The pathogenesis of poliovirus infection, responsible for the induction of a poliovirus-specific mucosal immune response following intraperitoneal (i.p.) inoculation of virus in mice transgenic for the poliovirus receptor (PVRTg mice), was studied. Following inoculation of poliovirus, replication was determined by increase in virus titre (TCID₅₀) and by PCR of poliovirus-specific negative-strand RNA in peritoneal macrophages, mesenteric lymph nodes, Peyer’s patches, duodenum, brain, kidney and liver. The presence of poliovirus antigens in several cell types was detected by immunolabelling. It was demonstrated that poliovirus replicated in the peritoneal macrophages of PVRTg mice, since the virus titre in peritoneal cells was increased compared to the titre in the inoculum. Negative-strand RNA was detected in these cells and most of the poliovirus-immunostained cells had the morphology of macrophages and expressed the macrophage-specific markers CD86 and M1/70 on their surface. Furthermore, in peritoneal lavage, poliovirus was also present in CD19⁺ B cells, but not in dendritic or T cells. Moreover, poliovirus was detected in macrophage-like cells in the lamina propria of the intestine, but not in epithelial cells. Replication of poliovirus in mesenteric lymph nodes, Peyer’s patches and brain was followed by excretion of virus in the faeces. This suggests that the virus is transported due to migration of macrophages from the peritoneal cavity to mesenteric lymph nodes and the lamina propria of Peyer’s patches. It is likely that this route is responsible for the induction of virus-specific IgA in the gut.

INTRODUCTION

Poliomyelitis is an acute paralytic disease in humans caused by infection of the central nervous system by poliovirus. When inoculated with virus intracerebrally, mice transgenic for the human poliovirus receptor (PVRTg mice) show clinical symptoms of poliomyelitis similar to those observed in humans and monkeys, which has led to their use for poliovirus neurovirulence tests (Abe et al., 1995; Horie et al., 1994). So far, attempts to demonstrate virus replication after oral inoculation with poliovirus in PVRTg mice have failed (Nomoto et al., 1994; Zhang & Racaniello, 1997).

We adapted the PVRTg mouse model to study faecal excretion and virus-specific mucosal immune responses, by use of an intraperitoneal (i.p.) route of inoculation, thus circumventing the oral route. We were able to induce a mucosal IgA response in both the intestine and the saliva of PVRTg mice, as well as faecal shedding of virus (Buisman et al., 2000). Our observations suggested that virus replication took place in the peritoneal cavity and/or the intestinal wall of PVRTg mice. Since poliovirus is reported to replicate in human monocytes (Eberle et al., 1995), we hypothesized that peritoneal macrophages might play a role in entry, or replication, of poliovirus in PVRTg mice, and in the induction of the mucosal immune response. The present study was undertaken to examine this hypothesis.

To detect virus replication, we developed a PCR to detect viral negative-strand RNA. This is indicative of poliovirus replication, since replication of the positive-sense genomic RNA of poliovirus proceeds via a negative-strand intermediate (Bishop et al., 1969). This negative-strand intermediate is present in low amounts compared to viral positive strands. The ratio of positive to negative strands in infected cells ranges from approximately 40 : 1 to 70 : 1, 2–6 h after replication (Novak & Kirkegaard, 1991). Therefore, a very specific method is needed to detect this intermediate strand. Several methods to detect the negative intermediate RNA strand have been described for some picornaviruses (Agnes et al., 1994; Chaves et al., 1994; Nanda et al., 1994). For poliovirus, positive and negative strands have been demonstrated previously by fluorescent in situ hybridization.
(Bolten et al., 1998). In this study we detected the negative RNA strand intermediate specifically via a strand-specific PCR. Using this method, in combination with virus titrations and immunolabelling of poliovirus-infected cells in different organs of PVRTg mice, we studied the pathogenesis of poliovirus infection likely to be responsible for induction of the mucosal immune response in these PVRTg mice.

This work was presented in part at the Sixth International Symposium on Positive-strand RNA Viruses (28 May–2 June 2001, Paris, France; abstract P2-135) and the Mucosal Immunology Satellite Meeting (International Congress of Immunology) (28–30 July 2001, Reykjavik, Iceland; abstract P12.)

**METHODS**

**Animals.** Homozygous transgenic mice carrying the human PVR gene (ICR-PVRTg21), generated as previously described (Koike et al., 1994), were obtained from A. Nomoto (University of Tokyo, Japan). They were bred and housed in isolator cages, according to World Health Organization guidelines (WHO, 1993). Kidneys from transgenic mice (PVRTg) were tested for the presence of the PVR gene by PCR (Koike et al., 1990). Non-transgenic ICR mice (Harlan) were used as controls. The study design was approved by the RIVM Ethical Committee for Animal Experiments.

**Virus.** The virulent Mahoney strain serotype 1 was used in this study. Poliovirus-infected cell lysates were purified on a CsCl gradient in PBS. Subsequently, the quantity of infectious virus was determined by virus titration assays on Hep2 cells (WHO, 1997) and expressed as TCID50.

**Experimental design.** Eight- to twelve-week-old control mice and PVRTg mice (both male and female) were inoculated with poliovirus serotype 1 Mahoney by i.p. injection of each mouse with 50 U heparin ml–1 (p.i.), mice were sacrificed by cervical dislocation. Peritoneal cells were recovered by centrifugation at 12000 × g for 10 min. Cells were counted, used for immunolabelling, and the rest of the cells were pelleted and immediately frozen in liquid nitrogen for RNA isolation.

Macroscopically visible Peyer’s patches were dissected from the small intestine. Mesenteric lymph nodes, Peyer’s patches, a part of the ileum, were brought to a concentration of 4 × 106 cells ml–1. Then, 10 μl of the cell suspension was spotted on 12-well adhesion slides (Bio-Rad), allowed to adhere for 30 min at room temperature (RT), air dried, fixed for 10 min in methanol at room temperature, and stored in ice-cold PBS overnight. Cells were stained for poliovirus antigens by incubation with polyclonal rabbit anti-poliovirus serotype 1 antiserum, diluted in PBS containing 1 % bovine serum albumin (Sigma) (PBS/BSA) for 30 min at room temperature and washed in PBS containing 0-5 % Tween 20 (Merck). Subsequently, the cells were incubated with peroxidase-labelled swine anti-rabbit IgG (Dako) diluted in PBS/BSA and staining was visualized with 3,3-diaminobenzidine (Sigma) with ammonium nickel sulphate (Fluka). Cells were counterstained with nuclear fast red (Sigma).

For double staining, the cells were incubated with 0-1 M glycine for 10 min and then permeabilized with 0-1 % saponin (Merck) in PBS/BSA. Immunostaining for poliovirus antigens was performed first with polyclonal rabbit anti-poliovirus serotype 1 antiserum for 30 min at room temperature. Subsequently, after washing, secondary staining was performed with Cy3-labelled donkey F(ab)2 anti-rabbit IgG (Jackson Immunoresearch) for 30 min at room temperature. Poliovirus staining was subsequently combined with staining for cell markers in a three-step process. The following cell markers were used: for leukocytes, rat anti-mouse CD44 (Pharmingen); for macrophages, rat anti-mouse M1/70 (Serotec); for costimulatory molecule B7-2, rat anti-mouse CD86 (Pharmingen); for B cells, rat anti-mouse CD19 (Pharmingen); for T cells, rat anti-mouse CD3 or rat anti-mouse CD4 (Pharmingen); for dendritic cells, rat anti-mouse CD205 (clone NLDC-145, Serotec); and for polymorph nuclear cells rat anti-mouse Gr1 (PharMingen). Cells were incubated for 30 min at room temperature. During the second step, after washing, the cells were incubated with peroxidase-labelled mouse anti- rat IgG (Jackson Immunoresearch). During the third step, after washing, the signal was amplified by incubation with biotinyl tyramide (NEN Life Sciences), and diluted in amplification solution (NEN Life Sciences) for 10 min at room temperature. Biotin precipitation was visualized by a 30 min incubation with FITC-labelled streptavidin (Roche Diagnostics) at room temperature. All antibodies described above were diluted in 0-1 % saponin in HEPES-buffered saline solution (Clonetics). TO-PRO-3 (Molecular Probes Inc.) was used for DNA counterstaining. Cells were mounted in Vectashield (Vector Laboratories).

Immunostaining was also performed on cryostat sections of Swiss-rolls of the ileum containing Peyer’s patches, and on sections of mesenteric lymph nodes, fixed in ice-cold acetone for 10 min. The sections were stained for poliovirus antigens and cell markers by double immunofluorescent staining using FITC-labelled swine anti-rabbit (Dako) for poliovirus antigens and Cy3-labelled donkey F(ab)2 anti-rat IgG (Jackson Immunoresearch) as secondary antibodies.

**RT-PCR.** Total RNA samples were extracted from Hep2 cells, peritoneal cells, mesenteric lymph nodes, Peyer’s patches, brain and kidney from PVRTg mice and control mice by using a total RNA extraction kit (Promega). For specific amplification of poliovirus, we used primers located in the 5’-nontranslated region (5’-NTR) of the genome: upstream primer ME-1 hybridizes with the negative-strand sequence 5’-CGGTACCTTTGTGGCGCTGGTTTTATCTCC-3’ (representing positions 65–94 of the poliovirus genome); downstream reverse...
primer ME-2 is complementary to the poliovirus positive-strand sequence 5'-CACGGACACCCAAAAGTAGTCGGTTCGCA-3' (the reverse complement of positions 558–529). This primer pair for the poliovirus-specific RT-PCR gives an amplicon of 494 bp.

For reverse transcription into both cDNA and PCR in a single buffer system, thermostable recombinant Thermus thermophilus (rTth) DNA polymerase was used (Perkin Elmer). The ability of rTth DNA polymerase to efficiently reverse transcribe RNA templates at elevated temperatures increases the specificity of primer hybridization and excludes self-priming of the viral RNA, as observed using AMVrt DNA polymerase (data not shown). The rTth DNA polymerase was also used for subsequent extension. Total RNA samples were denatured at 96°C for 10 min and chilled on ice. For cDNA synthesis, 10 μl of the RNA sample was added to 40 μl reverse transcription mixture containing the following at their final concentration: 50 mM Bicine, 115 mM potassium acetate, 8 % (w/v) glycerol, pH 8.2, 2 mM Mn(OAc)₅, 0.25 mM of each dNTP, 5 μM primers ME-1 or ME-2 and 5 U of rTth DNA polymerase, and overlaid with mineral oil. cDNA synthesis was performed at 55°C for 60 min.

In order to ensure the specificity of the negative-strand RT-PCR, an RNase A and H digestion was performed directly after the cDNA reaction to digest all RNA left after reverse transcription (final concentrations RNase A/H mixture: 0.6 mg RNase A ml⁻¹, 0.8 U RNase H ml⁻¹). To each tube 2 μl of the RNase A/H mixture was added and the tubes were then incubated for 90 min at 37°C, heated for 10 min at 95°C and chilled on ice. For PCR amplification, 0.5 μM of the opposite primer ME-1 or ME-2 was added to the cDNAs and PCR was carried out in a DNA thermal cycler 480 (Perkin Elmer) according to the following protocol: an initial step of 94°C for 3 min followed by 40 cycles of a two-temperature PCR (94°C for 1 min, 65°C for 2 min 30 s) and ending with 65°C for 7 min.

As a control for the RNA digestion, experiments using one primer and no enzyme during the cDNA reaction followed by ribonuclease A + H digestion and PCR with both primers ME1 and ME2 using rTth polymerase did not result in PCR products.

Nested PCR. For the nested PCR, upstream primer ME-3 [5'–ACTTTGTGTTCCCCCGTG–3' (equivalent to position 167–184)] and downstream primer ME-4 [5'–CTTTACACCTTTGTCACA–3' (the reverse complement of position 420–403)] were used. The ME-3/ME-4 primer pair for the poliovirus-specific nested PCR gives an amplicon of 234 bp. One μl of amplified product was added to 49 μl of PCR mixture overlaid with mineral oil, containing the following: 1× PCR buffer II (50 mM KCl, 10 mM Tris/HCl pH 8.3), 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 μM primer ME-3 and ME-4, 0.6 U Taq DNA polymerase (Perkin Elmer). The reaction mixture was then subjected to 30 consecutive cycles of 45 s at 95°C, 45 s at 50°C, and 1 min at 72°C. The PCR products were electrophoresed through 2% agarose gels and visualized by ethidium bromide staining.

RESULTS

High titres of virus present in different organs of PVRTg mice after i.p. inoculation of poliovirus

From day 1 to day 8 following i.p. inoculation of mice with 1×10⁶ poliovirus, virus levels were determined in peritoneal fluid, mesenteric lymph nodes, Peyer’s patches and brain of PVRTg mice and control mice (Fig. 1A–D). In addition, the amount of virus in the duodenal contents was expressed as TCID₅₀ per 100 mg faeces (Fig. 1E). The mean virus level per mouse in peritoneal fluid of pooled PVRTg mice and control mice (i.e. non-transgenic for the PVR) is shown in Fig. 1(A). Control mice did not show an increase in virus titres in the peritoneal fluid. However, a strong 50 000-fold increase in virus titre was observed from day 3 p.i. to day 5 p.i., reaching a peak of 10⁵ TCID₅₀ per mouse at day 3 p.i. After day 3 p.i., the virus titre declined, reaching the detection limit of 10⁻¹³ TCID₅₀ per mouse at day 5 p.i. (Fig. 1A). In the pooled mesenteric lymph nodes and Peyer’s patches of PVRTg mice, the same pattern of virus load was found as described for the peritoneal fluid. The mean virus level per mouse in the mesenteric lymph nodes increased 10³-fold from day 1 to day 3 p.i., reaching a peak of 10⁷ TCID₅₀ at day 3 p.i. (Fig. 1B), while only a 500-fold increase was found in the Peyer’s patches from day 1 to day 3 p.i., reaching a peak of 10⁶ TCID₅₀ virus at day 3 p.i. (Fig. 1C). From day 3 onwards, virus titres declined in these organs to the limit of detection at day 5 p.i. Control mice, however, had a very low virus titre at day 1 p.i. of 5×10² TCID₅₀ in the...
mesenteric lymph nodes and $10^2$ TCID$_{50}$ in the Peyer’s patches; these values reached undetectable values at day 2 p.i.

Virus titres in the brains and duodenal contents of six individual PVRTg mice p.i. are presented in Fig. 1(D, E). In contrast to the virus titres in the organs described above, virus titres in the brain and duodenum did not show a sharp peak, but values increased and declined more gradually. Poliovirus was detectable in the brain at day 2 p.i., increased 30-fold until day 5 p.i., reaching values of $10^3$ TCID$_{50}$ per organ and decreased until undetectable values at day 8 p.i. (Fig. 1D). In the faeces from the duodenal contents of the PVRTg mice, poliovirus was present at day 1 p.i.; the titre increased 30-fold until $10^5$ TCID$_{50}$ at day 3 and subsequently declined until undetectable levels at day 8 (Fig. 1E). Control mice, however, did not have any detectable virus in the brain and duodenal contents samples. Collectively, these results indicate replication of poliovirus in PVRTg mice.

**Development of a PCR specific for the negative intermediate strand of poliovirus**

In order to confirm and extend these observations, we developed a poliovirus-specific negative-strand PCR. Since poliovirus forms a negative polarized RNA intermediate strand during replication, detection of this negative strand in infected cells or tissues is evidence of replication. For detection of negative-strand RNA, we used a specific reverse primer for reverse transcription of the isolated RNA compared to that used for the detection of the positive virus strand. In order to ensure the specificity of the negative-strand RNA, RNase A and H digestion were performed after the cDNA reaction, in order to delete all viral RNA and RNA–cDNA hybrid RNA that could react with both primers in the PCR reaction.

Hep2 cell monolayers were inoculated with poliovirus at an m.o.i. of 10. The inoculum of 10 m.o.i. contained positive-strand viral RNA, but no negative-strand RNA (Fig. 2). At 3, 6, 22 and 30 h p.i., total RNA was extracted from the cells and both positive and negative poliovirus strand PCR reactions were done using strand-specific primers. At 3 h p.i. the positive poliovirus RNA strand (494 bp) was detectable in the infected Hep2 cells and no negative-strand RNA was found. At 6, 22 and 30 h p.i., this PCR product was more abundant (Fig. 2). From 6 h p.i. onwards the negative-strand-specific PCR product became detectable in the Hep2 cells, and after 22 and 30 h p.i. this negative-strand product was still detectable. The negative-strand PCR products were less abundant than the positive-strand PCR products at all time-points (Fig. 2).

**Fig. 2.** Presence of poliovirus-specific positive-strand RNA and negative-strand RNA, as measured by PCR, at 0, 3, 6, 22 and 30 h p.i. in a Hep2 cell monolayer inoculated with an m.o.i. of 10.
Detection of positive- and negative-strand RNA in organs of PVRTg mice

At different time points p.i. of $1 \times 10^6$ poliovirus, the presence of negative and positive poliovirus RNA was examined by nested PCR in peritoneal cells, mesenteric lymph nodes, Peyer’s patches and brain of PVRTg mice and control mice. In peritoneal cells of PVRTg mice, positive polioviral RNA strands were detectable at days 1, 2, 3, 4, 5 and 8 p.i. More importantly, in these cells negative poliovirus RNA strands were found at days 1 to 5 p.i., but not at day 8 p.i. (Fig. 3). Peritoneal cells of control mice also contained positive RNA strands at days 1, 2, 3 and 4 p.i. only, but these PCR products were less abundant compared to those of the PVRTg mice at each time-point (Fig. 3). Negative-strand poliovirus RNA, however, was not detectable in the peritoneal cells of control mice at any time-point after infection, suggesting that the positive RNA strand found in control mice originated from the virus inoculum.

In mesenteric lymph nodes of PVRTg mice, both positive and negative poliovirus RNA strands were detectable at days 1, 2, 3, 4, 5 and 8 p.i., while these organs from control mice only contained positive poliovirus RNA strands p.i. (Table 1). In Peyer’s patches from PVRTg mice, positive poliovirus RNA strands were found at days 2, 3 and 4 p.i. and negative-strand RNA was detectable only at day 3. At days 5 and 8 p.i., poliovirus RNA was no longer detectable in the Peyer’s patches. Peyer’s patches of control mice did not contain any viral RNA (Table 1). Brains from PVRTg mice contained both positive and negative poliovirus RNA strands at days 1, 2, 3, 4, 5 and 8 p.i., while no poliovirus RNA was found in brains from control mice (Table 1).

The kidneys and livers from some PVRTg mice contained virus after poliovirus inoculation, at titres ranging from $6 \times 10^1$ to $2 \times 10^6$ TCID$_{50}$ per organ. In these organs both positive and negative poliovirus RNA strands were found. Kidneys and livers from control mice did not contain any poliovirus RNA.

Immunodetection of poliovirus antigens in different organs after i.p. inoculation

To characterize the cell types that harbour poliovirus, immunolabelling studies were performed on peritoneal cells, mesenteric lymph nodes and small intestine. At different time-points p.i., the percentages of cells in peritoneal lavages immunostained for poliovirus were determined in both PVRTg mice and control mice (Fig. 4). In

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**Fig. 3.** Presence of poliovirus-specific positive-strand RNA and negative-strand RNA, as measured by nested PCR, in peritoneal cells of PVRTg and control mice at days 1 to 8 after i.p. inoculation with $10^6$ TCID$_{50}$ poliovirus.
PVRTg mice about 1 (± 0.8 SD)% of the cells were immunolabelled for poliovirus at day 1 p.i. The number of positive cells increased to 17 ± 7% at day 2, reached a peak of 43 ± 8% at day 3, and thereafter declined to 19 ± 13%, 2 ± 1% and 7 ± 9% at days 4, 5 and 8 respectively. In control mice only 2–3% poliovirus-positive cells were found during this period (Fig. 4).

Immunostaining of peritoneal cells of PVRTg mice at day 3 p.i. demonstrated that the poliovirus-infected cells were large leukocytes with a kidney-shaped nucleus and a granular staining pattern in the cytoplasm of the cell. Some poliovirus-infected cells were lymphocytes and showed a small rim of staining (Fig. 5). Double immunostaining for poliovirus antigens and cell markers for leukocytes (CD86; Fig. 6A) and macrophages (M1/70) confirmed that these large virus-positive cells were indeed peritoneal macrophages (Fig. 6B). Moreover, the poliovirus-stained lymphocytes appeared to be B cells, since double immunostaining was found for CD19, a B cell-specific marker (Fig. 6C). Peritoneal dendritic cells of these mice, indicated by staining with CD205, were not positive for poliovirus antigens (Fig. 6D). None of the poliovirus-positive peritoneal cells showed a positive signal for the T cell markers CD3 or CD4 (data not shown).

In the ileum of PVRTg mice at day 3 p.i., we found sporadic cells positive for poliovirus antigens in the lamina propria, but none in the epithelial layer. The morphology of these cells corresponded to that of macrophages. Because of the very low number of poliovirus-immunoreactive cells in the ileum, attempts to identify these poliovirus-positive cells phenotypically were not successful. A double immunofluorescence staining for CD4 and poliovirus is depicted in Fig. 7 and illustrates a poliovirus-positive macrophage in the lamina propria. The poliovirus-immunoreactive cells

Table 1. Presence of poliovirus positive strand-specific and negative strand-specific RNA detected by strand-specific PCR in mesenteric lymph nodes, Peyer’s patches and brain from either PVRTg mice or control mice after i.p. inoculation of poliovirus

<table>
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Fig. 4. Percentage of peritoneal cells from PVRTg mice (white bars) or control mice (black bars) positive for poliovirus antigens measured by single immunoperoxidase labelling from days 1 to 8 after i.p. inoculation with 10⁶ TCID₅₀ poliovirus. Peritoneal cells pooled from three mice were used for the immunolabelling. Data represent mean ± SD of six mice.
in the mesenteric lymph nodes of PVRTg mice at day 3 p.i. also showed the morphology of macrophages (data not shown).

**DISCUSSION**

PVRTg mice provide a useful animal model to study poliovirus neurovirulence after intracerebral inoculation of poliovirus, and to study polio-specific mucosal immunity as well as shedding of poliovirus into the stools after i.p. infection (Abe et al., 1995; Buisman et al., 2000). The presence of poliovirus in the brain of some mice after i.p. inoculation with Mahoney virus, and the observed faecal shedding of the virus, imply that replication of the virus and transport from the peritoneal cavity to the central nervous system and the intestinal lumen occur in PVRTg mice – leading to paralysis and death of some of these mice (Buisman et al., 2000). Although the i.p. route of inoculation is somewhat artificial, these mice provide us with a useful animal model to study both systemic and mucosal immunity against poliovirus in challenge experiments and to study shedding characteristics of different poliovirus strains.

In the present study, we examined the pathogenesis of an i.p. poliovirus infection in PVRTg mice in order to explain the induction of mucosal IgA and the shedding of virus in these mice. We demonstrated that poliovirus replicates in peritoneal cells of PVRTg mice by detecting a 1000-fold increase in virus titre compared to the inoculum and a 100 000-fold increase from day 1 to day 3 p.i. in peritoneal lavage; while in control (non-transgenic) mice poliovirus did not replicate. We also demonstrated by immunolabelling that PVRTg mice contained far more poliovirus-positive cells in the peritoneal lavage compared to control mice (Fig. 4). Moreover, we detected poliovirus-specific negative-strand RNA in peritoneal cells of the PVRTg mice during the first 5 days p.i. Since replication of the positive-sense genomic RNA of poliovirus proceeds through a negative-strand intermediate, the detection of viral negative-strand RNA in specific organs indicates replication, as described for several other positive-strand RNA viruses (Agnes et al., 1994; Bishop et al., 1969; Chaves et al., 1994; Nanda et al., 1994). The sensitivity of the nested PCR for detection of negative-strand RNA is illustrated by the detection of negative-strand RNA in peritoneal cells of PVRTg mice at day 5 p.i., when the virus was not detectable by virus titration. In control non-transgenic mice, however, only positive-strand RNA was detected during the first 4 days p.i. This product probably reflects the virus inoculum, since we were unable to detect negative-strand RNA in the peritoneal cells of the control mice. Nested PCR of 10-fold dilutions of organs of PVRTg mice with known virus titres demonstrated that a virus titre of approximately $4 \times 10^1$ TCID$_{50}$ was detectable (data not shown).

Previous work suggested that human monocytes support poliovirus replication (Eberle et al., 1995). In addition,
murine peritoneal cells, elicited by thioglycollate, support poliovirus replication ex vivo (Freistadt & Eberle, 2000). Since thioglycollate activates macrophages (Carrick et al., 1995) we extended these findings ex vivo by demonstrating an increase in virus titre within 48 h, as well as the presence of negative-strand poliovirus RNA after poliovirus inoculation of normal (unstimulated) peritoneal cells from PVRTg mice (data not shown). More importantly, in our study we found not only that a high percentage of 70–90% of the peritoneal cells were macrophages (data not shown), but also that cells immunostained for poliovirus had the typical morphology of macrophages and expressed the macrophage/monocyte-specific marker M1/70. From the virus titrations, PCRs and immunolabelling results, we conclude that poliovirus does indeed replicate in peritoneal macrophages of PVRTg mice after i.p. inoculation.

Until now, little was known about other cell types supporting poliovirus entry and replication. In humans, the PVR is found on the majority of primary blood monocytes, but not on other blood cells (Freistadt et al., 1993), while in the mouse spleen a minority of T cells express the PVR (Freistadt & Eberle, 2000). Our results, however, demonstrated that poliovirus is able to infect mouse B cells, since all peritoneal cells immunostained for CD19 were positive for poliovirus. We could not detect any poliovirus-positive T cells in the population of peritoneal cells, which suggests that T cells are not responsible for harbouring, transporting or replicating poliovirus. Furthermore, we also isolated peritoneal T cells after poliovirus inoculation with anti-CD3 magnetic beads. In this CD3-positive population we could not detect poliovirus by immunostaining (data not shown). Our results are in agreement with the finding that human blood T cells do not support PV replication (Eberle et al., 1995; Shaw et al., 1995).

Poliovirus also replicated in the mesenteric lymph nodes and Peyer’s patches of PVRTg mice after i.p. inoculation of
poliovirus, as we showed by virus titration and by detection of negative-strand PCR. From these results we can conclude that macrophages and/or B cells infected with poliovirus in the peritoneal cavity are likely to be responsible for the transport of poliovirus to the mesenteric lymph nodes and the Peyer’s patches (Sminia et al., 1995). For instance, it has been documented that B-1 cells from the peritoneal cavity are able to migrate into the lamina propria (Kroese et al., 1989a, b; Murakami & Honjo, 1995). In support of the notion that macrophages and/or B cells transport virus is the finding that Peyer’s patches of PVRTg mice showed an increase in virus titre and the presence of viral negative-strand RNA at day 3 p.i., while negative-strand RNA in peritoneal cells and mesenteric lymph nodes was already present at day 1 p.i. As in peritoneal lavages, immunolabelling of both mesenteric lymph nodes and ileum indicated that poliovirus-positive cells had the morphology of macrophages. In the ileum of PVRTg mice we found poliovirus in macrophage-like cells in the lamina propria, suggesting further transport of poliovirus from the Peyer’s patches into the lamina propria. It has been recently described that, within the ileum of PVRTg mice, the poliovirus receptor is expressed nearly exclusively in the tunica muscularis of the Peyer’s patches (Iwasaki et al., 2002), while it is only expressed at a low level in the lamina propria. This makes it conceivable that poliovirus enters the intestinal wall via peritoneal cells and that this route of infection is responsible for the excretion of poliovirus found in the faeces.

Poliovirus replication in peritoneal macrophages may contribute to the pathogenesis of the disease by facilitating poliovirus uptake into the CNS by transport via the circulation (Clatch et al., 1990; Pulliam et al., 1995; Rossi et al., 1997; Sasseville & Lachner, 1997), or by retrograde transport of the virus via peripheral neurons – as described for humans, monkeys and transgenic mice (Ren & Racianello, 1992; Ohka & Nomoto, 2001). These ideas are supported by the fact that the virus was already detectable in brains from PVRTg mice at day 2 p.i.; this was also the case for the other non-lymphoid organs kidney and liver (data not shown). However, the mechanisms by which poliovirus enters the CNS remain controversial. Interestingly, poliovirus can also enter the CNS of non-transgenic mice via a non-receptor-mediated mechanism (Yang et al., 1997).

Replication of poliovirus in macrophages probably results in antigen presentation to specific B and T cells. 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 generating an IgA response. We conclude that replication of poliovirus in macrophages and B cells in the peritoneal cavity, mesenteric lymph nodes, Peyer’s patches cells and ileum is probably responsible for the induction of a virus-specific IgA response, as demonstrated in our earlier studies (Buisman et al., 2000; Coffin et al., 1999).

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