Arch Virol (1995) 140: 2135–2150

Archives of Virology

Low levels of poliovirus replication in primary human monocytes: possible interactions with lymphocytes

K. E. Eberle, V. T. Nguyen, and **M. S. Freistadt**

Department of Microbiology, Immunology and Parasitology,
Louisiana State University Medical Center, New Orleans,
Louisiana, U.S.A.

Accepted August 12, 1995

Summary. To investigate the molecular mediators of poliovirus tissue tropism, the correlation between poliovirus replication and poliovirus receptor expression was examined in a primary human tissue system. Earlier work [M. Freistadt, H. Fleit, and E. Wimmer, Virology 195: 798–803 (1993)] showed that the cellular receptor for poliovirus is present in 87% of primary human monocytes and that peripheral blood mononuclear cells support poliovirus replication. In the current work, monocytes, obtained by adherence or by a novel negative selection procedure using specific monoclonal antibodies to lymphocyte surface antigens, supported poliovirus replication. However, total virus yield was low and infectious centers assays revealed that a minority (6%) of monocytes become productively infected. Viral yield from monocytes was lower than from the heterogeneous mononuclear cells; however, when uninfected lymphocytes were added back to infected monocytes, the higher viral yield was restored. The purity of the cells did not significantly affect the number of cells infected. These results suggest that more poliovirus is produced per cell from activated rather than unactivated monocytes. Furthermore, poliovirus replication in monocytes may reflect genuine in vivo replication and comprise a system in which to determine molecular mediators of poliovirus tissue tropism.

Introduction

Despite the control of poliovirus and poliomyelitis by vaccines, a complete understanding of pathogenesis during this debilitating human infection remains elusive. Important unsolved questions that may pertain to currently uncontrolled viral infections include: the identity of the cell type supporting the first round of replication in a natural infection, the mechanism of poliovirus tissue tropism and the nature of nonneural replication sites. The impact of the identification, cloning and sequencing of the poliovirus receptor (PVR) in 1989

[18, 26, 27] in terms of answering these questions has, so far, been disappointing [7, 10].

Precise knowledge of distribution of PVR in human tissues will be required in order to understand the role of PVR in mediating poliomyelitis. However, due to the surprising complexity of the PVR gene, mRNAs and proteins produced [18, 26, 27], this information is currently incomplete. Published reports suggest that PVR expression in human tissues is either ubiquitous [9, 18, 27] or greatly exceeds poliovirus replication sites [36]. However, the localization of cell-bound PVR in human tissues has not been precisely determined. Previous studies have relied upon homogenization methods that do not distinguish cell type within tissues and probably contain heterogeneous cell types [9, 18, 27], used probes that do not distinguish between the isoforms of PVR [36], some of which are secreted [18] or utilized transgenic mice tissues, rather than human tissues [20, 36]. The possibility that PVR expression exceeds the tissue-specific replication of poliovirus, led to the invocation of a model for another cellular factor limiting poliovirus replication in vivo. Identification of a variant of CD44 ($_{AF3}$CD44H) [40] as the cognate antigen of AF3 [41], a mAb that inhibits binding of poliovirus, types 1 and 2, to the cell surface, suggested that CD44 and PVR may be in a complex and that this complex would delineate poliovirus tissue-specific replication. However, preliminary studies suggest that while $_{AF3}$CD44H may have a role in poliovirus uptake, it is likely not to be the molecular determinant of poliovirus tissue tropism (Freistadt and Eberle, submitted).

The role of host factors in mediating poliovirus tissue tropism cannot be assessed in cultured cells because tissue-specific blocks to poliovirus replication are not maintained upon culturing. Most cultured primate cells support poliovirus replication, regardless of their tissue origin [15]. When primary primate cells or cells from transgenic mice expressing PVR are cultured, they are initially resistant to poliovirus replication, despite having virus-binding activity, but become susceptible within 24 h of culturing [36].

To address these issues, we have chosen to study poliovirus tissue tropism in primary human cells. PVR is expressed on the cell surface of 87% of CD14-positive mononuclear cells from human peripheral blood [8]. CD14 is the LPS receptor and, in this cell population, is a specific marker for monocytes [49]. The presence of PVR in human blood cells may explain the apparent ubiquity of PVR expression [9, 18, 27]. Earlier work demonstrated that peripheral blood mononuclear cells (PBMCs), a heterogeneous population consisting of lymphocytes, monocytes and natural killer (NK) cells, support poliovirus replication [8], although the specific cell type supporting poliovirus replication was not determined. Although PVR-positive cells have not been detected within primary lymphocytes in our work, it is possible that a small population of such cells escaped detection and contributes to poliovirus replication within PBMCs. Furthermore, it is theoretically possible that an alternate receptor was active for poliovirus replication in the earlier experiments [8]. However, D171, a blocking anti-PVR antibody [30], fully inhibited poliovirus replication in PBMCs (Freistadt and Eberle, submitted).

The present study was carried out to characterize poliovirus replication in primary human blood cells. This system may be useful to address the unsolved questions regarding the molecular mediators of poliovirus tissue tropism. We have deliberately chosen not to use specific growth factors to maintain the cultures, because this may induce PVR expression [10]. The disadvantage of this system is that because the cells are not transformed, many monocytes do not survive regardless of viral infection. Nevertheless, for a number of reasons, we believe this system is representative of poliovirus tissue tropism and that increases in viral titer represent genuine viral replication, not sloughing of bound virus. First, primary cells, resistant to poliovirus, require at least 24 h of culturing to become susceptible [36]. In contrast, poliovirus replication in PVR-positive, primary human blood cells occurs within 24 h of removal from the body, strongly suggesting that the cells were susceptible prior to the experimental infection. Second, if poliovirus was merely binding to PVR on monocytes and eluting off, it would not be detected by plaque assay because the specific interaction of PVR and poliovirus results in an irreversible conformational change that inactivates poliovirus [11, 13, 16]. Third, we have determined that there are significant strain-specific differences in the ability of poliovirus to replicate in monocytes in our system (unpubl. obs.).

The presence of PVR in monocytes and the ability of primary human blood cells to support poliovirus replication suggested that poliovirus replication in blood cells may be important in mediating pathogenesis by poliovirus. In the current work, we show that monocytes support poliovirus replication and we characterize the replication cycle. The results suggest that interactions between monocytes and other blood cell types affect poliovirus replication.

Materials and methods

Cells, viruses, plaque assays, abbreviations used

Type 1 (Mahoney) poliovirus, obtained from E. Wimmer, was used in the experimental infections presented here. Virus was purified by isopycnic centrifugation in CsCl and the serotype confirmed in a neutralization assay. Virions were titered in a standard plaque assay on Hela R19 cells [6]. Through a cooperative agreement with The Blood Center of Southeast Louisiana, healthy volunteers sign a consent form for the use of their blood for biomedical research under an LSUMC IRB approved protocol. Blood from anonymous donors was screened for HIV, HBV and other infectious agents prior to its release. No identifiers were maintained. Abbreviations used are: *PBMCs* peripheral blood mononuclear cells, *PBS* phosphate-buffered saline, *PVR* poliovirus receptor, *moi* multiplicity of infection, *FACS* fluorescence activated cell sorter, *PHA* phytohemaglutinin, *NK* natural killer cells, *MALT* mucosal-associated lymphoid tissue, *γ-IFN* gamma interferon, *mAb* monoclonal antibody.

Isolation of PBMCs

Blood cells and Ficoll-Hypaque (Pharmacia) were kept at room temperature for at least 2 h. Blood was carefully layered over one-half volume of Ficoll-Hypaque. The material was centrifuged at 300 **g** for 30 min and allowed to stop without the brake. Mononuclear cells

form a flocculent band in the upper half of the tube. The band was retrieved and the cells were rinsed two times in room temperature PBS to remove any residual anti-poliovirus antibodies form the donor. In order to obtain sufficient monocytes we found that it was important to: 1) have the Ficoll, PBS and cells at room temperature, 2) use a centrifuge that has been leveled for the Ficoll density centrifugation step. These factors appear to affect the formation of the Ficoll gradient.

Poliovirus infection of monocytes

10^7 PBMCs (or cell fractions obtained from 10^7 PBMCs) were resuspended in a 15 ml sterile, plastic tube with 0.5 ml of RPMI + 0.2% fetal calf serum containing poliovirus. Moi of 10 was used throughout. The tube was gently rocked for 30 min at room temperature. The cells were then washed 3 times in 37 °C RPMI to remove unbound virus and then resuspended in 5 ml of RPMI + 10% calf serum and Penicillin/Streptomycin. The cells were then plated in 6 cm dishes and a "Time zero" sample (0.4 ml) was taken. Subsequent samples were taken at approximately 24 h intervals.

Adherence fractionation

For each sample to be infected, 10^7 Ficoll-purified PBMCs were plated in 5 ml of RPMI + 10% calf serum and Penicillin/Streptomycin. The plates were incubated for 2 h at 37 °C in a CO_2 incubator. Similar results were obtained if cells were incubated overnight for adherence. Nonadherent cells were removed and the adherent cells were rinsed three times with RPMI or PBS.

Negative selection of cells using specific antibodies and paramagnetic beads

For each sample to be infected, 10^7 Ficoll-purified PBMCs were suspended in biotinylated-specific antibody (Harlan) in RPMI + 1% calf serum and Penicillin/Streptomycin at 10^6 cells/ml. Optimal concentration of antibody was determined empirically but was generally about 10 μg/10^6 cells. When using more than one antibody, the incubations were sequential, not concurrent. Cells and antibody were incubated for 20 min at 4 °C with gentle rocking. The cells were rinsed two times with RPMI + 1% calf serum and Penicillin/Streptomycin. The cells were then suspended in 1% streptavidin-paramagnetic beads (Promega) at 10^6 cells/ml and incubated for 10 min at 4 °C with gentle rocking. The tubes were then placed on a magnetic rack for 10 min. After separation, the supernatant (cells without beads) was carefully pipetted off and subjected to the magnetic separation again. This supernatant was used for infections.

Immunofluorescence staining and flow cytometric analysis

Washed blood cells were divided into two samples (10^6 cells each) and each sample was stained in three sequential staining and rinsing steps [8, 39]. One sample was used for a negative control (using isotype matched mAbs) and the other was the experimental. The three steps were: (i) primary mAb e.g., D171 [30] or control, (ii) Goat anti-mouse Ig-FITC to both samples and (iii) PE-conjugated mAb against the second marker or a negative control. Staining was carried out on ice for 30 min; antibody dilutions and rinses were carried out in PBS with 1 mg/ml BSA and 0.2% sodium azide (PBS/BSA/azide). After the third staining, the cells were rinsed in PBS/BSA/azide, fixed in 1% PBS-buffered formalin for 10 min at room temperature and stored at 4 °C until flow cytometric analysis was carried out.

Nonspecific esterase stain for monocytes

Air-dried smears of cells were fixed for 30 seconds at 4 °C in buffered formal-acetone, rinsed 5 times and air dried. The fixed, dried smears were incubated for 45 min at room temperature in filtered alpha-naphthyl butyrate/pararosanaline solution. This was made by diluting 2.0% alpha-naphthyl butyrate [Sigma] in ethylene glycol monoethyl ether into 0.04% pararosanaline (from 4% pararosanaline in 20% HCl), 0.04% sodium nitrite in phosphate buffer, pH 6.3. After 5 rinses with deionized water, the cells were counterstained with methyl green for 2 min and rinsed again. After drying, percentages of monocytes and lymphocytes were determined by counting multiple fields through a light microscope. In this assay, monocytes stain red while lymphocytes stain blue [22, 50].

Infectious centers assay

In this assay [14], the uncharacterized cells (blood cells) were infected with poliovirus. After adsorption and rinsing, the cells (or an aliquot of the supernatant) were serially diluted onto a monolayer of a Hela cells. The cells were overlayed with agar (similar to a plaque assay) and two or three days later, plaque number was determined. Since the only source of input virus is from viral replication in the uncharacterized cell type, plaques will be formed only where the surrounding Hela cells were subsequently infected by progeny virus. (Plaques formation from the supernatant was also assessed and subtracted from the number produced from cells.) The dilutions include a concentration such that if low number of cells were infected, each infected monocyte sample would yield countable plaques (about 50 per 6 cm dish). A comparison of the maximum number of possible plaques (if every cell produced one infectious center) to actual plaques yields the percentage of cells infected. Controls using infected Hela cells diluted into Hela cells were carried out.

Results

Increases in poliovirus titer from primary human monocytes

Earlier work showed that PBMCs, a heterogeneous population, support poliovirus replication [8]. To determine whether CD14-positive, PVR-positive, primary human blood cells support poliovirus replication, Ficoll-purified PBMCs were fractionated to separate monocytes from nonmonocytes and subsequently infected with poliovirus. Two methods of fractionation were separately carried out: (a) adherence to plastic culture dishes or (b) a novel negative selection procedure using specific monoclonal antibodies to lymphocyte surface antigens and secondary antibodies conjugated to paramagnetic beads, followed by magnetic separation.

Poliovirus replication in adherent PBMCs

Adherence to plastic culture dishes was used to separate monocytes from other cell types in PBMCs because monocytes adhere to plastic while the lymphocytes and NK cells do not. Each fraction of cells (PBMCs, adherent or nonadherent fraction) was separately infected with poliovirus at an moi of 10. A high moi was used to ensure that the maximum number of cells were infected. However, rigorous rinsing was then required to remove unbound virus, which was the majority of the input virus. The titers at time zero (residual virus, either

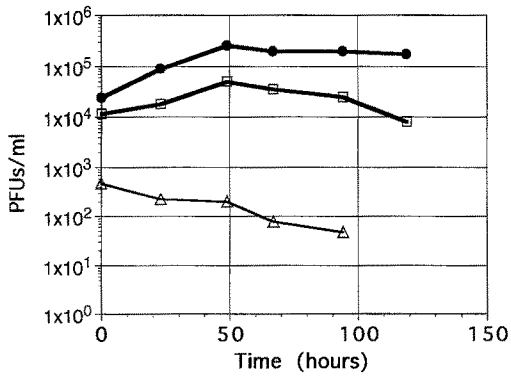

Fig. 1. Replication of poliovirus in Ficoll-purified PBMCs and in adherence-fractionated cells. Primary human peripheral blood mononuclear cells, adherent cells or nonadherent cells were infected with poliovirus, rinsed thoroughly to remove unbound virus and samples titered in a standard plaque assay on Hela cells at the indicated timepoints. Viral yield, expressed as pfus/ml, is depicted as a function of time in hours. Data are from 3 separate experiments: ● viral yield from 10^7 Ficoll-purified PBMCs; □ viral yield from adherent cells from 10^7 Ficoll-purified PBMCs; △ viral yield from nonadherent cells from 10^7 Ficoll-purified PBMCs

loosely bound or not yet penetrated) tend to differ for the various fractions. This is likely to be due to the differing number of cells that express PVR in the various fractions, since residual virus correlates with PVR levels (unpubl. obs.). Samples were withdrawn at daily intervals and titered in a separate plaque assay on Hela cells (Fig. 1). Control infections of unfractionated PBMCs supported poliovirus replication, as previously demonstrated [8]: the adherent cells also supported poliovirus replication, while the nonadherent cells did not support poliovirus replication. Although we are here concerned primarily with viral replication that occurs within 24 h of extravasation, a timecourse over 5 days is shown in Fig. 1: data for the first day only is presented for subsequent studies. Increases in poliovirus titer from PBMCs is generally 2–10 fold within 24 h, although there is significant donor-to-donor variation.

The extent of fractionation by adherence was assessed by immunofluorescence staining using lineage-specific markers followed by flow cytometric analysis. The starting material (Ficoll-purified PBMCs) was 32.9% CD14-positive (mean of 9 measurements, 23.3–41.6% range), the adherent cells contained 62.4% CD14-positive cells (mean of 5 measurements, 53.8–75.8% range), while the nonadherent cells contained 17.1% CD14 (mean of 4 measurements, 8.8–25.7% range). Therefore, adherent cells, which are greatly enriched for monocytes, can support poliovirus replication. However, the nonadherent cells, which contain a small amount of contaminating CD14-positive cells, occasionally supported a low level of poliovirus replication. This is likely to be due to the contaminating CD14-positive cells in the nonadherent fraction. However, it is possible that it may be due to other causes. It is possible that there is a small population of PVR-positive cells that are not monocytes: we have no evidence for this in

primary blood cells. In addition, it is theoretically possible that there is an alternate receptor for poliovirus replication in primary human tissues. However, D171, a blocking anti-PVR antibody [30], fully inhibited poliovirus replication in PBMCs (Freistadt and Eberle, submitted). To address these issues, an improved cell fractionation procedure was developed.

Poliovirus replication in monocytes obtained by negative selection with specific antibodies and paramagnetic beads

One method to separate cells on the basis of cell surface markers is FACS. Several attempts to use this method to derive a pure population of monocytes capable of supporting poliovirus replication were not successful. This was probably due to the long time periods that were required to fractionate the relatively low abundance monocytes and to the resulting reduced viability of the purified cells. An alternate procedure that uses negative selection by paramagnetic beads to separate specific antibodies was developed. A positive selection was not used to avoid possible interference of the antibodies with subsequent infection. The non-CD14-positive cell population in PBMCs consists of CD19 (pan B marker)- and CD2 (pan T and NK marker)-positive cells. Therefore, we used specific monoclonal antibodies to these lineage-specific markers in a negative selection procedure that leaves CD 14-positive cells intact. The cells were assessed for purity using a non-specific esterase histological stain [22, 50] because residual antibodies from the fractionation interfere with immunofluorescence staining. This histological stain is specific for monocytes. Using this method, negatively selected monocytes, that were 73% pure, were obtained (Table 1). These monocytes supported poliovirus replication, while lymphocytes (negatively selected using an mAb to CD 14) were not able to support poliovirus replication (Table 2). There was donor-to-donor variation in the initial titers (Day 0). Therefore, fold-increase was used to calculate and compare extent of viral replication. These data strongly support the interpretation that within PBMCs, CD14-positive cells exclusively support poliovirus replication.

Table 1. Fractionation of PBMCs by specific mAbs and paramagnetic beads

	PBMCs (starting material)	CD2, CD19-negative (monocytes)	CD2, CD19-positive (lymphocytes)	CD14-negative (lymphocytes)
NSE[a]-positive	40[b]	73[c]	16[d]	25[e]
NSE-negative	60	27	84	75

[a] Nonspecific esterase stain for monocytes
[b] Mean of 5 measurements, range: 35–43%
[c] Mean of 5 measurements, range: 63–88%
[d] Mean of 5 measurements, range: 0–37%
[e] Mean of 3 measurements, range: 14–37%

Table 2. Replication of poliovirus in Ficoll-purified PBMCs, monocytes and lymphocytes negatively selected with specific mAbs and paramagnetic beads

	Virus yield (PFUs/ml)		Fold increase	Average fold increase
	Day 0	Day 1		
PBMCS	500	1700	3.4	
	3400	12250	3.6	
				3.5
Monocytes	47.5	475	10.0	
	275	587	2.13	
				6.07
Lymphocytes	725	362.5	0.58	
	267.5	80	0.3	
	625	316	0.51	
				0.46

10^7 Ficoll-purified PBMCs, monocytes negatively selected with anti-lymphocyte mAbs or lymphocytes negatively selected with anti-monocyte mAbs (both from 10^7 Ficoll-purified PBMCs) were infected with poliovirus, rinsed thoroughly to remove unbound virus and the indicated samples titered in a standard plaque assay on Hela cells. Viral yield is expressed as pfus/ml; fold increase over the first 24 h was calculated by dividing pfus/ml for Day 1 by that for Day 0. For each fraction, the fold-increase is averaged

Table 3. Infectious centers assays on PBMCs and adherent cells

Cells	Infectious centers	Correct for % CD14+, PVR+[a]	Correct for IC efficiency[b]	Viral yield (in pfus) per infected cell[c]
PBMCs	0.32 +/− 0.12%	1.05%	1.20%	30
Adherent cells	3.15 +/− 1.55%	5.43%	6.21%	4.0

[a] 32.9% of PBMCs (23.3–41.6% in 9 flow cytometric measurements) and 62.36% (53–75.8% in 5 measurements) of adherent cells are CD14-positive and 92.9% of CD14-positive cells are PVR-positive. The percent of infectious centers has been adjusted to reflect these numbers

[b] In a control experiment, Hela cells yielded 87.5% IC. Therefore, the IC values were divided by 0.875 to correct for efficiency of the Infectious Centers assay

[c] To derive viral yield per cell when PBMCs were infected, viral yield (1.1×10^6 pfus: 2.2×10^5 pfu/ml from 5 ml culture; mean of 9 separate experiments) was divided by infected cells (3.7×10^4 [1×10^7 multiplied by 0.0032 and divided by 0.875]). For the adherent cells, viral yield (1.2×10^5 pfus: 2.45×10^4 pfu/ml from 5 ml culture; mean of 3 measurements) was divided by infected cells (3.0×10^4 [8.25×10^5 adherent cells {from 1×10^7 PBMCs} multiplied by 0.0315 and divided by 0.875])

Determination of percentage of cells infected

To determine the percentage of cells infected, infectious centers assays were carried out on PBMCs and adherent cells. In these assays, 0.32% of PMBCs were infected while 3.15% of adherent cells were infected (Table 3). After correction for the percentage of cells in each population that are PVR-positive and for the efficiency of the assay, it was determined that 1.20% of PBMCs and 6.21% of adherent cells were infected. The infectious centers data were used to calculate pfus produced per cell. For PBMCs, 30 pfus were produced per infected cell; for adherent cells, 4 pfus were produced per infected cell.

Effect of adding nonmonocytes

The percentage of cells infected in adherent cells was higher than the percentage infected in PBMCs, suggesting that more monocytes support PV replication when they are separated from nonmonocytes. However, when one calculates the number of cells infected from the same starting material (1×10^7 PBMCs), taking into account the difference in number of PVR-positive cells between the two populations, the values for number of infected cells within PBMCs and fractionated monocytes do not differ greatly (3.7×10^4 for PBMCs and 3.0×10^4 for adherent cells; see caption to Table 3). Of interest, however, is the higher virus yield per infected cell obtained from PBMCs. This suggested that nonmonocyte cells in PBMCs affect poliovirus replication. To test this, the uninfected nonadherent cell fraction was added back to an infected adherent fraction and assessed for poliovirus replication as before (Table 4). The addition of nonadherent cells restored viral replication levels to that for the unfractionated PBMCs.

Discussion

In this work, we developed two assays to identify the subpopulation within PBMCs that support poliovirus replication. Using these two assays, we identified monocytes as the exclusive cell type within PBMCs supporting poliovirus replication. Preliminary dual staining experiments of infected PBMCs for viral antigens and cell surface markers support this interpretation (unpubl. obs.). Virus yield from separated monocytes was lower than that from PBMCs. Infectious centers assays revealed while that a minority of cells, under either condition, supports poliovirus replication, more virus per cell is produced from the heterogeneous population. Higher total viral yield was restored when infected monocytes were mixed with uninfected lymphocytes, suggesting that interactions between monocytes and lymphocytes may affect poliovirus replication. Primary human cells were used in this study to avoid culture conditions that alter poliovirus susceptibility. However, primary cells are difficult to work with: there was donor-to-donor variability in the ability of monocytes to support poliovirus replication. Whether this is due to intrinsic differences between individuals or subtle differences is presently difficult to determine. It is possible

Table 4. Effect of adding nonadherent cells on poliovirus replication in adherent cells

	Virus yield (PFUs/ml)		Fold increase	Average increase
	Day 0	Day 1		
PBMCs	2800	7000	2.5	
	1300	2600	2.0	
	24000	90000	2.75	
	1000	2700	2.7	
				2.49 +/− 0.34
Adherent, separate	7500	10500	1.4	
	900	1500	1.67	
	11500	18000	1.75	
				1.54 +/− 0.13
Non-adherent separate	100	0	0	
	475	225	0.47	
				0.24 +/− 0.33
Infected adherent mixed with uninfected nonadherent	2200	5750	2.61	
	21700	47500	2.19	
				2.4 +/− 0.3

Various cell fractions were infected with poliovirus, rinsed thoroughly to remove unbound virus and samples titered in a standard plaque assay on Hela cells at Day 0 and Day 1. Primary human peripheral blood mononuclear cells, adherent cells, nonadherent cells were infected: the last set represents infected adherent cells mixed with uninfected nonadherent cells. Viral yield is expressed as pfus/ml; fold increase over the first 24 h was calculated by dividing pfus/ml for day 1 by that for day 0. Data are from 9 separate experiments. For each fraction, average and standard deviation of fold-increase is presented

that genetic differences mediating differential susceptibility to poliovirus in epidemics, if they exist, are mediated by differences in the ability of an individual's monocytes to support poliovirus replication. A possible area of difference may be levels of PVR; however, with only one exception, we have not detected donor-to-donor differences in the levels of PVR on monocytes. A retrospective study of poliomyelitis victims may be required to address this issue. The levels of viral replication are very low: both in terms of percentage of infected cells and yield per cell. It is possible that, if viral replication in humans occurs in monocytes and macrophages, it is at these low levels. This is in contrast to tissue culture systems that have been optimized for viral production. The poliovirus replication cycle in monocytes appears to be slower than in Hela cells (about eight hours): kinetic studies are planned for the future. Preliminary experiments suggest that during the timecourse of the experiments presented

here (1–5 days) multiple rounds of replication are not occurring (unpubl. obs.). Whether the infection is lytic has not been determined: too few cells are infected to observe lysis visually. If a persistent infection is being initiated, it would not be detected in these experiments because these primary cells are not immortal. However, persistent infection in macrophages may be relevant to the pathogenesis of Post-Polio Syndrome. It is of interest that poliovirus persistent infection has been reported in a hematopoietic cell line [23].

The infectious centers assays appear to indicate that more monocytes, when pure, rather than within PBMCs, support poliovirus replication. However, when one calculates the number of cells infected from the same starting material (1×10^7 PBMCs), taking into account the difference in PVR-positive cells between the two populations, the values for number of infected cells within PBMCs and fractionated monocytes do not differ greatly (footnote, Table 3). Of interest, however, is the higher virus yield per infected cells obtained from PBMCs, especially when virus yield per cell is considered (Table 3). Mixing infected adherent cells with uninfected nonadherent cells restored the higher level of viral replication. It is possible that under certain conditions, nonmonocytes support poliovirus replication, although there is no evidence for this. Alternatively, enhancement of virus yield per infected cell may be due to an interaction between monocytes and lymphocytes. Preliminary results suggest that PHA increases viral replication (unpubl. obs.). Since PHA is a lymphocyte mitogen, this could be due to the lymphocyte secretion of cytokines that activate monocytes. The finding that γ-IFN increases PVR expression in the absence of poliovirus [10] may provide a mechanistic explanation for this phenomenon. Recent reports that levels of receptor can mediate differences in susceptibility support this interpretation [19]. It was not possible to correlate PV susceptibility with PVR expression on a cellular level in the present work because D171 (an anti-PVR mAb recognizing the poliovirus binding site) does not detect PVR during a poliovirus infection. Other effects of activation may be responsible as well. This can be tested by adding activated lymphocyte culture supernatant or purified cytokines to monocytes and determining whether this affects virus yield per cell. Alternatively, the enhanced virus yield could be due to direct cell-cell contact between different cell types. Within PBMCs, it may be that susceptible monocytes are already activated: differences in monocyte activation between donors may explain the differences in extent of poliovirus replication between people.

Our experiments with primary human cells suggest that monocytes replicate poliovirus in a natural infection. There have been previous studies of poliovirus replication in hematopoietic cells, but these have generally been carried out with cultured cells [31]. Studies of poliovirus replication that used primary human blood cells did not use defined cell surface markers to identify the cell type supporting poliovirus replication [24, 46]. Our ability to detect poliovirus replication in PVR-positive, primary human blood cells within 24 h suggests that the cells were susceptible prior to the experimental infection. Although our studies have focused on monocytes, it is likely that macrophages

also support poliovirus replication in vivo. In vitro generated macrophages support poliovirus replication, but since changes occur in the cells during the culturing, this does not shed light on whether primary human macrophages support poliovirus replication. This may require primary human macrophages.

The ability of primary human blood cells to support poliovirus replication may explain several historically unresolved phenomena concerning poliomyelitis. Although it has long been known that poliovirus replicates in oropharyngeal and intestinal mucosa [3], the specific cell type carrying out the primary round of viral replication has not been identified. Furthermore, whether the replication occurs during or after crossing the gut barrier is not clear. There is recent, indirect evidence that poliovirus can be trans-cytosed across the epithelial layer by M cells, suggesting that the initial uptake is nonspecific and does not include a round of replication [42]. In this case, resident mononuclear phagocytic cells in the Peyer's Patches may support initial rounds of poliovirus replication, similar to a hypothesized scheme for reovirus pathogenesis [48]. This suggests that after nonspecific entry into mucosal associated lymph tissue (MALT), resident macrophages may be the specific cell type that carries out the primary round of poliovirus replication. The virus produced could be shed into the blood, causing plasma viremia [28]. However, it is possible that monocytes in the blood may also become infected and shed additional virus into the blood. Additionally, myeloid cells in MALT may be one of the elusive "extraneural" sites of viral replication that is responsible for continued shedding of poliovirus into feces after viremia has been cleared [4].

Myeloid cells may also be important in the dissemination of poliovirus through the body during an infection. Another unresolved question in poliovirus pathogenesis has been how the virus enters the CNS. There is evidence for two routes: direct crossing of the blood brain barrier [29] or retrograde transfer after replication in peripheral tissues [37]. Furthermore, there are strain-specific differences in the route viruses use [29]. Our work suggests a third possible, though not necessarily exclusive, route: in nonimmune individuals, monocytes may carry the virus into the CNS when they diapedese and differentiate into microglia. The early finding that specific antisera rapidly cleared plasma viremia [28] is not inconsistent with such a route because in the presence of antibody, poliovirus enters monocytes via Fc receptors and is rapidly degraded (unpubl. obs.). In an immune individual, presumably antibody would clear virus before significant numbers of blood monocytes are infected. Direct testing of this may require an animal model.

Although poliovirus has not been thought of as a "macrophage-tropic" virus, the ability of viruses to replicate in mononuclear phagocytes can be critical in establishing a natural infection [5, 12, 25]. Similar to other viral infections, (HIV and HCMV), blood monocytes or tissue macrophages may act as a reservoir for poliovirus. Replication in mononuclear phagocytes has been observed for: measles virus [14], coronavirus [2], arterivirus [21], arenavirus [1], alphaviruses [33], lentiviruses [5], poxvirus [38] and herpes viruses

[34,44]. Strain-specific differences in virulence of ectromelia may be mediated by their differential ability to replicate in mononuclear phagocytes [38]. Differences in susceptibility of mononuclear phagocytes to viral infection may account for variation in the age-related or other genetically conferred differences in susceptibility of animals to HSV, ectromelia or MHV [2,43,45].

Replication in macrophages may enhance antigen presentation. Since the efficacy of live, attenuated vaccines is due to their ability to induce IgA-mediated immunity, replication in gut-associated macrophages may be critical in generation of IgA response. Although attenuating mutations have been mapped in the viral genome [17,32,35,47], their functional significance during a natural infection is not known. If poliovirus replication in monocytes is important in poliovirus pathogenesis, a prediction would be that there are strain-specific (attenuated vs. neurovirulent) differences in poliovirus replication in monocytes. Preliminary results suggest that this is the case (unpubl. obs.). Continued studies on poliovirus replication in primary human blood cells may shed light on these various issues.

Acknowledgements

We gratefully acknowledge the help of The Blood Center for Southeast Louisiana, LSUMC Core Laboratories, Tulane Flow Cytometry Facility, Shannon Meadows and Miriam Corti. This work was funded, in part, by Louisiana Education Quality Support Fund grant #RD–A–12.

References

1. Aronson JF, Herzog NK, Jerrells TR (1994) Pathological and virological features of arenavirus disease in guinea pig: comparison of two Pichinde virus strains. Am J Pathol 145: 228–235
2. Bang FB, Warkwick A (1960) Mouse macrophages as host cells for the mouse hepatitis virus and the genetic basis of their susceptibility. Proc Natl Acad Sci USA 46: 1065–1075
3. Bodian D (1959) Poliomyelits: pathogenesis and histopathology. In: Horsfall FL Jr, Tamm I (eds) Viral and rickettsial infections of man. Lippincott, Philadelphia, pp 479–498
4. Bodian D, Horstmann DM (1965) Polioviruses. In: Horsfall FL Jr, Tamm I (eds) Viral and rickettsial infections of man. Lippincott, Philadelphia, pp 430–473
5. Clements J, Zink M, Narayan O, Gabuzda D (1994) Lentivirus infection of macrophages. Immunol Ser 60: 589–600
6. Emini EA, Jameson BA, Wimmer E (1983) Priming for and induction of anti-poliovirus neutralizing antibodies by synthetic peptides. Nature 304: 699–703
7. Freistadt MS (1994) Distribution of the poliovirus receptor in human tissue. In: Wimmer E (ed) Cellular receptors for animal viruses. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 455–461
8. Freistadt MS, Fleit HB, Wimmer E (1993) Poliovirus receptor on human blood cells: a possible extraneural site of poliovirus replication. Virology 195: 798–803
9. Freistadt MS, Kaplan G, Racaniello VR (1990) Heterogeneous expression of poliovirus receptor-related proteins in human cells and tissues. Mol Cell Biol 10: 5700–5706

10. Freistadt MS, Stoltz DA, Fberle KE (1994) Role of poliovirus receptors in the spread of the infection. In: The post-polio syndrome: advances in the pathogenesis and treatment. New York Academy of Sciences, Washington, pp 37–47
11. Guttman N, Baltimore D (1977) A plasma membrane component able to bind and alter virions of poliovirus type 1: studies on cell-free alteration using a simplified assay. Virology 82: 25–36
12. Ibanez CE, Schrier R, Ghazal P, Wiley C, Nelson JA (1992) Human cytomegalovirus productively infects primary differentiated macrophages. Virol 65: 6581–6588
13. Joklik WK, Darnell JE (1961) The adsorpton and early fate of purified poliovirus in HeLa cells. Virology 13: 439–447
14. Joseph BS, Lampert PW, Oldstone MBA (1975) Replication and persistence of measles virus in defined subpopulation of human leukocytes. J Virol 16: 1638–1649
15. Kaplan AS (1955) Comparison of susceptible and resistant cells to infection with poliomyelitis virus. Ann NY Acad Sc 61: 830–839
16. Kaplan G, Freistadt MS, Racaniello VR (1990) Neutralization of poliovirus by cell receptors expressed in insect cells. J Virol 64: 4697–702
17. Kohara M, Abe S, Komatsu T, Tago K, Arita M, Nomoto A (1988) A recombinant virus between the Sabin 1 and Sabin 3 vaccine strains of poliovirus as a possible candidate for a new type 3 poliovirus live vaccine strain. J Virol 62: 2828–2935
18. Koike S, Horie H, Ise I, Okitsu A, Yoshida M, Iizuka N, Takeuchi K, Takegami T, Nomoto A (1990) The poliovirus receptor protein is produced both as membrane-bound and secreted forms. EMBO J 9: 3217–3224
19. Koike S, Taya C, Aoki J, Matsuda Y, Ise I, Takeda H, Matsuzaki T, Amanuma H, Yonekawa H, Nomoto A (1994) Characterization of three different transgenic mouse lines that carry human poliovirus receptor gene – influence of the transgene expression on pathogenesis. Arch Virol 139: 351–363
20. Koike S, Taya C, Kurata T, Abe S, Ise I, Yonekawa H, Nomoto A (1991) Transgenic mice susceptible to poliovirus. Proc Natl Acad Sci USA 88: 951–955
21. Kuo LL, Harty JT, Erickson L, Palmer GA, Plagemann PG (1991) A nested set of eight RNAs is formed in macrophages infected with lactate dehydrogenase-elevating virus. J Virol 65: 5118–23
22. Li C, Lam K, Yam L (1973) Esterases in human leukocytes. J. Histochem Cytochem 21: 1–12
23. Lloyd R, Bovee M (1993) Persistent infection of human erythroblastoid cells by poliovirus. Virology 194: 200–9
24. Mastroeni P, Merendino RA, Bonina L, Arena A, Liberto MD, Gazzara D (1995) In vitro study on human mononuclear phagocytic cells behaviour versus DNA and RNA viruses. Giorn Batt Virol Immun 78: 262–273
25. McNearney T, Hornickova Z, Markham R, Birdwell A, Arens M, Saah A, Ratner L (1992) Relationship of human immunodeficiency virus type 1 sequence heterogeneity to stage of disease. Proc Natl Acad Sci USA 89: 10247–10251
26. Mendelsohn C, Johnson B, Lionetti KA, Nobis P, Wimmer E, Racaniello VR (1986) Transformation of a human poliovirus receptor gene into mouse cells. Proc Natl Acad Sci USA 83: 7845–7849
27. Mendelsohn CL, Wimmer E, Racaniello VR (1989) Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell 56: 855–865
28. Nathanson N, Bodian D (1962) Experimental poliomyelitis following intramuscular virus injection: I. The effect of neural block on a neurotropic and a pantropic strain II. Viremia and the effect of antibody. Bull Johns Hopkins Hosp 108: 308–333

29. Nathanson N, Bodian D (1961) Experimental poliomyelitis following intramuscular virus injection: III. The effect of passive antibody on paralysis and viremia. Bull Johns Hopkins Hosp 111: 198–220
30. Nobis P, Zibirre R, Meyer G, Kühne J, Warnecke G, Koch G (1985) Production of a monoclonal antibody against an epitope on HeLa cells that is the functional poliovirus binding site. J Gen Virol 66: 2563–2569
31. Okada Y, Toda G, Oka H, Nomoto A, Yoshikura H (1987) Poliovirus infection of established human blood cell lines: relationship between the differentiation stage and susceptibility or cell killing. Virology 156: 238–245
32. Omata T, Kohara M, Kuge S, Komatsu T, Abe S, Semler BL, Kameda A, Itoh H, Arita M, Wimmer E, Nomoto A (1986) Genetic analysis of the attenuation phenotype of poliovirus type 1. J Virol 58: 348–358
33. Peters CJ, Dalrymple JM (1990) Alphaviruses. In: Fields BN, Knipe DM, Channock RM, Hirsch MS, Melnick JL, Monath TP, Roizman B (eds) Virology. Raven Press, New York, pp 713–762
34. Plaeger-Marshall S, Willson LA, Smith JW (1983) Alteration of rabbit alveolar and peritoneal macrophage function by herpes simplex virus. Infect Immun 41: 1376–1379
35. Racaniello VR (1988) Poliovirus neurovirulence. Adv Virus Res 34: 217–246
36. Ren R, Racaniello VR (1992) Human poliovirus receptor gene expression and poliovirus tissue tropism in transgenic mice. J Virol 66: 296–304
37. Ren R, Racaniello VR (1992) Poliovirus spreads from muscle to the central nervous system by neural pathways. Infect Dis 166: 747–752
38. Roberts JA (1963) Histopathogenesis of mousepox. Br J Exp Pathol 44: 465–472
39. Salcedo TW, Fleit HB (1991) Plasma membrane and intracellular polls of transferring receptors decline during in vitro cultivation of U937 cells. Cell Prolif 24: 383–401
40. Shepley MP, Racaniello VR (1994) A monoclonal antibody that blocks poliovirus attachment recognizes the lymphocyte homing receptor CD44. J Virol 68: 1301–1308
41. Shepley MP, Sherry B, Weiner HL (1988) Monoclonal antibody identification of a 100 kDa membrane protein in HeLa cells and human spinal cord involved in poliovirus attachment. Proc Natl Acad Sci USA 85: 7743–7747
42. Sicinski P, Rowinski J, Warchol JB, Jarzabek Z, Gut W, Szczygiel B, Bielecki K, Koch G (1990) Poliovirus type 1 enters the human host through intestinal M cells. Gastroenterology 98: 56–58
43. Silverstein SC (1975) The role of mononuclear phagocytes in viral immunity. In: Van Furth R (ed) Mononuclear phagocytes in immunity, infection, and pathology. Blackwell, Oxford, pp 557–573
44. Soderberg C, Larsson S, Bergstedt-Lindquist S, Moller E (1993) Definition of a subset of human peripheral mononuclear cells that are permissive to human cytomegalovirus infection. J Virol 67: 3166–3175
45. Tyler KL, Fields BN (1990) Pathogenesis of viral infections. In: Fields BN (ed) Virology. Raven Press, New York, pp 191–235
46. Willems FTC, Melnick JL, Rawls WE (1969) Replication of poliovirus in phytohemagglutinin-stimulated human lymphocytes. J Virol 3: 451–457
47. Wimmer E, Hellen CUT, Cao X (1993) Genetics of poliovirus. Annu Rev Genet 27: 353–436
48. Wolf JL, Rubin D, Finberg R, Kauffman RS, Sharpe AH, Trier JS, Fields BN (1981) Intestinal M cells: a pathway for entry of reovirus into the host. Science 212: 471–472

49. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC (1990) CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 249: 1431–1433
50. Yam L, Li C, Crosky W (1971) Cytochemical identification of monocytes and granulocytes. Am J Clin Pathol 55: 283–290

Authors' address: Dr. M. S. Freistadt, Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112, U.S.A.

Received May 2, 1995