Human parvovirus B19: a mechanistic overview of infection and DNA replication

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ABSTRACT Human parvovirus B19 (B19V) is a human pathogen that belongs to genus *Erythroparvovirus* of the *Paroviridae* family, which is composed of a group of small DNA viruses with a linear single-stranded DNA genome. B19V mainly infects human erythroid progenitor cells and causes mild to severe hematological disorders in patients. However, recent clinical studies indicate that B19V also infects nonerythroid lineage cells, such as myocardial endothelial cells, and may be associated with other disease outcomes. Several cell culture systems, including permissive and semipermissive erythroid lineage cells, nonpermissive human embryonic kidney 293 cells and recently reported myocardial endothelial cells, have been used to study the mechanisms underlying B19V infection and B19V DNA replication. This review aims to summarize recent advances in B19V studies with a focus on the mechanisms of B19V tropism specific to different cell types and the cellular pathways involved in B19V DNA replication including cellular signaling transduction and cell cycle arrest.

KEYWORDS • B19V • cell cycle • DDR • DNA damage response • DNA replication • Epo/EpoR signaling • human parvovirus B19 • hypoxia • tropism

Human parvovirus B19 (B19V) was discovered in 1975 by Cossart and colleagues when screening for hepatitis B virus in a panel of human serum samples [1]. The virus was described as 23 nm in diameter, a typical capsid size of a parvovirus. The virus came from the serum sample coded as panel B number 19, and thereafter was named ‘Parvovirus B19.’ Most commonly, B19V infection causes erythema infectiosum or fifth disease (also named ‘slapped cheek syndrome’), which was first identified by Anderson et al. in the early 1980s [2]. In addition to infections in children, B19V is also highly infectious and spreads easily among adults with a seropositive rate ranging from 60 to 90% [3]. It causes a spectrum of clinical complications in vulnerable populations, including arthropathy in healthy adults (particularly in middle-aged women), persistent anemia in immunosuppressed patients, transient aplastic crisis in patients with increased erythropoiesis (such as sickle-cell disease patients), and hydrops fetalis and congenital anemia in pregnant women [4–6]. Noteworthy, in addition to these common manifestations, accumulating clinical reports indicate that B19V infection is associated with cardiovascular diseases, for example, myocarditis, in both adults and children [7–12]. The association of B19V infection to several liver diseases, for example, acute and chronic hepatitis, acute fulminant liver failure and autoimmune hepatitis, has been also reported [13–16]; however, only erythroid progenitor cells from fetal liver have been shown permissive to B19V replication [17]. And, therefore, the mechanism for B19V infection of liver cells will not be discussed in this review.

B19V is a member of the *Erythroparvovirus* genus within the *Parovirinae* subfamily of the *Paroviridae* family [18]. It has a linear ssDNA genome of 5596 nucleotides. It is flanked by two identical inverted terminal repeats (ITRs) that form an imperfect palindrome at each end (Figure 1A).
Figure 1. Structure of the human parvovirus B19 genome and the human parvovirus B19 genetic map. (A) Schematic diagram of the minus strand of the B19V ssDNA genome. Identical ITRs are present at each end of the genome, and these are depicted with unpaired or mismatched bases in the palindromes represented by ‘bulges’ or ‘bubbles,’ respectively. (B) Schematic diagrams of the duplex RF of the B19V genome (B19V J35 strain; GenBank accession number: AY386330), which has the capability to express viral genes, replicate and produce progeny virions. The left- and right-hand ITRs (L-ITR and R-ITR), P6 promoter, RNA initiation site, splice donors (D1 and D2), splice acceptors (A1-1, A1-2, A2-1 and A2-2) and proximal and distal polyadenylation sites ((pA)p1/2 and (pA)d) are indicated, along with the nine major mRNAs (R1-9) and three minor mRNAs (R1'-3'). The numbers of nucleotides (nt) are indicated in each case. The proteins encoded by each mRNA are shown on the right, while the question mark (?) denotes that the protein translated from the mRNA is currently unknown. The size of the ITRs and the NS1- and VP1-encoding regions diagrammed are not to scale. B19V: Human parvovirus B19; ITR: Inverted terminal repeat; NS1: Nonstructural proteins; RF: Replicative form; VP1: Capsid proteins.
Understanding the mechanism underlying B19V DNA replication, a critical step to developing antivirus strategies has been impeded by the difficulty of efficiently propagating B19V in an in vitro cell culture system. Several breakthroughs have been made recently to improve and expand B19V infection in in vitro cultures of cells, including permissive, semipermissive and nonpermissive cells, which have greatly advanced the understanding of B19V infection and DNA replication. This article will summarize these studies and discuss the cellular requirements for B19V tropism and B19V DNA replication in these different cell systems.

**B19V tropism for erythroid lineage cells**

B19V infection and viral DNA replication are restricted by the narrow tropism of the virus. In nature, B19V mainly infects human erythroid progenitor cells (hEPCs) from bone marrow and liver,[17,40–41], although restricted infection of other tissues has been frequently reported. B19V DNA replication was first observed in suspension cultures of human erythroid bone marrow from patients with hemolytic anemia,[41], and its replication efficiency was greatly enhanced during infection of isolated hematopoietic progenitor cells from normal human bone marrow.[40]. Identification of hEPCs as B19V target cells was first observed by Mortimer et al.[42] and Young and colleagues.[43] Further studies showed that B19V infection causes reduction of in vitro–cultured colony-forming unit erythroid and burst-forming unit erythroid cells, but only in the first few days in culture,[44], indicating that efficient B19V DNA replication requires certain differentiation stages of the hEPCs. In particular, B19V only infects hEPCs with surface marker CD36+ differentiated from CD34+ human hematopoietic stem cells (HSCs), but not the HSCs and myeloid lineage cells.[45–47]. The establishment of ex vivo–expanded CD36+ hEPCs from HSCs by Wong et al. has greatly advanced studies of B19V infection.[47]. CD36+ hEPCs are highly permissive to B19V infection with productive B19V DNA replication occurring on a few interval days during the period of post-differentiation from CD34+ HSCs to CD36+ hEPCs.[47]. Therefore, productive B19V infection only occurs for a short time during hEPC differentiation. It is not clear whether the differentiation status of CD36+ hEPCs affects B19V entry and virus trafficking and what are the cellular factors fluctuating during the differentiation to affect productive B19V infection.
Figure 2. Rolling-hairpin model of human parvovirus B19 DNA replication. (A & B) The B19V ssDNA genome as shown is first converted into RF DNA that is primed by the 3’OH of the L-ITR, a process that may not require viral proteins [39]. (C) The 3’ end of the newly synthesized complementary strand is likely ligated to the R-ITR, resulting in the formation of cRF DNA as the major conversion product cRF DNA.
A few megakaryocyte–erythroid lineage cell lines were documented to support B19V infection, including erythroid leukemic cell lines (KU812Ep6 and JK-11) [48–50], and human megakaryocytic leukemia cell lines (MB-02, UT7/Epo and UT7/Epo-S1) [51–53]. A comparison study evaluating their differences in permissiveness to B19V infection showed that the UT7/Epo-S1 cell line was the most sensitive cell line to B19V infection, based on detection of the viral NS1 protein and increased viral DNA production [53]. Although B19V infection in UT7/Epo-S1 cells is less efficient than that in hEPCs [54], it is particularly useful for the transfection of the B19V infectious clone and subsequent mutagenesis studies, since transfection is extremely difficult in hEPCs due to the nature of this type of primary cells.

At least two major factors have been identified to account for the tropism of B19V for hEPCs and these megakaryocyte–erythroid lineage leukemic cell lines. First, all these types of cells express globoside (erythrocyte P antigen), which is the primary receptor for B19V [55]. B19V capsid directly interacts with the globoside on erythroid cells, and pre-incubation antogloboside antibody or purified globoside prevents B19V infection of human bone marrow cells [55]. Individuals whose erythrocytes do not have globoside are naturally resistant to B19V infection [56]. Globoside is also present in red blood cells and some nonerythroid cells, such as fetal myocytes, placenta, megakaryocytes and some endothelial cells [57–60], which might explain the diverse clinical manifestations in different tissues. Besides the primary receptor globoside, integrin α5β1 and Ku80 were proposed to be coreceptors for B19V [61,62]; however, cell surface expression of Ku80 was shown to be very low in ex vivo-expanded CD34+ hEPCs (<5%) and other B19V-permissive cells [45,63]. Therefore, the erythroid tropism of B19V cannot be simply explained by the presence of the receptor/coreceptors. The second explanation is that all these megakaryocyte–erythroid lineage cells require erythropoietin (Epo) for growth, and Epo receptor (EpoR) signaling is required for B19V DNA replication [45]. In hEPCs, Epo binds to EpoR and activates the Janus kinase 2 (Jak2) by autophosphorylation [64]. Activated Jak2 further phosphorylates EpoR and initiates a kinase cascade with three major pathways, including the signal transducer and activator of transcription 5A (STAT5A), mitogen-activated protein kinase (ERK/MAPK) kinase (MEK) and phosphatidylinositol-3 kinase (PI3K).

B19V infection of hEPCs is Epo concentration-dependent [45]. Unlike globoside, EpoR is not required for B19V entry because Epo exposure after virus entry still enables B19V DNA replication. In fact, the EpoR signaling of Jak2 phosphorylation and the MEK/ERK activation play a key role in facilitating B19V DNA replication in hEPCs [45].

In addition to these findings, a recent study showed that the VP1 unique region (VP1u) of the B19V capsid protein VP1 is essential for B19V binding and internalization during B19V infection of in UT7/Epo-S1 cells [65]. Purified-recombinant VP1u can also be internalized in UT7/Epo-S1 cells. The N-terminal 29 amino acids of the VP1u have been shown to be essential for binding and internalization of the VP1u, which is independent of the PLA2 activity that was thought to be critical to B19V infectivity [66]. Interestingly, the VP1u-interacting cellular partner was uniquely expressed on UT7/Epo-S1 and KU812Ep6 cells, but not on nonerythroid lineage cells, such as HeLa, HEK 293 and HepG2 cells [65], indicating the unique role of VP1u in facilitating B19V binding and internalization in erythroid lineage cells. Although the specific VP1u-interacting molecule (receptor) has not been identified, this study presents a novel parvovirus internalization mechanism.
In conclusion, in in vitro cultures, B19V is mainly permissive to hEPCs and a few megakaryocyte–erythroid lineage leukemic cell lines. The tropism of B19V for erythroid lineage cells is largely due to the B19V receptor globoside and Epo/EpoR signaling, as well as VP1u that facilitates B19V internalization.

**B19V infection of myocardial endothelial cells & monocytic cells**

A few other nonerythroid lineage cells have been reported to support B19V infection [63,67–68]. B19V infection has been suggested to be associated with myocarditis and acute and chronic inflammatory cardiomyopathies, since B19V DNA was frequently detected in patients who have these symptoms [67,69–71]. B19V was also shown to be the most frequent pathogen detected in patients with normal coronary anatomy that clinically mimics acute myocardial infarction [69]. In addition, in vivo studies have demonstrated that B19V can also productively infect endothelial cells from heart [72] and placental tissues [73], indicating endothelial cells could be a natural target for B19V infection.

A recent study by Kietzell et al. identified a new route for the B19V infection of myocardial endothelial cells [63]. There are no major differences in surface expression of B19V receptor/coreceptors among UT7/Epo-S1 and primary endothelial cells, which are isolated from the pulmonary arteria (human pulmonary artery cells [HPAEC]), the umbilical vein (human umbilical vein endothelial cells [HUVEC]) and the aorta (human aortic endothelial cells [HAoEC]). B19V binds primary endothelial cells at a similar level as that of UT7/Epo-S1 cells. However, B19V internalization is deficient in these endothelial cells, a deficiency which is significantly enhanced by preincubation of the virus with anti-B19V antibodies. Mechanistically, the B19V-antibody complex might enter the cells through endocytosis mediated by the direct interaction of antibody-bound complement factor C1q with its receptor CD93 on the cell surface. Kietzell et al.’s study explains that the B19V genome and virus were detected in myocardial samples from patients with cardiac diseases [67], and provides an explanation for the frequent prevalence of B19V in endothelial cells from a variety of tissues, which may be related to the spread of B19V to other cell types [63]. However, the study does not provide evidence of B19V DNA replication in endothelial cells.

An early study has shown an antibody-dependent enhancement of B19V infection in monocytic cell line U937 cells [68]. B19V DNA was detected in infected U937 cells but with abortive B19V DNA replication. By addition of anti-B19V IgG, B19V DNA replication was significantly increased. However, the antibody-enhancement pathway appears to be different between endothelial and monocytic cells, as monocytic cells are speculated to be Fc receptor-mediated enhancement of B19V internalization [68].

In conclusion, collective evidence indicates that B19V enters myocardial endothelial cells, but lacks sufficient support to replicate in them. There is a long road ahead to prove B19V is a causative agent of myocardial diseases. It is attractive to speculate that the endocytosed B19V genome could be sensed by cytosol nor nuclear innate immunity DNA sensors, which induce proinflammatory cytokine secretion [74], and subsequently inflammatory cardiomyopathies. Additionally, the fact that B19V infects both the monocytic cell line and myocardial endothelial cells through an antibody-enhancement pathway suggests that the antibody-mediated B19V entry might be a common mechanism for B19V infection of nonerythroid lineage cells.

**Identification of the B19V minimum DNA replication origin**

Abortion of B19V infection was thought previously to be due to a block in full-length transcription maturation, as well as the in the conversion of viral ssDNA to double-stranded replicative intermediates [75–77]. With the available infectious DNA of B19V [27], recent studies have suggested that the abortive B19V infection in nonpermissive cells is largely due to the inefficient replication or nonreplication of B19V DNA in these cells. With the help of adenovirus, B19V DNA replicates in nonpermissive human embryonic kidney 293 cells (293 cells) [35,78]. 293 cells either infected with adenovirus or transfected with the Helper plasmid, which contains the adenovirus genes E2A, E4orf6 and VA RNA, support B19V DNA replication [35]. In line with this, adenovirus infection also enhances B19V DNA replication in UT7/Epo-S1 cells. One explanation for this phenomenon is that adenovirus E1A protein transactivates the B19V promoter in nonpermissive cells [79]. Also, both B19V and AAV have symmetric ITRs at each end of the viral genome; thus, helped by adenovirus, B19V DNA replication in 293 cells may share the same mechanism as that in adenovirus-helped AAV DNA replication. A detailed examination of the function...
of the adenovirus gene products in B19V DNA replication confirmed that the E4orf6 protein and VA RNA functioned similarly to help B19V DNA replication as they do during adeno-associated virus 2 replication, while E2A had no stimulatory effect on B19V DNA replication or gene expression [78]. More specifically, the E4orf6 protein serves as a scaffold to form a cullin 5-based E3 ubiquitin ligase complex that targets cellular proteins, such as p53 and Mre11, for degradation, while the VA RNA binds and inactivates protein kinase PKR, a (ds)RNA-dependent protein kinase [78]). Notably, adenovirus gene products are not required for the DNA replication of human bocavirus and the canine virus analogue, minute virus of canines (MVC) [80,81]. Both human bocaviruses and MVC have asymmetric terminal hairpin structures, suggesting the differences of terminal hairpin structures may account for the different replication mechanisms of parvoviruses.

By using the 293 cell transfection system, a B19V DNA replication origin has been identified [35]. The minimum origin of B19V DNA replication is only 67 nucleotides (nucleotide 5214 to 5280 of GenBank: AY386330), which covers a NS1 trs and four repeated NS1 binding elements (NSBEs) (Figure 3). B19V NS1 specifically binds to the NSBE1–NSBE2 region in an in vitro binding assay, while NSBE3 and NSBE4 may provide binding sites for potential cellular factors [82], which should assemble a nucleoprotein complex involving cellular factors to separate the dsDNA strand and enable NS1 to nick the top strand at the trs (Figure 2D).

Surprisingly, the mutant B19V infectious DNA with deletion of either the left-hand or the right-hand ITR still replicates in 293 cells with adenovirus infection or helper transfection. In addition, transfection of a B19V DNA fragment containing the NS1 expression cassette and only the Ori replicates in 293 cells in the presence of the three adenovirus gene products, as well as in UT7/Epo-S1 cells [35]. Based on these results, a hairpin-independent replication model has been proposed for B19V DNA replication [35]. It was expected that NS1 has the ability to reverse the direction of the replication (Figure 2F & G), regardless of the repairing/annealing of the ITR structure [35], for second-strand replacement synthesis (Figure 3H). However, the in-depth mechanism is not understood yet.

Despite of these interesting observations in B19V-permissive UT7/Epo-S1 cells and B19V nonpermissive 293 cells, it is not clear whether the hairpin-independent replication is employed during B19V infection of hEPCs. Also, little is known about its relevance to natural infection. The fact that B19V DNA replicates in nonpermissive 293 cells with the helper function of adenovirus provides a possibility that B19V coinfection with other viruses in nonerythroid lineage cells, for example, myocardial endothelial cells, facilitates a high level of B19V DNA replication, which may result in some disease outcomes and awaits more in vivo evidence.

**Hypoxic conditions promote productive B19V infection of erythroid lineage cells**

Although the ex vivo-expanded EPCs enable B19V DNA replication at a high efficiency, there is still a huge discrepancy in the production of progeny virions from infected hEPCs and during viremia of B19V-infected patients (10⁸ genomic copies per milliliter of plasma), indicating that other factors remain unidentified to recapture the in vivo B19V infection of human bone marrow in patients in vitro. One of these factors could be the oxygen level in human bone marrow, which is much lower than that in in vitro cell culture conditions [83].

In fact, a significant enhancement of B19V DNA replication as well as progeny virus production has been observed during B19V infection of hEPCs when they were cultured under 1% O₂ (hypoxia) [54,84–85]. Interestingly, hypoxia actually reduces the differentiation potential and the proliferation rate of the hEPCs [54]. The productive B19V infection under hypoxia is facilitated neither through an increase in virus entry or intracellular trafficking nor through the network regulated by hypoxia-inducible factor, a common signaling sensor for hypoxia-induced signaling transduction [54]. Strikingly, hypoxia regulates Epo/EpoR receptor signaling, which is essential for B19V DNA replication [49], and thereby, enhances B19V DNA replication. Two pathways mediated by the Epo/EpoR/JAK2 pathways, including upregulation of STAT5 signaling and downregulation of MEK/ERK signaling, boost B19V DNA replication in both hEPCs and UT7/Epo-S1 cells [54].

A promising application of this finding is to study B19V DNA replication by transfecting the B19V infectious clone in UT7/Epo-S1 cells precultured under hypoxia. The inoculated
Figure 3. Structure of the human parvovirus B19 right-hand-inverted terminal repeat and the human parvovirus B19 minimal DNA replication origin (Ori). The B19V right-hand ITR (R-ITR) at 365 nt is depicted in both the ‘Flip’ and the ‘Flop’ orientation, and the trs and NSBEs thought to comprise the NS1 binding site are indicated. The ITR is a nearly perfect palindromic structure; the exceptions are the three unpaired bases at two sites (shown as ‘bulges’) and three mismatched bases at three sites (shown as ‘bubbles’). The nucleotide sequences of the minimal B19V DNA replication origin (Ori) are indicated. The ITR sequence refers to the B19V J35 strain (GenBank accession number: AY386330).

B19V: Human parvovirus B19; ITR: Inverted terminal repeat; NSBE: NS1-binding element; trs: Terminal resolution site.

infectious clone replicated efficiently and produced progeny virions that were highly infectious in EPCs [54], which holds promise to perform mutagenesis study of B19V DNA replication.

DNA damage response-facilitated B19V DNA replication

A large number of DNA viruses have been shown to induce a DNA damage response (DDR), and some of them, including parvovirus [86], require the DDR for efficient viral DNA replication [87,88]. DDR is triggered by damaged DNA structures, such as ssDNA breaks, dsDNA breaks and stalled replication forks. The signaling transduction is conducted by a set of host defense machinery that is composed of a number of signaling sensors, transducers and effectors. Three major DDR sensors have been identified, including ataxia telangiectasia-mutated kinase (ATM), ATM- and Rad3-related kinase (ATR) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs). Each of these DDR sensors recognizes specific types of damaged (cellular) DNA structures and transduces a kinase cascade to numerous downstream mediators/effectors, which result in either cell cycle arrest for DNA repairing or apoptosis [89].

B19V infection of hEPCs activates the DDR signaling for the facilitation of viral DNA genome replication [90]. The B19V genome has two identical ITRs separated by a large ssDNA gap (Figure 2A), which is a perfect trigger to activate the ATR signaling [91]. In addition, B19V infection activates ATM and DNA-PKcs, possibly due to the intermediate replicative viral genome (Figure 2G&H) during B19V replication or the cross-interaction between DDR signaling. Notably, B19V hijacks ATR and DNA-PKcs, but not ATM, for viral DNA replication [90]. The DDR activation is associated with viral DNA replication status but not individual B19V structural or nonstructural proteins [92], suggesting that the DDR-involving proteins directly interact with the replicating B19V genome.

It is not clear how B19V replication is facilitated by the DDR signaling. Possibly, the ATR signaling plays a role in the first-strand DNA synthesis, which could be a DNA repair-associated DNA replication, and the DNA-PKcs activation recruits DNA ligase IV to ligate the RF DNA (Figure 2C).
Late S-phase-dependent B19V DNA replication

Cell-cycle arrest is required for a number of DNA viruses to modulate host microenvironment in such a way that favors viral DNA replication. Early studies have shown that B19V infections of both EPCs and UT7/Epo-S1 cells induced G2/M arrest, a status of 4N DNA content [53,92–94]. However, a more careful examination of B19V-infected cells using 5-bromodeoxyuridine (BrDU) incorporation combined with 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI) staining demonstrated that B19V infection actually induces a cell-cycle status with 4N DNA content as well as with BrDU-incorporation, suggesting a late S phase arrest [30]. Expression of NS1 alone induces a true G2/M arrest, a status with 4N DNA content and without BrDU incorporation [30]. The NS1-induced G2/M arrest has been reported to be caused by the deregulation of E2F family transcription [93], and is not caused by B19V infection-induced DDR [92]. B19V infection-induced late S-phase arrest suggests that replication of the B19V DNA genome, as with other autonomous paroviruses whose DNA replication depends on host cells arrested at S phase [31–33], requires cellular replication factors expressed in S phase. Similarly, during early infection of parovirus MVC, MVC DNA replication induces a DDR, which in turn arrests the cells at S phase [95,96]. The S phase arrest further facilitates MVC DNA replication. We speculate that B19V DNA replication-induced DDR causes cells arrested at S phase, while expression of the NS1 solely arrests cells at a status with 4N DNA content, and that the compromising of these two arrests confers the infected cells at late S phase.

Specific cellular replication factors, such as the DNA polymerase, have not been identified for B19V DNA replication, largely because of the essential role of these factors for proliferation and survival, and the lack of an in vitro DNA replication system of B19V. Nevertheless, several S phase factors, including DNA polymerase delta, proliferating cell nuclear antigen (PCNA), replication factor complex 1 (RFC1), Cyclin A and minichromosome maintenance complex 2 (MCM2) are found to appear in the B19V DNA replication center [30]. Knockdown of MCM2 and MCM5 to a level that does not affect cell proliferation blocks B19V DNA replication, confirming that the S-phase cellular DNA replication factors are recruited by an unknown mechanism for B19V DNA replication. It would be important to know whether these factors are recruited through a DDR-dependent pathway [97].

Conclusion

B19V is the only member of the Erythroparvovirus genus of the Paroviridae family, which infects humans. Although initially B19V was identified to infect erythroid lineage cells in bone marrow or fetal liver and to cause several mild to severe human hematological disorders, accumulating clinical reports indicate that B19V also infects nonerythroid lineage cells and may be associated with other diseases, such as myocarditis.

The selective B19V infection of erythroid lineage cells is due to the primary receptor globoside and Epo/EpoR signaling. B19V also infects myocardial endothelia cells and a monocytic cell line through an antibody-mediated enhancement pathway. In the presence of adenovirus gene products, B19V replicates in nonpermissive 293 cells and produces infectious progeny virions.

Mechanistic study of B19V DNA replication in erythroid lineage cells is greatly facilitated by the establishment of ex vivo-expanded CD34⁺ hEPCs and hypoxic conditions for B19V infection. B19V replicates in late S phase of infected cells by recruiting both cellular DNA replication/repairing factors (possibly through DDR signaling) to facilitate first strand synthesis. Thereafter, the dsDNA genome becomes competent for gene expression, NS1 binding and nicking at the replication origin, which initiates strand displacement of viral DNA synthesis, likely, through a hairpin-independent mechanism. Meanwhile, EpoR signaling, which is further enhanced under hypoxia, is crucial for B19V DNA replication and progeny virion production.

Future perspective

Studies of B19V infection and DNA replication have greatly increased our understanding of the B19V life cycle, which will shed light on identifying anti-B19V strategies and eventually a therapeutic approach to B19V infection-caused severe hematological disorders.

Although an in vitro cell culture system of B19V has been improved recently, the system still does not recapitulate B19V infection in vivo, which produces progeny virions at a high yield. A number of questions about...
the detailed mechanisms involved in B19V infection await exploration. For example, it is necessary to understand how EpoR signaling (STAT5 and MEK) affects B19V replication, what minimal cellular replication factors are involved in B19V DNA synthesis and whether these replication factors are recruited through a DNA repair- and S phase-dependent pathway. B19V infection arrests erythroid lineage cells at late S phase. Whether this represents a compromised condition forced by the NS1 protein and DDR signaling requires further study. Also, the transmission for transmission of B19V from the respiratory tract to human bone marrow, along with its infection of the myocardial system, liver system and possibly even more unidentified organs, warrants further investigation.

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EXECUTIVE SUMMARY

B19V infection of erythroid lineage cells

- The primary receptor globoside and likely the coreceptor integrin α5β1 have been indicated for the Human parvovirus B19 (B19V) tropism for erythroid lineage cells.
- Erythropoietin/erythropoietin receptor signaling is essential for B19V tropism and productive DNA replication.
- Hypoxia boosts productive B19V infection through enhancement of erythropoietin receptor signaling.
- B19V infection hijacks DNA damage response signaling to facilitate virus DNA replication.
- B19V infection induces late S phase, which favors virus DNA replication.

B19V infection of nonpermissive cells

- In the presence of adenovirus gene products, B19V DNA replicates in 293 cells.
- A 67-nucleotide region of the B19V minimum DNA replication origin has been identified and a hairpin-independent model of B19V DNA replication has been proposed.
- B19V infects myocardial cells and monocyctic cell line U937 through an antibody-mediated entry pathway.

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Papers of special note have been highlighted as:
• of interest; •• of considerable interest


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The B19V transcription map was revealed during infection of human bone marrow cells.


The first B19V infectious clone was constructed.


B19V genome replicates in nonpermissive 293 cells with the help of adenovirus gene products, and the B19V minimum replication origin was identified.


The first study to establish B19V infection in vitro-cultured cells.


The Epo/EpOR signaling is required for B19V infection of human erythroid progenitor cells (hEPCs).


Ex vivo-expanded CD34+ hEPCs are highly permissive for B19V infection during interval differentiation.


**Hypoxia conditions significantly enhance B19V DNA replication in hEPCs through STAT5A and MEK signaling.**


**B19V infection of myocardial endothelial cells is mediated through an antibody-dependent route.**


The N-terminus of VP1u is essential for B19V internalization of UT7/Epo-S1 cells.


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