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Review

Persistent parvovirus B19 infection in non-erythroid tissues: Possible role in the inflammatory and disease process

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ABSTRACT

Parvovirus B19 (B19V) is a small non-enveloped DNA virus of the *Parvoviridae* family. It is an obligate human pathogen that preferentially replicates in erythroid progenitor cells. B19V is the causative agent of multiple erythroid-related diseases due to replication-induced cytotoxicity. Despite its strong erythroid tropism and related acute disease association, B19V has been determined to persist in many other non-erythroid tissues. This review summarizes and appraises what is known about concomitant B19V DNA persistence and non-acute viral gene expression in various, particularly non-erythroid, tissue types. The methods utilized for B19V detection are described, focusing on the discrepancies in outcomes among the employed assays. The studies where investigations focused on the impact of persistent B19V expression on cellular signaling pathways are also summarized. These studies demonstrate the expanse of the types of cells capable of *in vivo* B19V expression as well as the possible effect of persistent viral infection on the cellular microenvironment. Overall, these reports indicate that B19V commonly persists in a wide range of both erythroid and non-erythroid tissues, and that low-level viral gene expression can be detected in some persistently infected cells. B19V capsid RNA or proteins have been reported in bone marrow, colon, heart, liver, lymphoid, synovial, testicular, and thyroid tissues. In a sub-set of these cases, B19V capsid mRNA or proteins have been associated with increased inflammatory-related gene expression. The development of standard protocols to assay for B19V infection and expression in the context of non-erythroid, non-acute disease is warranted, and with further targeted studies, may begin to elucidate the impact of persistent B19V infection *in vivo*. These studies may determine the most conducive cellular environment for persistent gene expression and possible impact on disease pathogenesis.

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1. Introduction

Human parvovirus B19 (B19V) is a small, approximately 20–25 nm virus that has been reported in a large percentage of the human population (Cossart et al., 1975). The B19V genome is a single-stranded DNA, 5596 nucleotides (nts) in length, with identical inverted repeats on both ends. A single promoter located

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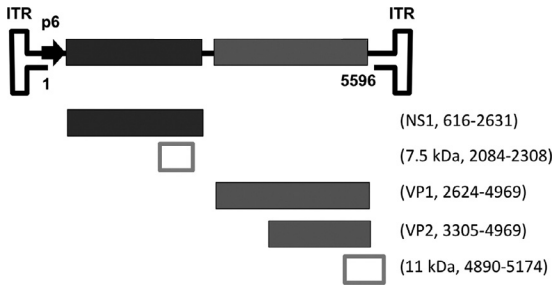

Fig. 1. Genome organization of B19V. Numbers represent nucleotide positions for each gene. GenBank accession number NC_000883.

at map unit 6 (p6) directs transcription of a major non-structural gene (NS1) as well as a gene encoding capsid proteins produced by alternative splicing (VP1 and VP2) (Heegaard and Brown, 2002). In addition, B19V encodes three minor non-structural proteins – 11 kDa, 7.5 kDa, and putative protein X (Amand and Astell, 1993; Luo and Astell, 1993). Visual representation of the B19V genome and organization of transcripts are shown in Fig. 1 (adapted from Zhi et al., 2004). B19V NS1 is thought to facilitate virus replication by exerting helicase, DNA-nickase, and transcriptional activities (Doerig et al., 1990; Sol et al., 1999; Wilson et al., 1991). Functions of the other non-structural proteins are less well characterized, although 11 kDa has been shown to participate in cell signaling and induce apoptosis in erythroid cells (Chen et al., 2010; Fan et al., 2001). Proteins VP1 and VP2 form viral capsids at a 5–95% ratio, respectively (Kawase et al., 1995). They are identical except for 227 amino acids at the amino-terminus of VP1 called the VP1-unique region (VP1u) (Saikawa et al., 1993).

B19V infection occurs through its main cellular receptor, P-antigen (Brown et al., 1993). B19V also has two reported co-receptors: α5β1 integrin and Ku80 (Munakata et al., 2005; Weigel-Kelley et al., 2001). The presence of virus-specific IgM antibodies in the serum and high titers of circulating viral genomes is typically indicative of acute infection. This is associated with a multitude of symptoms that can vary widely based on factors including age, immune status, and pregnancy. IgM antibodies appear around 10 days post infection and last for about 3 months (Anderson et al., 1986). IgG antibodies appear 2 weeks after infection and may persist indefinitely (Erdman et al., 1991). Because the primary site of replication for B19V is erythroid progenitor cells [*e.g.* erythroid burst forming units (BFU-Es) and colony forming units (CFU-Es)], diseases caused by B19V infection are often blood- or bone marrow-related including transient aplastic crisis, red cell aplasia, and hydrops fetalis in fetuses (Young and Brown, 2004). The immune response against the virus can also be a causative factor itself in disease, including rash development diagnosed as Fifth disease, as well as B19V-associated arthritis (Brown and Young, 1996).

Persistence of B19V DNA has been reported and thoroughly discussed in an expanding number of tissues not associated with acute infection or disease (Norja et al., 2006; Söderlund-Venermo et al., 2002). While most people may harbor the virus asymptomatically, the studies summarized here suggest that persistent B19V may impact disease progression in a sub-set of patients. Despite the presence of B19V DNA in multiple tissue types, studies correlating viral DNA, RNA, and protein detection in these tissues to a change in the cellular microenvironment or a specific disease state are limited and often contradictory. This article details methods used in assessing B19V persistence, as well as the link between persistent B19V infection, expression, and alterations in the cellular microenvironment of some tissues and suggests possible mechanisms.

2. Methods for the detection of persistent B19V

The main diagnostic marker of previous B19V infection is detection of B19V IgG in the serum. The Biotrin enzyme immunoassay is the most common method for evaluating the presence of B19V specific IgG and IgM antibodies in the serum (Biotrin International, Blackrock, Co. Dublin, Dublin, Ireland). Strip-Immunoassays containing multiple B19V antigens provide an additional method for evaluation of antibodies against different B19V antigens in serum samples, including VP1-specific antibodies, N- or C-terminal specific antibodies against capsid proteins, antibodies recognizing conformational or linear epitopes of the capsid protein, or NS1 specific antibodies. These assays may provide a more complete picture of B19V infection for research purposes. Previous studies have demonstrated increased presence of B19V NS1-specific IgG in serum from patients with chronic B19V infection or prolonged B19V infection-related symptoms (Hemauer et al., 2000; Kerr and Cunniffe, 2000). Furthermore, von Poblotzki et al. (1995a,b) demonstrated a link between B19V DNA persistence, chronic disease, and the presence of circulating B19V NS1 IgG antibodies. These reports indicate that more thorough methods may be useful in evaluating the presence of B19V-specific antibodies in studies examining persistent B19V infection.

Both commercially available and "in-house" real time assays have been employed to quantify B19V DNA in persistently infected tissue. Further validation of real time assays will be imperative to determine the relationship between quantities of B19V genome equivalents and disease diagnosis. In testicular DNA, no significant difference in genome equivalent amounts was associated with disease but a difference in viral quantities was reported depending on the commercial assay used (Ergunay et al., 2008). Standardization of reagents for B19V DNA detection has been established and is available from the World Health Organization (WHO) and the National Institute for Biological Standards and Control (NIBSC, UK). While the ease of acquiring these samples can vary based on the country in which the research is conducted, a push toward using a standard sample or method may decrease some of the discrepancies seen among studies.

Detection of persistent, low-level B19V expression has been reported by either RT-PCR or immunohistochemical (IHC) staining. There are often inconsistencies in assessment by these methods. This is especially demonstrated by cases of IHC examination of B19V in testicular tissue. One study reported only 19% of testicular tissues positive for B19V DNA and no viral IHC staining of the B19V VP2 protein or histological signs of viral infection (Tolfvenstam et al., 2002). In contrast, a second study detected B19V capsid proteins by IHC in greater than 74% of cases, but also did not report histological signs of viral infection (Polcz et al., 2012). While reports have demonstrated that some B19V antibodies can bind cellular proteins, the epitopes used to demonstrate auto-antigen binding are different from those detected by antibodies commonly employed for IHC. Lunardi et al. (1998) developed antibodies against a 24-mer peptide representing amino acids 57–80 on the VP2 capsid protein to demonstrate auto-antigen binding. The main antibody used in B19V IHC studies, MAB8293, also called RF92F6, recognizes amino acids 328–344 on the VP2 capsid protein. Escher et al. (2008) reported confirmation of VP1/VP2 binding of the RF92F6 antibody in B19V transfected 293T cells, and further showed a significant association between B19V DNA detection and IHC staining with this antibody in cardiac tissue. In an attempt to identify peptides unique to B19V for vaccine development, a majority of the peptides recognized by MAB8293 were shown to be highly unique to the virus and not present in human protein sequences (Fasano and Kanduc, 2011). IHC methods can be extremely variable, and differences in antigen retrieval, antibody concentration, incubation time, and temperature, and sensitivities of secondary conjugates