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Human Parvovirus B19 Can Infect Cynomolgus Monkey Marrow Cells in Tissue Culture

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The human pathogenic parvovirus B19 cannot be grown in standard tissue culture but propagates in human bone marrow, where it is cytotoxic to erythroid progenitor cells. We now show that parvovirus B19 can replicate in cynomolgus bone marrow. Cynomolgus monkeys may be a suitable animal model for pathogenesis studies of parvovirus B19.

Parvovirus B19, the only pathogenic member of the *Parvoviridae* family known to cause diseases in humans, is selectively tropic for human erythroid progenitor cells (4). Viral tropism is mediated by the cellular receptor for parvovirus B19, the neutral glycolipid globoside (blood group P antigen) (1). Globoside present on P antigen-positive erythrocytes mediates B19 virus hemagglutination (HA), and in bone marrow globoside mediates attachment of virus to the cell surface and is therefore necessary for viral infection. We have previously shown that P antigen-negative erythrocytes cannot be hemagglutinated (1) and that human bone marrow cells that do not have globoside on the cell surface are resistant to parvovirus B19 infection (3).

Globoside is also present on the erythrocytes of some nonhuman primates (10), and we hypothesized that the presence of globoside on cells of erythroid lineage of different species of primates might make these cells susceptible to parvovirus B19 infection. We therefore performed parvovirus B19-mediated HA (2) on a variety of primate erythrocytes. Human or nonhuman primate erythrocytes were collected in Alsevier's solution, washed three times, resuspended in saline solution at 0.5% (vol/vol), and tested with the HA assay (1). Baculovirus-expressed recombinant parvovirus B19 capsids were used as the antigen (7). HA at high titer was observed with erythrocytes from humans (P antigen positive; HA titer, 1:128,000), baboons (1:256,000), and rhesus (1:256,000) and cynomolgus (1:1,024,000) monkeys; no HA was shown with chimpanzee erythrocytes. Cynomolgus cells showed the highest HA titer and were chosen for further studies.

Cynomolgus bone marrow cells were tested for the presence of globoside by immunofluorescent flow cytometric analysis. On staining with anti-P mouse monoclonal antibody, about 3% of cynomolgus bone marrow cells were positive for P antigen, similar to the number of cells positive for P antigen in human bone marrow (data not shown). We therefore tested cynomolgus marrow for its ability to support B19 virus infection.

B19 virus infection of human erythroid progenitor cells produces a cytotoxic effect that can be assayed as the selective inhibition of formation of erythroid colonies in methylcellulose cultures, with sparing of myeloid colonies (8). Inhibition of erythroid colony formation was therefore used to assay parvovirus B19 infection in cynomolgus bone marrow cells. Bone marrow was obtained from human volunteers after informed consent was obtained according to a protocol approved by the National Heart, Lung, and Blood Institute Institutional Review Board and from cynomolgus monkeys by aspiration into preservative-free heparin and hematopoietic colony culture assays performed as previously described (8). Late erythroid progenitor cell (CFU-E) colony formation was assayed by microscopic examination at 7 days, and early erythroid progenitor cell (BFU-E)- and granulocyte-macrophage (CFU-GM)-derived colonies were assayed at 10 days. As with human bone marrow, parvovirus B19 infection of cynomolgus monkey bone marrow produced marked inhibition of erythroid colony formation (BFU-E- and CFU-E-derived colonies) but no inhibition of myeloid colony formation (Table 1).

We also performed in vitro infection experiments to see whether parvovirus B19 could replicate in bone marrow mononuclear cells from cynomolgus monkeys. Mononuclear cells from human and cynomolgus bone marrow were obtained as described above and cultured in Iscove's modified Dulbecco's medium–20% fetal calf serum with 10 U of erythropoietin per ml and 100 U of interleukin-3 per ml at a starting concentration of 10^6 cells per ml. For infection, 5 μl of viremic serum (10^9 viral genomes per liter) were added to 1 ml of cultured cells. The cells were harvested at 2 or 48 h after inoculum and washed in phosphate-buffered saline to remove nonadherent virus. Harvested bone marrow cells were lysed by sodium dodecyl sulfate-proteinase K treatment, total DNA was purified by phenol-chloroform extraction and ethanol precipitation (9), and the DNA was then quantitated by determination of absorbance.

The amount of parvovirus B19 DNA present in samples was measured by dot blot hybridization. Tenfold dilutions of normalized amounts of samples were immobilized on a nylon membrane (Hybond N+; Amersham) by filtration, using a dot blot apparatus. Viral DNA was hybridized to a digoxigenin-labelled (DIG DNA labelling kit; Boehringer Mannheim) pYT103 insert probe, an almost full-length clone of parvovirus B19 DNA, and the probe was detected by anti-digoxigenin alkaline phosphatase and Lumi-Phos 530 (Boehringer Mannheim) chemiluminescent detection. There was a significant increase in the amount of parvovirus B19 DNA present at 48 h postinfection as compared with that present at 2 h postinfection in the infected cynomolgus cell cultures (Fig. 1). The increase of 2 to 3 orders of magnitude was analogous to that usually seen with infected human cell cultures.

Southern blot analysis of the DNA purified from both cynomolgus and human cells at 48 h also indicated parvovirus B19

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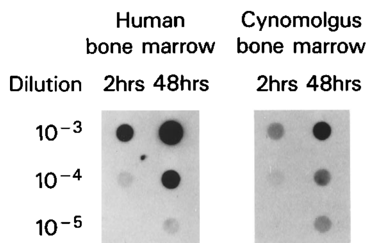


FIG. 1. Dot blot analysis of DNA purified from human and cynomolgus parvovirus B19-infected bone marrow cells at 2 and 48 h postinfection.

DNA replication. Purified DNA was cleaved with the restriction endonuclease *Eco*RI (no cuts in the parvovirus B19 genome) or *Bam*HI (a single cut in the parvovirus B19 genome), the fragments were separated by electrophoresis in a 1% agarose gel and transferred to a nitrocellulose membrane (Nytran; Schleicher and Schuell) by alkaline downward blotting (5), and parvovirus B19 DNA was detected as described above. Parvovirus B19 double-stranded DNA monomer and dimer and single-stranded DNA were present in both the human and cynomolgus marrow cultures. *Bam*HI restriction endonuclease cleavage of the double-stranded DNA replicative intermediates showed distinct doublets of terminal-extended and turnaround forms (Fig. 2), characteristic of the parvovirus B19 replicative process.

The infected cultures were also analyzed by in situ hybridization for the presence of parvovirus B19 in infected cells and by indirect immunofluorescence for the production of parvovirus B19 capsid proteins. Cells were harvested 2 days postinoculum, and in situ hybridization and immunofluorescence were performed as previously described (3, 6). Both viral nucleic acids (Fig. 3A) and capsid proteins (Fig. 3B) could be detected in the nuclei and cytoplasm of infected cells, in a

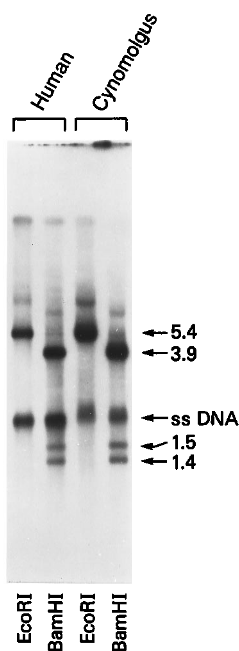
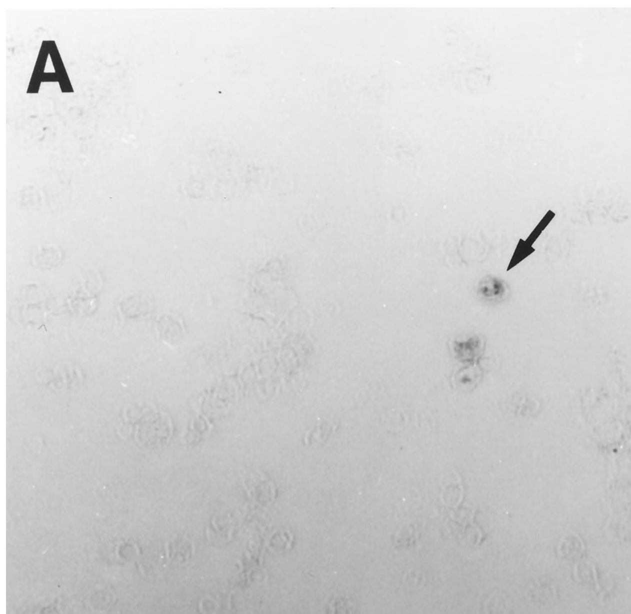


FIG. 2. Southern blot analysis of DNA purified from human and cynomolgus parvovirus B19-infected bone marrow cells at 48 h postinfection and cleaved by *Eco*RI or *Bam*HI restriction enzymes. ssDNA, single-stranded DNA.



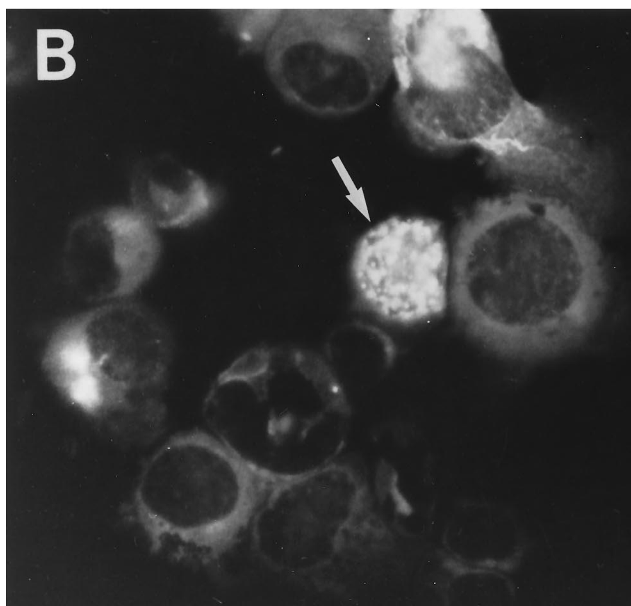


FIG. 3. (A) In situ hybridization of parvovirus B19 nucleic acids (low magnification); (B) immunofluorescence detection of parvovirus B19 capsid proteins in infected cynomolgus bone marrow cells (high magnification). Arrows point to parvovirus B19-infected cells.

pattern similar to that observed in infected human bone marrow cells, indicating replication and expression of the parvovirus B19 genome within the cynomolgus bone marrow cells.

Our observations show that parvovirus B19 replication is not restricted to human tissue; the virus appears to be capable of replication in nonhuman primate cells, specifically, cynomolgus erythroid progenitor cells. Parvovirus B19, to date, has not been shown to infect any cells apart from human erythroid cells, and studies of its pathogenesis have therefore been limited. The in vitro infection of cynomolgus bone marrow cells raises the possibilities of achieving experimental infection in cynomolgus monkeys and developing an animal model for further study of parvovirus B19 infection.

TABLE 1. Inhibition of hematopoietic colony formation with parvovirus B19

Monkey no.	No. of colonies[a]					
	CFU-E		BFU-E		CFU-GM	
	No virus (control)	Parvovirus B19	No virus (control)	Parvovirus B19	No virus (control)	Parvovirus B19
5685	29 ± 3	1 ± 0	7 ± 1	0 ± 0	59 ± 4	79 ± 0
5789	59 ± 1	11 ± 1	13 ± 4	0 ± 0	94 ± 9	84 ± 0
7706	20 ± 4	6 ± 1	9 ± 1	0 ± 0	41 ± 9	41 ± 2
7886	28 ± 5	1 ± 1	7 ± 0	0 ± 0	99 ± 1	77 ± 0

[a] Data are means ± standard deviations.

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