Human parvovirus B19-induced cell cycle arrest and apoptosis

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Introduction

The human parvovirus B19 has recently been reclassified into a new genus called Erythrovirus of the Parvoviridae [60]. Although it behaves like other autonomous parvoviruses, such as those of the Parvovirus genus which include minute virus of mice (MVM) and rat H1 parvovirus, its genomic organization and extremely limited host range suggest a closer relationship to the adeno-associated viruses (AAV), which belong to the Dependvirus genus [12]. In humans, B19 virus is the only pathogenic member of the Parvoviridae [12]. Five major clinical manifestations related to B19 virus infection occur: (1) aplastic crisis in patients with chronic hemolytic anemia, (2) erythema infectiosum, (3) arthralgia/arthritis mainly in woman, (4) persistent infections manifesting as chronic anemia in immunocompromised patients, and (5) after maternal infection, nonimmune hydrops fetalis, which can lead to intrauterine fetal death [3, 4, 12, 33, 82]. The mechanistic basis for these B19 virus-associated diseases is poorly understood; however, most symptoms appear to be related to the unique tissue tropism of B19 virus [12]. Cells permissive for B19 virus replication are proliferating erythroid precursors in human bone marrow and fetal liver tissues [12, 55].

Parvovirus B19 is also able to agglutinate human erythrocytes. In 1993, Brown et al. [11] demonstrated that the receptor for B19 hemagglutinin (HA) is a blood group P antigen globoside (Gb4) which is predominantly present on red blood cell membranes [11]. Furthermore, the purified P antigen or monoclonal antibody to Gb4 blocked the in vitro infectivity of B19 virus to human erythroblasts, suggesting that the globoside is a functional receptor for B19 virus entry into its target cells [11]. However, since the P antigen is also expressed on B19 virus-non-permissive tissues such as heart, liver, lung, kidney, endothelium and synovium [18], other unknown cellular factors may determine the narrow tissue tropism of B19 virus infection [80].

B19 virus is a single-stranded DNA virus that lacks an envelope, and has genome length of 5.4 kb with hairpin structures at each extremity [12, 21]. The symmetric hairpin structures have palindromic sequences at both ends of the viral DNA [21].
Two major open reading frames extend throughout almost the entire genome of B19 virus [70]. A non-structural protein (NS1) on the left side of the genome with a molecular mass of 70–77 kDa appears as two bands on Western blot analysis [46, 70]. The functional difference between these two NS1 molecules is still unknown. Two capsid proteins (VP1 and VP2) derived from overlapping reading frames are present on the right side of the genome and share substantial amino acid similarity [63]. VP1 is the minor capsid protein of 83 kDa, and VP2 is the major capsid protein of 58 kDa [65]. Smaller peptides of 7.5 and 11 kDa have been identified as possible products of short and abundant RNA species transcribed from the middle and far right of the genome [38]. Their functions, however, have not yet been clarified. All transcripts are synthesized from a strong left side promoter at map unit 6 (P6) [36]. mRNAs and proteins detected by their specific probes showed an earlier appearance of NS1 compared to the capsid proteins [34, 72].

Comparatively little is known about the molecular pathogenesis of B19 virus infection, since B19 virus is particularly difficult to cultivate in cell cultures. While primary cell cultures from bone marrows and fetal livers are well-known host cells for B19 virus propagation [55, 56, 84], it is particularly difficult to prepare these materials. In 1992, Shimomura et al. [71] first reported that B19 virus can replicate in a megakaryoleukemia cell line, UT7/Epo, which was selected to grow in the presence of erythropoietin (Epo) from UT7 cell line dependent on a granulocyte colony-stimulating factor (GM-CSF). Using B19-positive serum as a virus seed, they confirmed the amplification of the viral genome up to 50-fold in the multiplicity of infection (MOI) of 0.05, and detected infectious virus in the culture supernatant [71]. Following UT7/Epo, the erythroleukemia cell lines, MB-02 [47], JK-1 [74], and KU812Ep6 [39], were identified as susceptible for the B19 virus infection and virus genome amplification. However, we cannot prepare sufficient amounts of the virus for use in in vitro experiments using all these cells. Furthermore, virus antigens were detected in less than 10% of cells after infection, even with highly concentrated seed of virus. The other reason is that infectious plasmid DNA clones have not been established. Why it has not been possible to obtain an infectious recombinant B19 DNA, while recombinant infectious virus clones have been successfully established with other parvovirus, MVM and AAV, may lie with the hairpin structures at both ends of the B19 virus genome which are longer in length compared to other parvoviruses and obstruct the cloning of viral DNA in bacterial cells [21].

Recently we established a highly susceptible cell line, UT7/Epo-S1, for B19 infection, which was subcloned from UT7/Epo by limiting dilution and screened by immunostaining using an anti-VP1/2 mAb [46]. We observed that more than 40% of cells were positive for anti-VP1/2 immunostaining 96 h postinfection with B19 virus [46]. This cell line allowed us to analyze cellular events occurring in the B19-infected cells. Here, we present the recent results concerning B19-induced cytopathology from the study using UT7/Epo-S1 cell line.

**Parvovirus B19 induces cytotoxicity of erythroid precursor cells with apoptotic features**

In the case of transient aplastic crisis, an unexpected interruption of erythropoiesis after B19 virus infection leads to sudden severe anemia [33]. Intrauterine fetal death in B19 virus-infected pregnancies is thought to be caused by fetal anemia resulting