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Invited critical review

Serologic and molecular detection of human *Parvovirus B19* infection

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Abstract

Following its identification by Yvonne Cossart in 1975, human *Parvovirus B19* has been recognized as the causative agent of a wide range of diseases. In childhood, the most common disease is a typical exanthema called "fifth disease". In adults, viral infection may be responsible for fetal loss and for aplastic anaemia in immuno-compromised patients. Because persistent viral infection may induce an autoimmune response, *Parvovirus B19* is emerging as an environmental factor linked to the pathogenesis of autoimmunity. As a result of its expanding disease spectrum, *Parvovirus B19* is the subject of intense efforts to clarify the pathogenesis of virus-related disorders as well as improve diagnostic laboratory testing including standardization of serological and nucleic acid-based detection assays. Enzymatic immunoassays based on conformational antigens have proven to be the most important tools for accurate diagnosis in the majority of cases. In other selected clinical cases, the detection of *Parvovirus B19* infection can be complemented by PCR and, more recently, by the real-time PCR.

Keywords: Human *Parvovirus B19*; Autoimmunity; Diagnosis; Serological tests; Polymerase chain reaction

Contents

1. Introduction

Human *Parvovirus B19* was identified in 1975 by Yvonne Cossart [1] and classified as a member of the Parvoviridae family in 1985. It is the only virus of this family known to be pathologic in humans. The members of the large family of Parvoviridae, common animal and insect pathogens, were the smallest DNA-

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containing viruses able to infect mammalian cells until the recent identification of circoviruses [2]. The Parvoviridae family is currently divided into two sub-families, Parvovirinae and Densovirinae based on their ability to infect vertebrate or invertebrate cells, respectively. Parvovirinae subfamily is divided into three genera according to the ability to replicate autonomously (genus *Parvovirus*), with helper virus (genus *Dependovirus*) or efficiently and preferentially in erythroid cells (genus *Erythrovirus*). *Parvovirus B19* is currently the only accepted member of the genus *Erythrovirus* [3,4].

Parvovirus B19 is a small and simple virus composed of a non enveloped capsid of 22–24 nm in diameter. The genome of *Parvovirus B19* consists of a single DNA strand of 5596 nucleotides, composed of an internal coding region of 4830 nucleotides flanked by terminal palindromic sequences of 383 nucleotides. These palindromes can acquire a hairpin configuration and serve as primers for complementary strand synthesis. The genome encodes two structural proteins, VP1 (nucleotides 2444–4786) and VP2 (nucleotides 3125–4786), as well as a major non-structural protein NS1 (nucleotides 436–2451) [5–7]. *Parvovirus B19* can undergo sequence variability as demonstrated by restriction nuclease enzyme digestion analysis, polymorphism analysis of polymerase chain reaction (PCR) amplification products and sequencing [8,9]. VP1 and VP2 regions show a greater sequence variation in contrast to the NS1 region that is highly conserved [10]. Sequence heterogeneity in the VP region of isolates from distinct epidemiological and geographical settings ranged from 0.5% and 4.8% base substitution corresponding to an amino acid variability of 0 to 1.7% [10]. In the study of Hemauer et al. [11] isolates from persistently infected subjects showed high variability of 4% and 8% at the DNA and amino acid level, respectively. This was particularly true for the VP1 unique region. Interestingly, these variations do not appear to affect the immunologic properties of *Parvovirus B19* or its clinical manifestations [12,13].

Recently, a number of novel genotypes have been reported. A *Parvovirus* variant, termed *V9*, was identified in a child with transient aplastic anemia. In this patient the serological assay was negative for acute *Parvovirus B19* infection. Subsequent sequence analysis, however, demonstrated an 11% difference from the known *Parvovirus B19* sequence [14,15]. A second virus variant, *A6*, was later described [16] and finally, the strain *K71* was described in 2002 [17].

Servant et al. [18] performed a systematic analysis of *Parvovirus* variants based on sequence alignment and phylogenetic analysis of the NS1 and VP1 unique sequences. This analysis clearly indicated the presence of three phylogenetic clusters. As such, the authors proposed that the Erythrovirus genus should be classified into three distinct genotypes: genotype 1 corresponding to human *Parvovirus B19* (prototype strain Pvaua), genotype 2 (prototype Lali) comprising the new identified strains A6 and K71, and genotype 3 with the V9 strain as prototype. The prevalence and clinical significance of these variants is, however, unknown, and has recently been reviewed [19].

The capsid proteins, arranged with icosahedral symmetry, consist of 60 capsomers predominantly (95%) composed of VP2 [20]. The other viral structural protein VP1 makes up the remaining 5%. The VP2 protein has a molecular weight of 58 kDa and is encoded by the nucleotide sequence 3125 to 4786. The minor structural protein VP1, with a molecular mass of 84 kDa, is encoded by the sequence from nucleotide 2444 to 4786. VP1 is identical to the carboxy-terminus of VP2 with an additional 227 amino acids at its amino-terminus. This amino-terminal domain, the VP1 "unique region", is located largely outside the virion and is therefore accessible to antibody binding.

The major non-structural protein NS1 has a molecular weight of 77 kDa, but its function is not well characterized. NS1, however, appears to be involved in several regulatory functions in viral life cycle including control of transcription as well as viral replication and packaging [21]. Moreover, NS1 protein shows some properties that play a role in the virus-host cell interaction, i.e., it has site specific DNA-binding, ATPase and helicase activities which can explain its cytotoxicity [22,7,23] and induction of growth arrest in target cells [24–26].

2. Pathogenesis of *Parvovirus B19* infection

Parvovirus B19 infection is very common in humans. Seroprevalence increases with age and more than 70% of adult population is seropositive [27]. *Parvovirus B19* infection has been associated with an expanding range of clinical disorders. This pattern is influenced by the age and haematological and immunological status of the host. It is believed that the virus is transmitted to the human host by inhalation of virus containing aerosol droplets. Viremia occurs one week after exposure, leading to infection of cells through the P antigen or globoside (Gb4) [28]. This receptor is expressed on erythroid cells and on other cells including synoviocytes, platelets, endothelium, vascular smooth muscle cells and fetal myocytes [29]. However, Weigel-Kelley et al. [30] have demonstrated that the P antigen is necessary for the binding of the virus to cell surface, but it is not sufficient for the virus entry into human cells. These authors have suggested that *Parvovirus B19* requires the presence of a cell surface co-receptor for successful infection [31]. This receptor was subsequently identified as $\alpha5\beta1$ integrin. Interestingly, these findings may clarify the reason why virus replication is restricted to progenitor cells within the erythroid lineage, because these cells express high levels of both P antigen and co-receptor. In contrast, a number of P antigen-positive non-erythroid cells are non-permissive for efficient infection because they do not express the co-receptor.

The viremic period is accompanied by mild illness characterized by pyrexia, malaise and myalgia. Reticulocytes disappear from peripheral smear analysis with slight reduction in haemoglobin levels. In healthy subjects this is a temporary and limited phenomenon, with general recovery within a week. During the second week, the viremia titer decreases and specific IgM antibodies are detected. In the third week, a second phase of symptoms appears characterized by erythematous rash, itching or arthralgia coincident with an IgG antibody response.

In children the most common clinical presentation of *Parvovirus B19* infection is "fifth disease" or "erythema infectiosum", an illness characterized by a non-specific prodromal phase, followed by the typical "slapped cheek" rash. Although joint symptoms are rare in children, they are more common in