was never found in the spinal cord of these mice (Jubelt and Meagher, 1984b; Miller, 1981). It has thus been difficult to investigate PV persistence in the mouse spinal cord following the acute phase of the disease.

Previously, we isolated mouse-adapted PV-1 mutants after a single passage of the PV-1/Mahoney strain in the murine CNS, by intracerebral inoculation (Couderc *et al*, 1993) and remarkably, most animals inoculated with these mutants survived after the onset of paralysis (Destombes *et al*., 1997). We investigated whether or not PV is capable of persisting in the spinal cord following paralysis by studying the surviving paralyzed mice for 12 months after the onset of paralysis (Destombes *et al*, 1997). Histopathological examination of the spinal cord revealed, throughout the whole observation period, neuronal damage in a limited area of the ventral horn, which corresponds to the distribution of clinical paralysis. In this area, different cytopathological stages could be observed at each time point, ranging from mild to severe chromatolyzed motoneurons with the typical appearance of neuronophagia. In the same area, inflammatory cell infiltration was pronounced at 10 days and 3 months after the onset of symptoms, then decreased, but did not disappear entirely at later time points, including the 12-month point. Viral antigens as well as PV particles were detected by immunocytochemistry in the motoneuron cytoplasm of the mouse spinal cord, up to 12 months post-paralysis. Moreover, both plus and minus-strand products of viral RNA synthesis were detected in the spinal cord of paralyzed mice but not in infected mice which remain asymptomatic, suggesting that PV persis-

tence is associated with viral replication. However, infectious virus could not be recovered from mouse spinal cord homogenates isolated later than 10 days after the onset of the disease. Similar observations have been reported for other neurotropic viruses such as the measles virus (Schneider-Schaulies and ter Meulen, 1992) and mouse hepatitis virus (Kyuwa and Stohlman, 1990) for which viral antigens and genomes were detected, although infectious virus could rarely, if ever, be isolated from the CNS during persistent infection. The absence or scarcity of infectious particles could be due to restricted replication in the CNS, which would allow the virus to persist beyond the acute phase of the disease. In fact, a prerequisite for the establishment and maintenance of a persistent infection by a lytic virus is the limited expression of the viral genome, which thus allows the virus to escape the immune system (Ahmed *et al*, 1997; Oldstone, 1989).

The mechanisms by which non-retroviral RNA viruses of positive polarity persist *in vivo* with restricted replication include abnormal regulation of minus-strand RNA production and the generation of defective interfering particles. This mouse model now makes it possible to study the molecular mechanisms of PV persistence in the CNS and, in particular, in motoneurons. We are currently investigating whether one or several of the aforementioned mechanisms are involved in PV persistence in this mouse model.

Our results showing continuing motoneuron damage in the spinal cord of paralyzed mice could be analogous to the histopathological observations made in humans. Actually, inflammation, neuronal atrophy and chromatolysis were observed in spinal cord sections of patients 9 months to 44 years after acute poliomyelitis, even in the absence of new muscular symptoms. Taken together, these observations suggest that there is some degree of ongoing neuronal necrosis in the spinal cord after the onset of paralysis in both mice and humans. Despite the relatively short life expectancy of the mouse, this model of PV persistence in the spinal cord of paralyzed animals provides a new tool for studying the evolution of poliomyelitis after the onset of paralysis, and could shed light on the etiology of the PPS.

Conclusions

PV research continues to be a broad field of study. Several recent advances have focused on the interactions between PV and nerve cells. The development of murine models of paralytic poliomyelitis has permitted a better understanding of the host restriction of PV in the mouse. In particular, most of the determinants of mouse-adaptation which have been identified are localized in the capsid and likely play a role in virus-receptor interactions. In fact, it is surprising that the PV host range is so restricted, given that the presence of merely one point mutation in the PV capsid is sufficient to permit PV to extend its host range to the mouse. However, the molecule(s) which play the role of the PV receptor in the murine CNS remain to be identified. In addition, the demonstration that murine determinants, aside from the receptor, are capable of moderating viral replication at the level of transcription and translation, opens the way to a new field of investigation in the study of PV host range determinants.

These murine models, in association with the monkey model, have turned out to be precious tools in the identification of numerous determinants of attenuation. Among these determinants, those localized in the 5′NCR of the PV genome are well characterized, while on the contrary, the role of the other determinants, and notably most of those localized in the region encoding for the capsid proteins, remains to be elucidated. Nevertheless, it seems that these mutations confer a reduced capacity to multiply not only in neural cells but also in extra-neural target cells such as monocytes, to the vaccine strains, thereby affecting their pathogenesis. Unfortunately, an ideal animal model for studying the different steps of pathogenesis following natural infection via the oral route does not yet exist. Such a model would be a great help towards understanding why attenuated PV strains and also, to a certain extent, neurovirulent strains, so rarely reach the CNS.

The studies of PV persistence *in vitro* have overturned the generally accepted belief that PV is an exclusively lytic virus. Different models including persistent PV infection of human epithelial and neuroblastoma cells, and more recently, of primary cultures of human post-mitotic neurons have now been developed. Preliminary studies clearly indicate that the modification of different viral and cellular factors affecting the early steps of PV infection are implicated in persistent infection in cell culture. These studies open the way to promising genetic approaches for studying the viral and cellular aspects of viral infection in greater detail.

The isolation of mouse-adapted PV mutants able to induce a non-fatal paralytic poliomyelitis in mice comparable to that seen in humans, provides an *in vivo* model for studying the long term evolution of acute paralytic poliomyelitis. The demonstration that PV persists in the spinal cord of the mouse after the onset of paralysis raises, to an even greater extent, the question of PV persistence in the human CNS and its eventual link with the etiology of the PPS.

In conclusion, the recent advances in the field of neurovirulence and attenuation presented in this review could contribute to optimizing the chances

of success of the goal of global PV eradication. Moreover, although many questions remain to be answered, the tremendous progress made in the techniques of molecular biology in recent years has lead to an increased understanding of the interactions between PV and nerve cells at the molecular level, opening the way to the investigation of new aspects of PV pathogenesis.

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