In Vitro Myelosuppressive Effects of the Parvovirus Minute Virus of Mice (MVMi) on Hematopoietic Stem and Committed Progenitor Cells

By Jose C. Segovia, Almudena Real, Juan A. Bueren, and Jose M. Almendral

The interaction of two strains of the parvovirus minute virus of mice (MVM) with the mouse hematopoietic system has been studied. The immunosuppressive strain MVMi, but not the prototype virus MVMp, inhibited hematopoiesis in vitro, as judged by colony-forming assays of the erythroid burst-forming unit and granulocyte-monocyte colony-forming unit (CFU-GM) progenitors. Interestingly, primitive hematopoietic cells of the stem compartment (CFU-S_total) were equally susceptible to the MVMi cytototoxic infection, unravelling an unprecedented feature of virus-hematopoiesis interactions. The replication of both strains of MVM virus was evaluated in primary myeloid cells of long-term bone marrow cultures. A high viral DNA synthesis and maturation was observed in MVMi-infected myeloid cells, but it was undetectable in MVMp infections; moreover, the expression of the cytotoxic nonstructural NS-1 protein, a more reliable parameter of cell permissiveness to MVM infection, was only detected in MVMi-infected cells. Correspondingly, MVMi was propagated to high titers of infectious virus and it mediated an acute myelosuppression in these cultures. We conclude that MVMi has a wider tropism than was previously suspected and it is proposed that cytotoxic infection of hematopoietic stem cells, besides that of committed progenitors, may provide an additional basis to understand the pathogenesis of certain animal and human bone marrow failures of viral etiology.
production) in myeloid cells of mice. To our knowledge, the results presented here are the first evidence showing that one virus is capable of cytotoxicity and also the hematopoietic stem cells. The implications of these data for virus tropism and for pathologic aspects in infection by these strains are discussed.

**MATERIALS AND METHODS**

**Cell lines and viruses.** MVMi and the T-cell lymphoma line EL-4 used to grow it were kindly provided by B. Hirt (Swiss Inst for Exp Cancer Res, Epalinges, Switzerland). MVMp, the A9 cell line of mouse fibroblasts used to propagate and assay this strain, and the human SV40 transformed fibroblast cell line NB324K for plaque assay MVMi, were generously supplied by J. Rommelaere (Institut Pasteur, Lille, France). Cells were cultured in Dubecco’s Modified Eagle Medium (DMEM; GIBCO Laboratories, Grand Island, NY) supplemented with 5% inactivated fetal bovine serum (FBS). Virus stocks were prepared by low-multiplicity infection (5 × 10<sup>-3</sup> plaque forming units [PFU]/cell) of the corresponding cell line. In all the experiments performed in this work we have used purified viruses banded in density gradients as described below.

**Virus titration and purification.** Reference titers for MVMp and MVMi viruses were obtained by respective plaque assays on A9 and NB324K indicator cell lines as described. To visualize the plaques, monolayers were fixed overnight with 10% formaldehyde and stained with 0.2% crystal violet. Virus titers were expressed as PFU per milliliter and the multiplicity of infection (MOI) as the number of PFU inoculated per cell. The plaque efficiency of the MVMp strain was between 10 to 20 times higher in NB324K than in A9 cell lines (not shown), in agreement with previously published data.

The virus purification method was based on published procedures. Briefly, cells were scraped off the plates directly into the medium, collected by low-speed centrifugation, and resuspended in 20 mmol/L Tris pH 8.5, 1 mmol/L EDTA (TE buffer). Intracellular virus was released by lysing cells with three freeze-thaw cycles, followed by centrifugation to remove debris. Extracellular virus was recovered from the medium by overnight precipitation with 3.4% PEG-6000, 0.5 mol/L NaCl at 4°C, and centrifugation at 8,000g for 30 minutes at 4°C. The virus was resuspended in TE buffer and pooled with intracellular virus. Clumps were disaggregated by gentle sonication, the suspension was made 5 mol/L in MgCl<sub>2</sub> and digested with 20 μg/mL DNaseI and RNaseA for 30 minutes at 37°C. The virus suspension was then adjusted to 1% sodium dodecyl sulfate (SDS), virus titration and purification. Reference titers for MVMp and MVMi viruses were obtained by respective plaque assays on A9 and NB324K indicator cell lines as described. To visualize the plaques, monolayers were fixed overnight with 10% formaldehyde and stained with 0.2% crystal violet. Virus titers were expressed as PFU per milliliter and the multiplicity of infection (MOI) as the number of PFU inoculated per cell. The plaque efficiency of the MVMp strain was between 10 to 20 times higher in NB324K than in A9 cell lines (not shown), in agreement with previously published data. The virus purification method was based on published procedures. Briefly, cells were scraped off the plates directly into the medium, collected by low-speed centrifugation, and resuspended in 20 mmol/L Tris pH 8.5, 1 mmol/L EDTA (TE buffer). Intracellular virus was released by lysing cells with three freeze-thaw cycles, followed by centrifugation to remove debris. Extracellular virus was recovered from the medium by overnight precipitation with 3.4% PEG-6000, 0.5 mol/L NaCl at 4°C, and centrifugation at 8,000g for 30 minutes at 4°C. The virus was resuspended in TE buffer and pooled with intracellular virus. Clumps were disaggregated by gentle sonication, the suspension was made 5 mol/L in MgCl<sub>2</sub> and digested with 20 μg/mL DNaseI and RNaseA for 30 minutes at 37°C. The virus suspension was then adjusted to 1% sodium dodecyl sulfate (SDS), virus titration and purification. Reference titers for MVMp and MVMi viruses were obtained by respective plaque assays on A9 and NB324K indicator cell lines as described. To visualize the plaques, monolayers were fixed overnight with 10% formaldehyde and stained with 0.2% crystal violet. Virus titers were expressed as PFU per milliliter and the multiplicity of infection (MOI) as the number of PFU inoculated per cell. The plaque efficiency of the MVMp strain was between 10 to 20 times higher in NB324K than in A9 cell lines (not shown), in agreement with previously published data. The virus purification method was based on published procedures. Briefly, cells were scraped off the plates directly into the medium, collected by low-speed centrifugation, and resuspended in 20 mmol/L Tris pH 8.5, 1 mmol/L EDTA (TE buffer). Intracellular virus was released by lysing cells with three freeze-thaw cycles, followed by centrifugation to remove debris. Extracellular virus was recovered from the medium by overnight precipitation with 3.4% PEG-6000, 0.5 mol/L NaCl at 4°C, and centrifugation at 8,000g for 30 minutes at 4°C. The virus was resuspended in TE buffer and pooled with intracellular virus. Clumps were disaggregated by gentle sonication, the suspension was made 5 mol/L in MgCl<sub>2</sub> and digested with 20 μg/mL DNaseI and RNaseA for 30 minutes at 37°C. The virus suspension was then adjusted to 1% sodium dodecyl sulfate (SDS), 5 mmol/L NaCl and centrifuged 18 hours at 16 Krpm and 15°C in a Beckman SW40 rotor (Beckman Instruments, Inc, Fullerton, CA) through a 20% sucrose cushion in 50 mmol/L Tris pH 8.0, 0.1 mol/L NaCl, 1 mmol/L EDTA, and 0.2% SDS. The virus pellet was resuspended in TE buffer plus 0.2% Sarkosyl, adjusted to a density of 1.38 g/mL in CsCl, and centrifuged at equilibrium for 42 hours at 48 Krpm and 5°C in a Beckman Ty56 rotor (Beckman Instruments). Gradients were fractionated and the virus protein distribution was determined by hemagglutination. Leading fractions of infectious virus, free of defective particles were pooled, dialyzed against TE buffer, and stored at 4°C as reference stocks of MVMp and MVMi viruses.

**BM cells and MVM infections.** BM cells were obtained by flushing Iscove’s Modified Dulbecco’s Medium (IMDM; GIBCO) through the shaft of the femora and tibiae of at least three mice. Cells were dispersed with a 16 × 5 mm needle and the number of nucleated cells was determined in a ZM Counter (Coulter Electronics, Luton, UK).

When hematopoietic cells, essentially free from MVMi-susceptible T cells and MVMp potentially susceptible fibroblastic stromal cells were needed, nonadherent cells were collected from long-term BM cultures (LTBMCs) established 3 weeks earlier in Fischer’s medium plus 20% horse serum (HS), 10<sup>−6</sup> mol/L hydrocortisone, and antibiotics and fed on a weekly basis. Before performing the infection of these nonadherent cells, they were further incubated for 24 hours in the presence of 10% WEHI-3b as a source of stimulating factors to facilitate the virus progression in the infected cells.

Infection of BM or stimulated nonadherent LTBMC cells with MVM was performed at a concentration of 1 to 2 × 10<sup>5</sup> cells/mL in IMDM with 2% FCS for 1.5 hours at 37°C and constant shaking (similar results could be obtained in a 4°C infection, data not shown). Afterwards, cells were washed with IMDM plus 10% FCS to remove most adsorbed virus and resuspended in IMDM to perform further clonal assays of the BM progenitors, or in medium supplemented with 10% FBS and 10% WEHI-3b to analyse the virus growth in the myeloid LTBMC cells.

**Myeloid progenitor cell assays.** Hematopoietic progenitor cells committed to the granulocyte/monocyte (CFU-GM) or erythroid lineage (BFU-E) were assessed in semisolid cultures, essentially as described. The culture media consisted of IMDM, 30% FBS, 1% donorized bovine serum albumin (Fraction V, Sigma Chemical Co, St Louis, MO), 10% WEHI-3b Conditioned medium, 0.8% methylcellulose (Dow Chemical Co, Midland, MI), 2 U/mL of erythropoietin (Terry Fox Laboratory, Vancouver, Canada), 10<sup>−5</sup> mol/L α-thioglycerol, 2 mmol/L L-glutamine and 10<sup>−7</sup> mol/L Na<sub>2</sub>SeO<sub>3</sub>. Nucleated cells (10<sup>5</sup>) in a final volume of 0.9 mL were dispensed in three wells of a multowell culture dish (24-well Costar plate, Costar Corp, Cambridge, MA) and colonies were scored under an inverted microscope 7 days after incubation at 37°C in a 1% 5% CO<sub>2</sub> humidified atmosphere.

**Assessment of mouse hematopoietic stem cells.** For the determination of hematopoietic precursors of the stem compartment, exogenous spleen colony-forming unit (CFU-S) assays were performed, under conditions allowing the expression of primitive CFU-S. Recipient mice were total-body irradiated with 10.5 Gy in fractions of 5.0 Gy and 5.5 Gy spaced 4 hours apart, with a Philips MG332 X-ray equipment (Philips GmbH, Hamburg, Germany) at 300 kV, 12.8 mA (HVL:3.2 mmCu) delivering a dose rate of 1.05 Gy/min. This irradiation regimen totally ablated the endogenous CFU-S that originates colonies on day 12 and allowed the survival of about 90% recipient mice. An appropriate number of BM cells to obtain about 10 colonies per spleen was injected into the recipients through the lateral tail vein. At day 12 after transplantation, mice were killed, their spleens excised, fixed in Tely’s solution, and the number of colonies representing the CFU-S<sub>100</sub> counted under a dissecting microscope.

**Spleen-seeding efficiency of colony-forming cells.** The spleen-seeding efficiency (f) of the spleen colony forming cells (CFC-S) from normal and MVMi-infected BM, was measured by the retransplantation technique, as modified as described. Cells, 4 × 10<sup>5</sup>, from mock-infected BM or 1.6 × 10<sup>5</sup> BM cells infected with MVMi at MOI 1 PFU/cell, were injected into each recipient for the CFU-S<sub>100</sub> assay (10 recipients per group). Two parallel groups of five primary recipient mice were injected with 100 times more cells
(4 × 10⁶ uninfected, 1.6 × 10⁷ MVMi-infected cells), killed 24 hours later, and spleen cell suspensions made in Hanks' solution. Fractions of one fifth and one twentieth of each spleen were injected into groups of 10 secondary recipients for further CFU-S assay. The spleen-seeding efficiency was calculated from the ratio of secondary colonies obtained to primary CFU-S injected.

Viral DNA and protein analyses. Nonadherent myeloid cells from LTBMCs, A9, or EL-4 cells were infected at MOI 4 PFU/cell with MVMP or MVMi. At the indicated hours postinfection (hpi) cells were pelleted, total protein extracts were prepared, and MVM replicative intermediates were purified by a modified Hirt procedure, with carrier tRNA to ensure quantitative yields. DNA was fractionated by agarose gel electrophoresis, blotted to nylon membranes (GeneScreen-plus; Du Pont, New England Nuclear, Boston, MA), and hybridized with MVMP DNA (very similar in sequence to MVMi) gel-purified from a recombinant clone containing the entire virus genome (J.C. Ramirez and J.M. Almendral, unpublished results). The probe was labeled by random-priming to a specific activity of 1 to 2 × 10⁹ cpm/μg and hybridized overnight at 42°C in 50% formamide, 5X SSC (1X SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate), 5 × Denhardt solution, 10% dextran sulphate (Pharmacia Fine Chemicals, Uppsala, Sweden), 1% SDS, and 200 μg of denatured salmon sperm DNA/mL. Final washing of filters was at 56°C in 0.1X SSC, 0.5% SDS. Kodak X-Omat films (Eastman Kodak, Rochester, NY) were exposed to the filters with an intensifying screen at -70°C. Proteins were polyacrylamide gel electrophoresis (PAGE) fractionated, electroblotted to nitrocellulose filters, and detected with antibodies and a color reaction with peroxidase following conventional procedures. Antiserum against purified MVMP capsid proteins (serologically undistinguishable from MVMi) was raised in rabbit and NS-1-specific antiserum was obtained by immunizing mice with a purified NS-1/Bgal fusion protein (to be described elsewhere).

RESULTS

Susceptibility of murine committed myeloid progenitors to MVMp and MVMi infection. To assess the effects of MVM infection on the proliferation and differentiation capacity of hematopoietic progenitors, BM cells were infected with purified MVMi or MVMP viruses and thereafter cultured in vitro to allow the expression of the CFU-GM and BFU-E progenitors. Because the susceptibility of cell lines to parvovirus infection is defined in terms of cell viability in relation to input virus, the infection was performed at different MOI and the percentage of these progenitors with proliferative capacity of the multipotential spleen colony forming cell CFU-S that represents one of the earliest progenitors of the stem cell compartment might be potential target cells for MVMi or MVMP infections. Therefore, we have analyzed the effect of MVMi or MVMP infection at different MOI on the proliferative capacity of the multipotential spleen colony forming cell CFU-S, that represents one of the earliest progenitors of the stem cell compartment for which a clonal assay is available. Figure 3 shows that the number of CFU-S decreased in a dose-related manner to the infectious MVMi input and with similar kinetics to that found in the committed progenitor assays, which suggests a similar susceptibility of the CFU-S to MVMi, CFU-GM, and BFU-E to the MVMi infection. Here again, a slight reduction in the CFU-S numbers (up to 80% survival) were observed at the higher MOIs of MVMP.

To further support that this inhibition of in vitro hematopoiesis is a direct consequence of the MVMi infection of the progenitors, the viability of these cells was assessed after neutralizing the virus infectivity with antiserum raised against purified MVMi capsids. Figure 2 shows that the inhibitory effect of MVMi was completely abrogated when the virus was preincubated with anti-MVMi serum, but not with the control serum. This result confirmed that MVMi infectivity is responsible for the inhibition of the colony formation ability of the CFU-GM and BFU-E progenitors.

Susceptibility of mouse hematopoietic stem cells to MVM infection. Because parvovirus multiplication relies on cellular functions expressed at appropriate differentiation states, other hematopoietic progenitors like those of the stem cell compartment might be potential target cells for MVMi or MVMP infections. Therefore, we have analyzed the effect of MVMi or MVMP infection at different MOI on the proliferative capacity of the multipotential spleen colony forming cell CFU-S that represents one of the earliest progenitors of the stem cell compartment for which a clonal assay is available. Figure 3 shows that the number of CFU-S decreased in a dose-related manner to the infectious MVMi input and with similar kinetics to that found in the committed progenitor assays, which suggests a similar susceptibility of the CFU-S to MVMi, CFU-GM, and BFU-E to the MVMi infection. Here again, a slight reduction in the CFU-S numbers (up to 80% survival) were observed at the higher MOIs of MVMP.
IN VITRO MVMi MYELOSUPPRESSION

Fig 2. Reversion of the MVMi myelosuppressive effect with neutralizing MVM antiserum. BM cells were infected at 4 PFU/cell with MVMi preincubated for 1 hour at 37°C with serial dilutions of either nonimmune rabbit serum (open symbols), or a hyperimmune antipurified MVM(p) capsids serum (solid symbols). Samples were diluted and centrifuged to remove unadsorbed virus and antibody, and semisolid cultures of CFU-GM (○) and BFU-E (△) progenitors were performed.

producing splenic colonies, either in mock-infected and in MVMi-infected (1 PFU/cell) BM. As shown in Table 1, the seeding efficiency of surviving MVMi-infected CFC-S was similar to that obtained for uninfected BM, what conduces to a CFC-S survival fraction analogous to that observed for CFU-S (Fig 3).

Macromolecular parameters of MVMi and MVMp growth in myeloid cells of mice. As the susceptibility of hematopoietic progenitors and stem cells to MVMi correlated with the input of infectious virus, one would expect this inhibition of proliferation to be a consequence of cytotoxic mechanisms underlying MVMi replication and not operating in MVMp-infected cells. To test this hypothesis and to determine the myeloid cells permissiveness to the replication of both MVM strains, we monitored the extension of two macromolecular parameters of virus growth in infected nonadherent cells from LTBMCs.

Myeloid cultures were infected at MOI 4 PFU/cell with MVMi or MVMp to ensure the infection of most cells and viral DNA replication and the synthesis of the nonstructural protein NS-1 were studied at early and late times of the viral cycle (5 and 17 hpi, respectively). In parallel, reference infections of the respective MVMi and MVMp permissive and restrictive cell lines were performed. Southern blots of Hirt supernatants of infected cultures showed (Fig 4A) a high DNA synthesis of intermediate replicative forms (RF1 and RF2) as well as single stranded mature viral DNA (ss DNA) at late time of the viral cycle in MVMi-infected myeloid cells that were not detected in overexposed films of MVMp infections, where only an ss DNA band of the high-input virus genome was seen at 5 hpi. As previously established, infection of permissive cell lines led to high DNA replication of the corresponding strain of MVM (EL-4 infected by MVMi and A9 by MVMp) while infections of restrictive cells led to poor replications of viral DNA (EL-4 infected by MVMp and A9 by MVMi).

The expression of the nonstructural NS-1 protein was chosen as a second and main parameter of MVM multiplication in myeloid cells, given that NS-1 is a multifunctional nuclear phosphoprotein involved in several critical steps of virus cycle. Immunoblots developed with specific NS-1 antiserum showed (Fig 4B) that NS-1 expression was high at late time of the viral cycle in MVMi-infected myeloid cells as well as in MVMp and MVMi infections of the respective permissive cell line. Nevertheless, NS-1 expression was not detected in restrictive infections and in myeloid cells infected by MVMp. Therefore, these data demonstrate that mouse myeloid cells are permissive to MVMi NS-1 expression as well as to viral DNA replication and maturation, but not significantly to any of these parameters of MVMp growth.

Propagation of MVMi in myeloid cells of mice in culture. We further analyzed whether the permissiveness of myeloid cells to MVMi gene expression and DNA replication yields infectious virus and whether the virus is subsequently able to propagate the infection in myeloid cells. With this aim, nonadherent LTBMC myeloid cells were infected at low
MOI (0.5 PFU/cell) with MVMi or MVMp and the number of viable cells, proliferatively viable CFU-GM and BFU-E progenitors and the virus titers, were quantified twice daily over the time. The results of a representative experiment are shown in Fig 5. Cultures inoculated with MVMi showed a marked reduction in the cellularity with time beyond 48 hpi (Fig 5A) that was not manifested in uninfected or MVMp-infected cultures. Earlier and more pronounced responses were observed at the progenitors level; the number of CFU-GM (Fig 5B) and BFU-E (Fig 5C) cells decreased sharply beyond 24 hours post-MVMi infection, but again no effect was observed in MVMp-infected cultures. Finally, MVMi titer increased up to 100-fold in the culture, with a production rate concomitant to the progressive progenitors depletion (Fig 5D). These results demonstrate that MVMi is highly propagated in myeloid cells in culture. On the contrary and in agreement with the lack of cytotoxic infection in BM progenitors and NS-1 expression in myeloid cells, only a slow increase in MVMp titer was observed that did not correspond to the myeloid cells’ depletion.

**DISCUSSION**

The experiments presented in this report were conducted to investigate the characteristics of the interaction between murine hematopoietic progenitors and two strains of the parvovirus MVM in terms of cell susceptibility and virus gene expression and multiplication.

Our results demonstrate that, under the assayed conditions of infection, the proliferative capacity of committed progenitors (BFU-E and CFU-GM) and pluripotent stem cells (CFU-S), is susceptible to MVMi virus. This effect of MVMi is inherent to virus infectivity as was mediated by purified virus, was reversible with neutralizing MVM antisera, and correlated with the input of infectious virus. The

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**Table 1. Spleen-Seeding Efficiency of Mock- and MVMi-Infected CFC-S**

<table>
<thead>
<tr>
<th>Cells</th>
<th>CFU-S/10⁵ cells</th>
<th>Spleen-Seeding Efficiency (fl CFU-S/10⁵ cells)</th>
<th>CFC-S/10⁵ cells</th>
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<tr>
<td>Mock-infected BM</td>
<td>22.9 ± 1.8</td>
<td>0.057 ± 0.006</td>
<td>401.7 ± 52.8</td>
</tr>
<tr>
<td>MVMi-infected BM (1 PFU/cell)</td>
<td>5.2 ± 0.4 (22.7)</td>
<td>0.046 ± 0.005</td>
<td>113.0 ± 15.1 (28.1)</td>
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Results are shown as mean ± SE. Values in parenthesis indicate percent of survival.

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**Fig 4. Analysis of MVMp and MVMi replication in cell lines and myeloid cells. A9, EL-4, or stimulated nonadherent myeloid cells (MC) of LTBMCs were infected at 4 PFU/cell with MVMp or MVMi and 5 and 17 hpi DNA and protein samples were prepared. (A) Hirt supernatants of 8 x 10⁴ A9 cells, 5 x 10⁴ EL-4 cells, or 2 x 10⁴ myeloid cells loaded per gel lane, electrophoresed on 1% agarose gel, blotted, and hybridized with an MVM ³²P-DNA probe as described in Materials and Methods. Exposure was for 8 hours with intensifying screen for MC and EL-4 and without screen for A9. The positions of the intermediate replicative forms (RF1, RF2) and the single-stranded viral DNA (ss), are indicated. MVMi, viral genomes isolated from purified virions. (B) Western blot of extracted proteins from 10 times more cells than in (A), incubated with NS-1 antiserum. The clasp indicates the position of the normal and hyperphosphorylated protein forms. (i, MVMi infection; p, MVMp infection; 5, 5hpi; 17, 17hpi; c, uninfected cells).**
proliferative inactivation kinetics of these precursors were in fair accord with statistical predictions of the Poisson distribution for cell-virus interactions, suggesting a direct cytotoxic infection of these precursors and that the MVMi-infective unit be the same for BFU-E, CFU-GM, and CFU-S as well as for NB324K cells, currently the most susceptible cell line used to titrate the MVMi. Although other mechanisms like the reinfection of developing colonies in the CFU-S assays could mediate and modulate in part the observed effects, the *in vitro* incubation of BM cells 4 hours postinfection with mouse-neutralizing MVM antisera, or the periodical injection of this antiserum into recipients of the CFU-S assay, did not vary the number of spleen colonies (not shown). Other possible misleading effects on the CFU-S susceptibility curve, like a selective decrease in the seeding efficiency of infected CFC-S, was also ruled out by the finding of a seeding efficiency (Table 1) close to reported values that implies a CFC-S survival analogous to that obtained for CFU-S at the same MOI (Fig 3).

In marked contrast to the MVMi effect, hematopoietic progenitors and stem cells were not susceptible to MVMp. The slight reduction in viability observed at high MOI of MVMp virus is analogous to that found in infected resistant cells that do not support the replication of parvovirus, and it could be by means of a cell cycle perturbation mechanism mediated by high concentration of incoming virus particles. This MVMp cytopathogenicity at high MOI is even less significant if the MVMp titer obtained in NB324K instead of A9 cells is considered because then we would have been using 10 to 20 times more infectious MVMp than MVMi virus.

MVMi, but not MVMp, replicated DNA and expressed detectable levels of the nonstructural protein NS-1 in infected myeloid cells from LT-BMCs. DNA replication has been shown to occur at substantial levels even in nonpermissive cells, independently of protein production, gene expression, and cell sensitization to the parvovirus attack. However, the nonstructural NS-1 polypeptide possesses several roles in the MVM life cycle, like viral DNA replication and maturation, transcriptional regulation of the P38 promoter that governs capsid proteins synthesis and of its own P4 promoter and likely in viral DNA packaging. Therefore, its expression in myeloid cells is a good indicator of true virus replication in this system. Moreover, parvoviral nonstructural proteins are suspected of being cytotoxic for cell lines in vitro, and their expression increase viral cytopathic effects and cell killing. Thus, the inhibition of colony formation by MVMi infection may be a consequence of cell permissiveness to
the onset of parvovirus gene expression and NS-1 induction that would operate in MVMi-infected mouse hematopoietic progenitors and stem cells.

The MVMi infection of myeloid cells from LTBMs shown in Fig 5 demonstrates a progressive decrease in the proliferative capacity of progenitor cells concomitant with infectious virus production in the cultures; meanwhile the total cell number is only brought down some days later. As expected by the above experiments, none of these effects were observed in MVMp infection and the virus was not appreciably propagated in this system. The ninefold increase of MVMp titer may rather be a consequence of a low virus replication in some residual stromal fibroblastic cells that were progressively apparent in these cultures.

Taken together, these experiments demonstrate that the strain MVMi, previously described as immunosuppressive, is highly cytotoxic to the myeloid system. So, in addition to its ability to inhibit various functions of T cells in vitro, the myelotropism should be considered now a relevant property of MVMi biology, characterized by a wide myelosuppressive potential within the hematopoietic system that includes erythroid and granulomacrophage progenitors as well as cells of the stem compartment. Thus, the term MVMim could be a more accurate designation for this strain.

Many viruses infecting hematopoietic cells of animals and humans and causing BM failure by diverse mechanisms have been described. Flaviviruses, like dengue virus, propagate in human BM cells. Although not cytopathic, this virus alters the morphology of the colony and slows cell proliferation. Hematopoietic precursors were shown to be decreased in humans and simians infected with immunodeficiency retrovirus, and indirect manifestations of no cytopathic macrophage infection or immune-mediated suppression, were proposed. Other retroviruses, the feline leukemia virus, in addition to immunodeficiency and proliferative diseases, causes rapid depletion of early erythroid precursors when injected into cats. Viruses with liver tropism, like the mouse hepatitis virus (MHV-3) or hepadna viruses, are associated with suppression of hematopoiesis and the hepatitis B virus inhibits colony formation of human hematopoietic progenitor cells. Myelosuppression mediated by cytomegalovirus implies the perturbation of stromal cells functions that support hematopoiesis, as well as a direct effect on myeloid cells. Other herpes viruses may incite a host cytotoxic immune response against hematopoietic cells. Panleukopenia and erythropaenia leading to eventual aplastic crises have been described in human and animals as a direct consequence of parvovirus cytotoxic infection, but to our knowledge this report on MVMi infection is the first evidence of a cytotoxic interaction of a virus with a hematopoietic pluripotent progenitor with self-renewing capacity. Our data provide new insights to explain BM failures in animals and humans infected by parvoviruses. Effects on the stem cell compartment may unleash pathologic manifestations and clinical symptoms that are the consequences of impaired myelopoiesis and lymphopoiesis or BM aplasia in immunocompromised hosts.

The susceptibility to MVMi infection by the CFU-S stem cell may be regarded as an unexpected result because these cells have a proliferative rate that is characteristically low in normal healthy mice, and cell proliferation is essential both for parvovirus DNA replication and for the expression of cytotoxic proteins in the infected cells. In our experiments, although hematopoietic stem cells should be quiescent when exposed to MVMi, they were further stimulated to proliferate when transplanted into lethally irradiated mice to assess the CFU-S precursor. This conditional susceptibility of these cells opens the possibility that the stem compartment may be a reservoir of parvovirus under normal conditions that would be manifested when such cells were triggered into cycle in response to hematologic damage or physiologic requirements. Latent or persistent infections would have epidemiologic relevance as a mechanism of parvovirus permanence in nature, as well as in pathologic processes like chronic BM failures of inapparently infected immunodepressed patients. Experiments are currently in progress to evaluate these hypotheses in vivo in the MVMi-mouse model.

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