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Correlation between Poliovirus Type 1 Mahoney Replication in Blood Cells and Neurovirulence

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Poliovirus (PV) is not often described as a monocyte- or macrophage-tropic virus; however, previous work indicated that neurovirulent PV type 1 Mahoney [PV(1)Mahoney] can productively infect primary human monocytes. To determine whether this replication has a functional role in pathogenesis, primary human mononuclear blood cells were infected with pairs of attenuated and neurovirulent strains of PV. Two neurovirulent strains of PV, PV(1)Mahoney and PV(2)MEF-1, replicated faster and to higher titers than attenuated counterparts PV(1)Sabin and PV(2)W-2, respectively, in primary human monocytes, suggesting that this replication may contribute to pathogenesis. PV(3)Leon grew weakly, while PV(3)Sabin, PV(2)Sabin, and PV(2) P712 did not replicate in these cells, perhaps because of their slow replication cycle. In U937 cells, a monocyte-like cell line, PV(1)Mahoney replicated but PV(1)Sabin did not, while both grew well in HeLa cells. When molecular recombinants of PV(1)Mahoney and PV(1)Sabin were assessed, a correlation between neurovirulence and the ability to replicate in primary human mononuclear blood cells was found. Surprisingly, infectious centers assays with primary human mononuclear blood cells and U937 cells indicated that despite the lower overall viral yield, more cells are initially infected with the attenuated viruses. These results indicate that there are virulence-specific differences in the ability of PV(1)Mahoney to replicate in monocytes and suggest that there may be factors in monocytes that virulent strains of PV require.

Despite the widespread, successful use of vaccines against poliovirus (PV) (30, 44), poliomyelitis remains a world health problem. Testing the safety of vaccine strains involves the costly use of primates. The development of safer and more stable vaccines as well as antiviral agents requires an understanding of the molecular mediators (both viral and host-encoded) of PV pathogenesis. Important unresolved questions regarding host-encoded determinants of PV pathogenesis include the type of cell supporting the first round of replication in a natural infection, the localization of the cell surface PV receptor (PVR) in primary human tissues, the mechanism of PV tissue tropism, and the nature of nonneural replication sites. Despite the identification, cloning, and sequencing of PVR (25, 31, 32), the impact of this information, in terms of understanding the molecular mechanisms of PV pathogenesis, has been disappointing (13, 16). This is, in part, due to the surprising complexity of the PVR gene, mRNAs, and proteins (25, 31, 32). Some reports describe ubiquitous expression of PVR (15, 25, 32), while another study suggests a much more limited expression (40): a comprehensive description of cell surface PVR protein in primary human cells has not been carried out (13). Nevertheless, it seems likely that PVR expression exceeds PV tissue tropism. In human tissues that express PVR but are resistant to PV, there may be postreceptor blocks to PV replication. CD44 is unlikely to function as a necessary coreceptor (45), because cell lines lacking CD44 can support PV replication (13a). Because tissue-specific blocks to PV replication in primate cells are lost after 24 h of culturing (22, 40), identification of the molecular mediators of PV tissue tropism cannot be addressed with existing tissue culture systems.

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Immunofluorescence staining and flow cytometric analysis revealed that PVR is expressed on primary human monocytes but is absent from neutrophils, lymphocytes, and platelets (14, 48). PVR expression in blood cells may explain the apparent ubiquity of PVR expression upon analysis by homogenization techniques as well as the apparent contradiction in the published literature between ubiquitous (15, 25, 32) and a more limited expression (40). Peripheral blood mononuclear cells (PBMCs), a heterogeneous population consisting of lymphocytes, monocytes, and natural killer cells, support PV replication (14). Fractionation experiments revealed that monocytes are the sole cell type supporting PV replication in PBMCs (11). PV replication is observed within 24 h of the removal of blood cells from the body, suggesting that the cells were susceptible in vivo. The finding that blood cells support PV replication provides a mechanistic explanation for early reports that some PV is cell associated in the blood (18, 29). Replication in phagocytic cells by several other pathogenic viruses may be a critical step in establishing a natural infection (10, 19, 21, 28). Understanding this replication may be important in the development of novel vaccines for other viruses. We hypothesize that PV replication in monocytes may be a requisite step in PV pathogenesis. To test this hypothesis, pairs of attenuated and neurovirulent strains of PV were tested for differences in their abilities to replicate in PBMCs. Here, we show that for some pairs of PV strains, the virulent type is more efficient than its attenuated counterpart in its ability to replicate in PBMCs and that there is a general correlation between viral segments mediating neurovirulence and those mediating replication in PBMCs. Traits that do not cosegregate may represent monocyte-specific determinants.

Comparison of pairs of virulent and attenuated PV strains for replication in PBMCs and U937 cells. Within PBMCs, PV replicates exclusively in monocytes; however, higher yields of virus are obtained when replication in PBMCs is compared with replication in purified monocytes (11). For this reason,

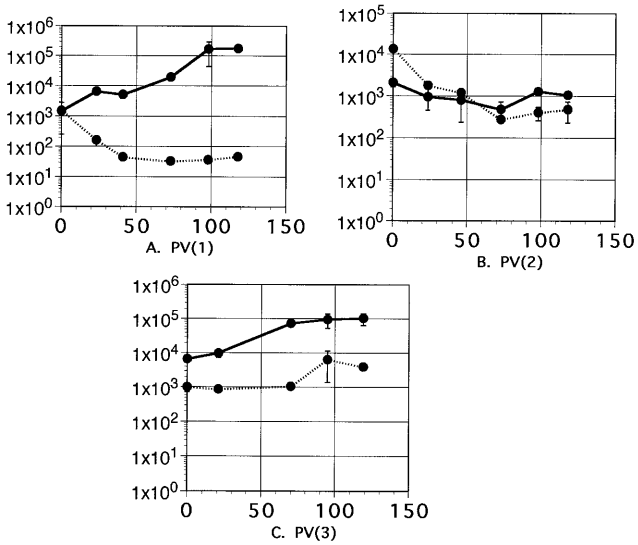

FIG. 1. Replication of matched sets of Sabin strains and their neurovirulent parent strains in PBMCs. PV(1)Mahoney, PV(1)Sabin, PV(2)P712, PV(2)Sabin, PV(3)Leon, and PV(3)Sabin (obtained from O. Kew, Centers for Disease Control) were purified by centrifugation at 30,000 rpm in a Beckman TV 50.3 rotor and used to infect aliquots of Ficoll-purified PBMCs as described previously (11, 14). (A multiplicity of infection of 10 was used in the experiments described here.) Blood (from the Blood Center of Southeast Louisiana) from anonymous donors was screened for human immunodeficiency virus, hepatitis B virus, and other infectious agents prior to its release. No identifiers were maintained. Samples were withdrawn at daily intervals, and viral titers were determined in an independent plaque assay in HeLa cells (12), except that plaque assays for PV(2) and PV(3) isolates were stained after 3 days rather than after 2 days, as was done for the PV(1) assays. Duplicate titrations were carried out for each infection sample. Viral serotypes were confirmed in a strain-specific antibody neutralization assay. In each panel, the solid line represents the neurovirulent strain while the dotted line represents the attenuated vaccine strain. Virus yield (in PFU per milliliter; *y* axis) is plotted as a function of time (in hours; *x* axis) after inoculation. (A) Mahoney and Sabin 1. (B) P712 and Sabin 2. (C) Leon and Sabin 3. The datum points shown are the averages of duplicate titers, with standard deviations being shown as error bars. The reason that some points appear not to have error bars is that the size of the error bar is smaller than the point on the graph.

PBMCs were used in the studies presented here. To test whether there are virulence-specific differences in the abilities of PV strains to grow in PBMCs, the abilities of pairs of neurovirulent and nonneurovirulent (Sabin) strains to replicate were compared. Purified virus was used to infect Ficoll-Hypaque-purified PBMCs; the resulting replication titers are shown in Fig. 1. Since culturing cells can alter the susceptibility to PV (22, 40), our earlier work demonstrating that primary monocytes are susceptible to PV emphasized that replication within 24 h is likely to represent preexisting susceptibility (11, 14). In this system, which contains no exogenous growth factors other than those present in bovine serum, the majority of the primary monocytes are destined to die and not become transformed. It appears that PV replication in these primary cells is significantly slower (1 to 3 days) than the rapid 8-h cycle found with tissue culture systems. It is very likely that a single round of replication is occurring (unpublished data). Although our early work focused on replication that occurred within 24 h, in this study, data for 5 days are presented to emphasize the differences in monocyte replication potentials for various PV strains. If increases in viral titers did not occur within 24 h of culturing, the data were not considered relevant to the current study. As was noted earlier (11, 14), there is some donor-to-donor variability in PV replication in monocytes. In the present study, comparisons of strains are presented only when the data were obtained from blood from the same donor. Because of donor-to-donor variation, it was not possible to average values for samples from different donors; therefore, each figure shows duplicate titer values for just one donor. Despite donor-to-donor variation in the final titers, with only one exception discussed below, strain comparisons between donors were qualitatively similar.

For PV type 1 [PV(1)], Mahoney grew well while Sabin 1 did not replicate in blood cells. The increase in viral titer at 24 h for PV(1)Mahoney is statistically significant ($0.05 > P > 0.02$ by Student's *t* test [47]). These two viruses were tested nine times with blood from five different donors, and qualitatively similar results were obtained (data from one experiment are shown). Different isolates of PV(1)Mahoney (from E. Wimmer, A. Nomoto, and O. Kew) behaved similarly. PV(2)P712 and PV(2)Sabin did not replicate in PBMCs, although titers comparable to those of the PV(1) strains were obtained for these two strains with HeLa cells. Slight differences in initial titers were not reproducible. PV(3)Leon showed a slight elevation in titer over 5 days while PV(3)Sabin did not; however, this result does not reflect replication. PV(3)Leon and PV(3)Fox (Wy 3) obtained from the American Type Culture Collection and PV(2)Lansing (from E. Wimmer) did not replicate in PBMCs (data not shown). These experiments demonstrate that for PV(1), neurovirulence is correlated with replication in PBMCs. The lack of PV(2)P712 and PV(3)Leon replication in PBMCs may be due to the fact that their replication cycles are longer (33) than the life span of these primary cells in culture in medium lacking specific growth factors. It is possible that PV(2) and PV(3) may show this correlation in a different monocyte replication system.

Since the Sabin 2 parent strain, PV(2)P712, is itself attenuated (39, 43, 49), we obtained a more virulent isolate of PV(2), MEF-1, as well as attenuated PV(2)W-2 (8). As was expected, PV(2)MEF-1 replicated faster and to higher titers than PV(2) W-2 in PBMCs (Fig. 2).

To test whether these differences in replication are maintained in cultured monocytes, replication in U937 cells, a monocyte-like transformed cell line, was tested. PV(1)M grew rapidly and to high titers, while PV(1)S replicated to a very low level (Fig. 3). Both these strains replicated to high titers within 24 h in HeLa cells. Nonneurovirulent recombinant virus PV1(SM)IC8a (see below) grew to a low titer of 5.5×10^5 PFU/ml in U937 cells (data not shown). These results support the interpretation that strain-specific replication by PV in monocytes correlates with neurovirulence.

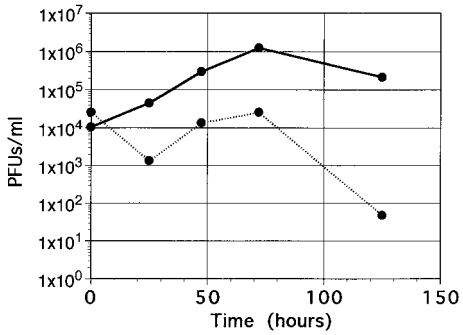

FIG. 2. Replication of different PV(2) isolates in PBMCs. PV(2)MEF-1 (solid line) and PV(2)W-2 (dotted line) were obtained from B. Jubelt (State University of New York at Syracuse) and used to infect PBMCs. Samples were withdrawn at daily intervals, and viral titers were determined in an independent plaque assay in HeLa cells.

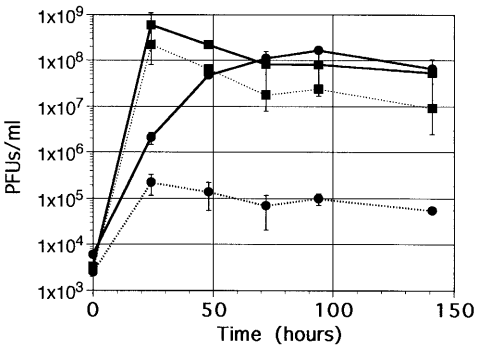

FIG. 3. Replication of virulent and attenuated PV(1) in U937 and HeLa cells. Purified PV(1)Mahoney (solid lines) and PV(1)Sabin (dotted lines) were used to infect U937 cells (circles) and HeLa cells (squares). Samples were withdrawn at daily intervals, and viral titers were determined in an independent plaque assay in HeLa cells. The datum points shown are averages of titers from duplicate samples.

Replication of Sabin 1-Mahoney recombinants in PBMCs. Sequence analysis of virulent and vaccine strains of PV has revealed 10- to 55-nucleotide differences between virulent and attenuated PV strains (2, 3, 35, 37, 38). The use of recombinant viruses (allele replacement experiments) and the phenotypic evaluation of neurovirulence in monkeys (1, 24, 36) or attenuation in mice (7) has delineated which genetic differences are functionally relevant. Initial experiments indicated that strong neurovirulence determinants reside in the 5′ half of the genome (1). Molecular genetic studies revealed that for PV(1), neurovirulence determinants reside 5′ of nucleotide 1122 as well as between nucleotides 1122 and 3664 (near the 3′ end of P1) (9, 24, 36). Other work identified nucleotide 480 in the 5′ noncoding region (NCR) and nucleotides 935, 2438, and 2795 in VP4, VP3, and VP1, respectively, as mediators of attenuation (7, 9, 23). Residues in the P2 region (nucleotides 3664 to 5601) and P3 region (nucleotide 5601 to the 3′ end) may each contain weak neurovirulence determinants (9, 36). Since attenuation is mediated by tissue-specific differences in viral replication, attenuating mutations may represent specific interactions between viral factors and host factors that differ depending on the cell type.

To determine which regions of the PV genome mediate the differences in monocyte replication, we obtained 10 Sabin 1-Mahoney recombinants as well as the parental viruses from A. Nomoto (Fig. 4). The restriction sites used to generate the recombinant viruses, *Aat*II (position 1122), *Kpn*I (position 3664), and *Bgl*II (position 5601), roughly divide the genome into four functional segments: 5′ NCR, P1, P2, and P3-3′ NCR. Each of these recombinants [except PV1(SM)13a and PV1 (SM)13b] has been tested for intracerebral neurovirulence in monkeys (24, 36). Each virus was used to infect PBMCs. Resulting titers are shown in Table 1. It is very likely that infection of purified monocytes would yield qualitatively similar results. The experiment with 10 recombinants was carried out three times with blood from three different donors. Data shown are from one experiment. A replication ratio (Table 1) was calculated for each virus. The lesion score for each virus (24, 36) is also shown. There are striking differences in the abilities of these viruses to replicate in PBMCs. At day 5, the highest titer (7.6×10^5 PFU/ml) and replication ratio (2.74) were obtained with PV1(M)IC (Mahoney), while the lowest titer (1.2×10^2 PFU/ml) and replication ratio (−0.74) were obtained with PV1(SM)IC8a. This virus contains Mahoney sequence 3665 to 5601 (approximately equivalent to P2) in a Sabin 1 background. The PV1(Sab)IC (Sabin 1) titer at day 5 was 4.0×10^2 PFU/ml, and its replication ratio was −0.45. In two experiments, results qualitatively similar to those shown in Table 1 were obtained. However, in one experiment, each of the viruses, except for PV1(SM)IC4b and PV1(Sab)IC, replicated at rates 1 to 2 logs higher than those in other experiments and yet otherwise retained their relative rates of replication. PV1(SM)IC4b (Mahoney sequence 0 to 1122 [5′ NCR] with the remainder of the Sabin sequence) replicated to a final titer that was a log lower than normal. In this single experiment, the titer at day 3 for PV1(Sab)IC was 1.9×10^4 PFU/ml (compared with 7.2×10^2 PFU/ml) (Table 1), but at day 5, PV1(Sab)IC reached a level of 2×10^6 PFU/ml (usually 10^2 PFU/ml [Table 1]) while PV1(M)IC was 6×10^6 PFU/ml. It is very likely that in this experiment, the donor's monocytes were particularly susceptible to PV1(Sab)IC replication. Since in nine other experiments with blood from five independent donors PV(1)Sabin failed to replicate in PBMCs, we interpret the result with this particular donor as being unusual. This discrepancy may reflect donor variability in the ability of blood cells to support the replication of PV. This variability may, in turn, reflect individual differences, possibly genetic, in susceptibility to PV.

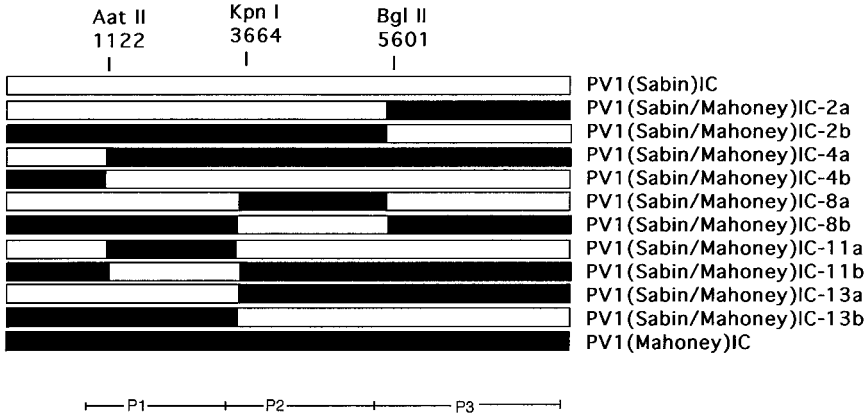

FIG. 4. Diagram of PV(1) mutants used in this study. PV(1)Mahoney, PV(1)Sabin, and Mahoney-Sabin recombinants derived from infectious clones (obtained from A. Nomoto, University of Tokyo) are diagrammed here (not to scale). Construction and characterization of the recombinant viruses, except for PV1(SM)13a and PV1 (SM)13b, have been described previously (24, 36). Solid regions represent genome segments from Mahoney, while open regions represent Sabin 1 segments. The juncture sites at *Aat*II (position 1122), *Kpn*I (position 3664), and *Bgl*II (position 5601) roughly divide the genome into functional segments 5′ NCR, P1, P2, and P3-3′ NCR.

TABLE 1. Replication of Sabin-Mahoney recombinant viruses in PBMCs

Virus	Titer[a]						Replication ratio[b]	Lesion score[c]
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5		
PV1(Mahoney)IC	1,000	1,700	14,000	150,000	725,000	760,000	2.74	2.48
PV1(SM)IC2a	950	500	330	235	320	230	−0.42	0.05
PV1(SM)IC2b	1,000	800	2,300	13,000	19,000	19,000	1.3	2.03
PV1(SM)IC4a	1,700	780	1,200	3,150	21,000	34,000	1.35	0.72
PV1(SM)IC4b	1,300	830	600	900	1,400	3,400	0.35	0.80
PV1(SM)IC8a	1,300	375	430	190	1,900	120	−0.74	0.12
PV1(SM)IC8b	2,300	1,600	9,000	37,000	37,000	60,000	1.4	1.56
PV1(SM)IC11a	2,500	775	1,400	2,300	2,500	2,500	0.18	0.08
PV1(SM)IC11b	1,500	2,000	3,000	6,750	11,000	11,000	0.81	1.73
PV1(SM)IC13a	4,350	3,000	2,650	7,000	11,000	9,250	0.43	NA[d]
PV1(SM)IC13b	1,800	850	3,100	13,000	8,750	6,250	0.75	NA
PV1(Sabin)IC	2,000	1,300	1,350	720	750	400	−0.45	0.07

[a] Titers are expressed in PFU per milliliter. Each value is an average of two or three independent titrations.
[b] Relative replication in PBMCs for each virus is expressed as the $\log_{10}$ of the ratio of the combined PFU per milliliter at days 4 and 5 to the combined PFU per milliliter at days 0 and 1.
[c] From references 24 and 36. Viruses PV1(SM)IC13a and PV1(SM)IC13b were not tested for neurovirulence.
[d] NA, not available.

In Fig. 5, the genomes of the recombinant viruses, ordered according to results from the functional assays, are shown. In the top portion of Fig. 5, the viruses are ordered from top to bottom by lesion score (a low lesion score indicates low neurovirulence). The replication ratio in PBMCs at each day for each virus is then displayed beside the lesion score. In the bottom portion of Fig. 5, the same values are displayed but are ordered from top to bottom in order of increasing replication in PBMCs, and the corresponding lesion score is displayed. To a first approximation, replication by the Sabin 1-Mahoney recombinants in PBMCs correlated with previously determined neurovirulence. Substitution of any Sabin 1 segment for a Mahoney segment resulted in reduced replication in PBMCs relative to that of PV1(M)IC. For example, the virus replicating to the second highest titer after PV1(M)IC, PV1(SM)IC-8b (Mahoney with Sabin sequence 3664 to 5601 [approximately P2]), replicated to a 26-fold higher titer than its original titer but was nonetheless reduced 29-fold relative to PV1(M)IC. Loss of any two Mahoney fragments did not fully abrogate replication in PBMCs but did strongly reduce it. Therefore, for PV(1), there is a good correlation between lesion scores and ratios for replication in PBMCs, suggesting that the

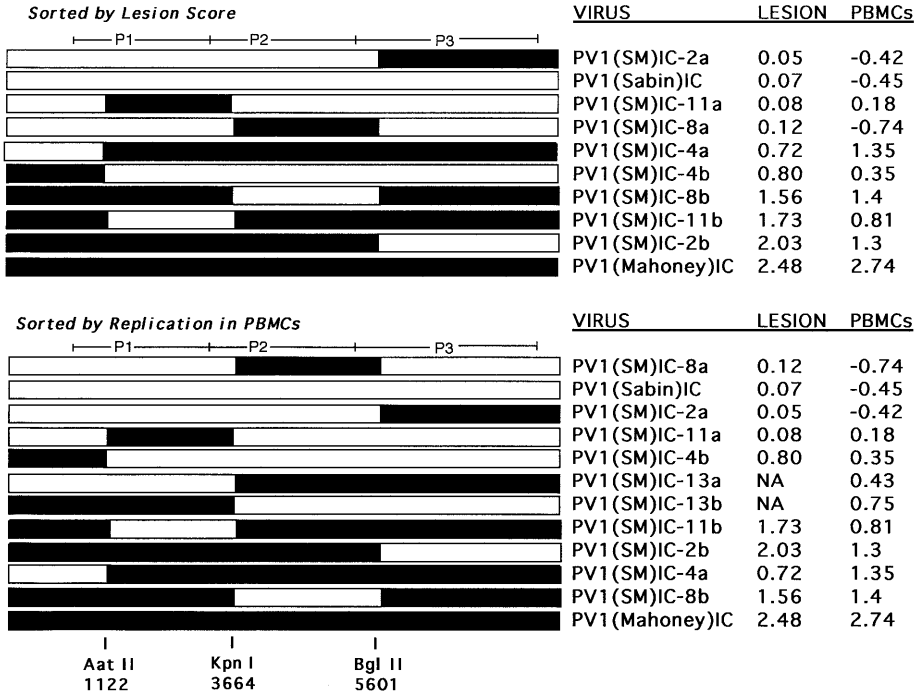

FIG. 5. PV(1) mutants ordered by neurovirulence and ability to replicate in PBMCs. In the top portion of the figure, the Sabin 1-Mahoney recombinants (not to scale) are arranged from top to bottom in order of increasing neurovirulence on the basis of published lesion scores (24, 36). Viruses PV1(SM)13a and PV1(SM)13b have not been assessed for neurovirulence. To the right, the virus name, corresponding lesion score, and replication ratio in PBMCs (Table 1) are listed. In the bottom portion of the figure, the viruses are arranged from top to bottom in order of increasing ability to replicate in PBMCs, and the corresponding lesion scores are displayed.

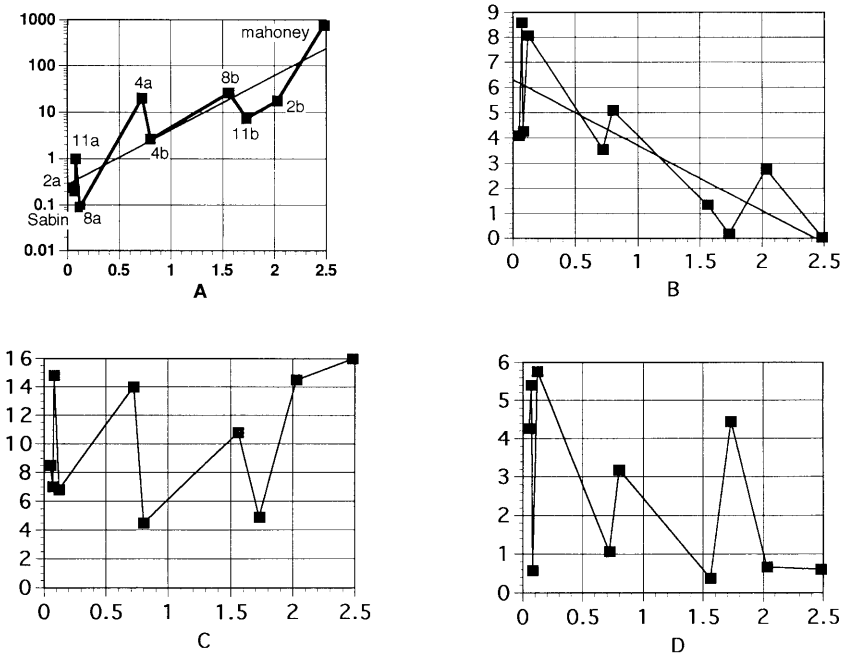

FIG. 6. Comparison of different in vitro correlates of PV neurovirulence. PV(1)Sabin 1-Mahoney recombinant viruses were arranged in order of increasing neurovirulence [PV1(SM)IC-2a, PV1(Sab)IC, PV1(SM)IC-11a, PV1(SM)IC-8a, PV1(SM)IC-4a, PV1(SM)IC-4b, PV1(SM)IC-8b, PV1(SM)IC-11b, PV1(SM)IC-2b, and PV1(M)IC] as determined by lesion scores after intracerebral injection in monkeys (24, 36). For each virus, its PBMC replication ratio (A), its *rct* marker value (B), its plaque size (C), and its *d* marker value (dependence on bicarbonate) (D) (*y* axes) were plotted as functions of its lesion score (*x* axis). Curve fitting for panels A and B was carried out with Delta Graph Pro 3, version 3.1.

more neurovirulent the virus, the better the replication in PBMCs.

The ability to replicate in the alimentary tract and, subsequently, in the central nervous system has been termed "oral neurovirulence" (39), which contrasts with neurovirulence determined by intracerebral injection. Sabin recognized that each PV strain has numerous genetically separable traits and that each trait can display a wide spectrum of variation (42). In the present study, some differences between loci mediating neurovirulence and loci mediating replication in PBMCs were noted. For example, in neurovirulence testing, reciprocal recombinants around residue 1122 (in the 5′ part of P1) yielded attenuated viruses that both had similarly lowered lesion scores relative to that for Mahoney, suggesting that neurovirulence determinants reside in both fragments (36). However, PV1 (SM)IC-4a, which lacks the Mahoney 5′ noncoding neurovirulence determinant at nucleotide 480, replicated to a moderate titer in PBMCs (a replication ratio of 1.35). The reciprocal recombinant, PV1(SM)IC-4b (with the Mahoney 5′ noncoding fragment in Sabin 1), replicated poorly in PBMCs (a replication ratio of 0.43). This result suggests that in the Mahoney background, Sabin residues 0 to 1122 are less disadvantageous in PBMCs than other Sabin fragments. Segments 5′ NCR and P2, when either was the only recombinant fragment, behaved differently in PBMCs than in neurons. Within a gene segment, loci mediating replication in PBMCs may be different from those mediating neurovirulence in the same segment. It is possible that the differences between neurovirulence and the ability to replicate in PBMCs that we observed may represent oral neurovirulence determinants. This possibility could be tested by assessing strains that have maintained intracerebral neurovirulence but lost oral neurovirulence (39, 42). These strains would be expected to have reduced capability to replicate in PBMCs relative to that of their orally neurovirulent parental strains. PV replication in primary neuronal cells probably requires specific factors. Nonneural sites of PV replication may require additional specific factors. Despite recent important advances in in vitro viral replication systems (34) and cell-free translation (17) and the implication of host-contributed factors (27), few molecular factors have been demonstrated to mediate tissue-specific PV replication differences. Factors from neuroblastoma cells may interact with the internal ribosome entry site (5, 46). Since p57/PTB is critical in cap-independent translation of PV (20) and p57/PTB may bind close to the attenuating mutations in domain V of the internal ribosome entry site (49), differential levels of active p57/PTB may regulate tissue-specific PV replication. It will be interesting to determine p57/PTB levels in monocytes.

Recombinant virus PV1(SM)13a (the 5′ end to position 3664 [near the end of P1] is from Sabin and the remaining 3′ sequence is from Mahoney) and its reciprocal construct, PV1(SM)13b, have not been tested for neurovirulence. However, their structures may be similar to those of recombinant viruses a3/v1-15 and v3/a1-25, respectively, which were generated by in vivo recombination (1). Virus a3/v1-15 contains the 5′ half of an attenuated virus and is attenuated; virus v3/a1-25 contains the 5′ half of a neurovirulent virus and is neurovirulent. In our studies, both viruses showed an intermediate phenotype [replication ratios of 0.35 and 0.75 for PV1(SM)13a and PV1(SM)13b, respectively]. PV1(SM)13b replicated better than PV1(SM)13a, showing that sequences in the 5′ half of PV(1)M are more critical for replication in PBMCs than those in the 3′ half.

In Fig. 6A, the day 5/day 0 ratio for replication in PBMCs is plotted as a function of the lesion score. The curve is close to being a line, which is suggestive of a linear relationship. In Fig. 6B, C, and D, the other published in vitro correlates for neurovirulence for each of the recombinant viruses (*rct* markers,

TABLE 2. Infectious centers assays of Mahoney and Sabin 1 in different cell types[a]

Virus strain	% Infected ± SD		
	HeLa cells	U937 cells	PBMCs
Mahoney	10.4% ± 0.96%	5.08% ± 0.84%	0.25% ± 0.05%
Sabin 1	1.47% ± 0.5%	22.7% ± 2.05%	0.50% ± 0.08%

[a] In this assay (21), the cells to be characterized (blood, U937, and HeLa cells) were infected with the indicated strains of PV. After adsorption for 30 min at room temperature and three rinses in medium without serum at room temperature, an aliquot of cells was serially diluted onto an independent monolayer of HeLa cells. For HeLa control infections, the cells were lifted by trypsinization. Twofold dilutions were plated so that 5 to 50 plaques per well of a six-well dish would be observed. The cells were overlayed with agar, and 2 days later, the plaque number was determined. Since the only source of input virus was from viral replication in the uncharacterized cell type, plaques were formed only where the surrounding HeLa cells were subsequently infected by progeny virus. To distinguish internalized virus from loosely associated or residual unbound virus, an aliquot of supernatant was obtained by centrifuging a separate equivalent volume of cells three times at 2,500 × *g* and visually checking for the absence of cells. This supernatant fraction was then similarly analyzed for infectious centers. Plaque formation from the supernatant was subtracted from the number of plaques produced from cells. A comparison of the maximum number of possible plaques (if every cell produced one infectious center) to actual plaques yielded the percentage of cells infected. Data presented are averages for three countable wells.

plaque size, and *d* markers [bicarbonate concentration dependence of growth] [24, 36]) are similarly plotted against lesion scores. Of these three parameters, *rct* yields the values closest to a straight line, and yet there is still much deviation (24, 36). In fact, molecular determinants for some of these traits have been mapped to regions genetically separable from those of neurovirulence determinants (7, 24, 36). When the PBMC replication ratio was plotted as a function of lesion score (Fig. 6A), a curve close to being a straight line was obtained, suggesting that replication in PBMCs may be a genuine correlate of neurovirulence. It may be possible to replace or complement testing of vaccines in animals with testing in PBMCs.

The temperature sensitivity of the Sabin strains is mediated by mutations in the capsid protein and in $3D^{pol}$ (7, 26). It is possible that some of the differences between PV(1)Mahoney and PV(1)Sabin replication in PBMCs are due to temperature sensitivity mutations in PV(1)Sabin. However, this possibility is unlikely for two reasons. First, in our results, there was no clear clustering of viruses containing these mutations. Second, the differences in HeLa replication were significantly less than those with U937 cells (Fig. 3), even though both infections were carried out at 37°C.

Infectious centers assays. To determine whether the differences in titers that we observed are due to differences in virus yield per cell or to different numbers of cells becoming infected, we carried out infectious centers assays in various cell types (Table 2). In HeLa cells (10^6 cells), PV(1)M replicates to a slightly higher titer than PV(1)S [5.9×10^8 PFU/ml compared with 2.2×10^8 PFU/ml for PV(1)S, or about threefold higher], and more cells are infected by PV(1)M than by PV(1)S (10.4% compared with 1.47%, respectively, or about sevenfold higher). The fact that less than 100% of the HeLa cells were infected may be because trypsin was used to lift the cells for the infectious centers assay. However, when cells of the myeloid lineage were studied, the opposite result was obtained. Despite a significantly higher final virus yield with PV(1)M, Sabin 1 infected more cells. With U937 cells, 5.08% of the cells were infected with PV(1)M and 22.7% of the cells were infected with Sabin 1 (for a 4.5-fold higher infection rate). With PBMCs, 0.25% of the cells were infected with PV(1)M and 0.50% of the cells were infected with PV(1)S (for a twofold higher infection rate). These results suggest that the virus yield of Sabin 1 per cell is extremely low. Earlier work with PV(1)M showed that despite the presence of PVR in >90% of the monocytes, a very low percentage (~6% of monocytes or 0.3% of PBMCs) is infected by PV (11). This result suggested that there are postreceptor blocks to the replication of neurovirulent PV and that monocytes may present a good cell culture system to identify the blocks. These postreceptor blocks to PV replication may reflect similar blocks in other PVR-positive human cells. There may be two blocks to PV replication in monocytes: PV(1)Mahoney may have one and Sabin 1 may have another. These blocks may represent host-encoded factors mediating tissue-specific PV replication. Future work may involve determining at what point these blocks fall in the viral life cycle. Knowledge of such blocks may lead to the development of antiviral agents.

Our finding of a correlation between neurovirulence and the ability of PV(1) to replicate in primary human monocytes strongly supports our hypothesis that replication in monocytes may have a functional role in PV pathogenesis. This finding may have implications for several important issues regarding PV pathogenesis. Monocytes or macrophages in Peyer's patches may be the first cell type to support replication in the gut. It has been noted that the extent of viremia correlates with virulence, with PV(1)Mahoney having highest levels of both (6). Our finding that PV(1)Mahoney replicates to very high titers in monocytes may provide a mechanistic explanation for this phenomenon. Low levels of viremia have been reported for the Sabin strains and for Sabin 2 in particular (18, 29). If PV replication occurs in monocytes in a natural infection, then our data show that neurovirulent strains would be more likely than attenuated strains to be associated with or carried by monocytes into the central nervous system of a nonimmune individual. There are a few studies in which the differences in the abilities of virulent and attenuated strains (of other viruses) to grow in monocytes have been addressed. In these systems, attenuated strains of poxvirus and arenavirus grew less well in monocytes than virulent strains (4, 41). Continued studies of PV replication in monocytes should yield further insights into the role of replication in myeloid cells in neurotropic viral pathogenesis.

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