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Parvovirus B19: Insights and implication for pathogenesis, prevention and therapy

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ABSTRACT

Parvovirus B19 (B19V) is a small ssDNA non-enveloped virus, member of *Parvoviridae* family. The infection is widely diffused and is responsible for a broad range of clinical manifestations including fifth disease in children, transient aplastic crisis in patients with haematological disorders, non-immune hydrops fetalis in pregnant women, persistent anaemia in immunocompromised patients, arthropathy and inflammation of various other tissues. B19V infects and replicates in erythroid progenitor cells (EPCs) in the bone marrow. The depletion of infected EPCs represents the pathogenetic mechanisms of some haematological B19V-associate diseases.

Following a primary infection, the virus can establish lifelong persistence in several tissues. Currently, the pathological potential of persistent virus on the cellular signalling pathways remains unclear. In non-erythroid tissues, the infection is usually, abortive, and the virus seems to exert its pathological role through indirect mechanisms, such as induction of inflammatory and autoimmune processes, or through virus-induced apoptosis mediated by viral proteins. In addition to the diseases for which the etiological role of B19V has been fully demonstrated, there are several clinical conditions, including autoimmune diseases, that are presumably, but not certainly, associated with B19V infection.

In this review, we describe recent findings that may give us new insight into the pathogenic role of B19V in systemic sclerosis, an autoimmune disease of unknown multifactorial aetiology. Furthermore, we describe the latest findings on the intrauterine B19V infections. Moreover, since there are some ongoing interesting studies focused on vaccine development and antiviral drug discovery for the prevention and treatment of parvovirus B19 infection we described some advances in this field of research.

1. Introduction

Human parvovirus B19 (B19V) is a small, non-enveloped, single-stranded DNA (ssDNA) virus belonging to Erythroparvovirus genus of the *Parvoviridae* family (Qiu et al., 2017). The viral genome, 5.596 nucleotides in length, has two major open reading frames (ORF) (Fig. 1). In the left half, an ORF encodes the major non-structural NS1 protein involved in viral replication and in the pathogenesis of some B19V-associated diseases. In the right half, a unique ORF encodes two structural proteins, VP1 and VP2, which form the viral shell (Agbandje et al., 1994). The VP2 account for 95% of capsid proteins. The VP1, localized to the surface of the viral capsid, shares the same C-terminus with VP2, and has additional 273 amino acids in the N-terminal region (VP1-unique region or VP1u) (Ozawa and Young, 1987). The VP1u region exhibits phospholipase A2 activity (Zadori et al., 2001; Dorsch et al., 2002), which is possibly used to evade lysosomal fusion and ensure nuclear entry of the virions. Other minor ORFs encode for additional non-structural protein, among them a better characterised 11 kDa protein that is involved in viral pathogenesis (Ganaie and Qiu, 2018) (see Fig. 1).

There are three distinct genotypes of B19V (Servant et al., 2002; Gallinella et al., 2003). Genotype 1 is the predominant circulating genotype, with worldwide distribution. In contrast, the circulation of genotype 3 is limited to sub-Saharan Africa and South America (Hubschen et al., 2009; Norja et al., 2006). Genotype 2 seems to be ancestral to genotype 1 (Gallinella et al., 2003) Norja et al., 2006 and is detected in tissues from older persons (Pyoria et al., 2017). No correlation between

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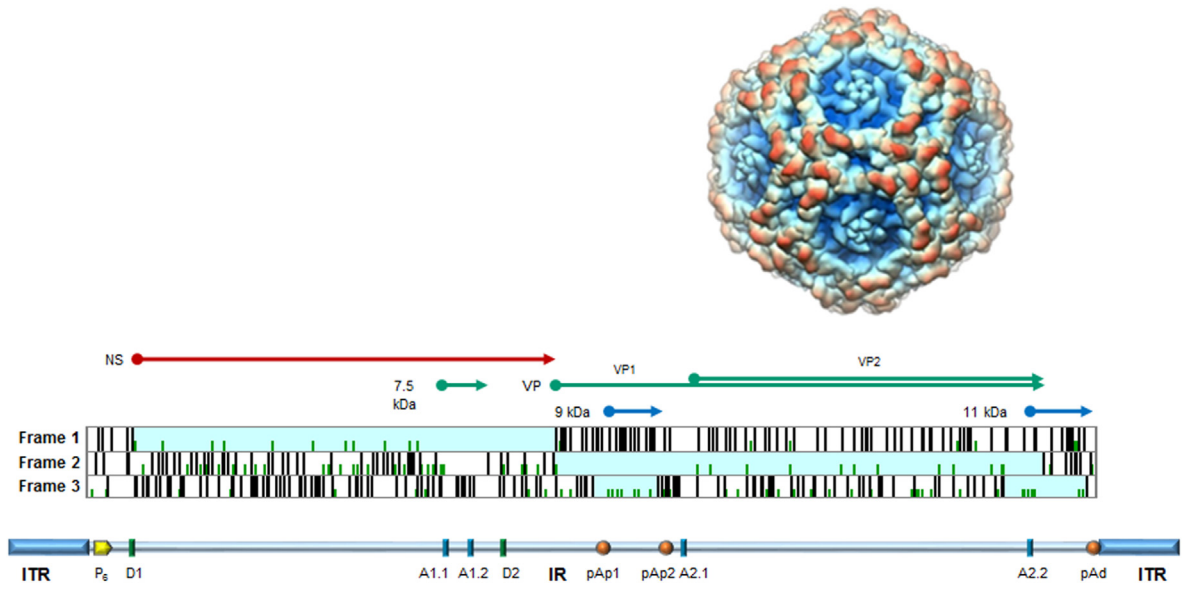

Fig. 1. B19V genome organization.
Bottom: Schematic diagram of B19V genome. ITR, inverted terminal regions; IR, internal region; *cis*-acting functional sites: P6, promoter; pAp1, pAp2, proximal cleavage-polyadenylation sites; pAd, distal cleavage-polyadenylation site; D1, D2, splice donor sites; A1.1, A1.2, A2.1, A2.2, splice acceptor sites. Center: Major open reading frames identified in the positive strand; arrows indicate the coding sequences for the viral proteins. NS, non-structural protein; VP, structural proteins, colinear VP1 and VP2, assembled in a T = 1 icosahedral capsid (above); 7.5 kDa, 9.0 kDa, 11 kDa: minor non-structural proteins. Top: structural model of Parvovirus B19 capisd shell. The shell is composed of 60 protein subunits, arranged in twelve pentons (T = 1 symmetry). The core is composed of the VP1/2 common region, while the less abundant VP1u region is close to the penton vertex. Modified from (Manaresi and Gallinella, 2019). Capsid shell image from PDBJ (www.pdbj.org; EMD-1467) © Protein Data Bank Japan (PDBj) licensed under CC-BY-4.0 International.

disease symptoms and genotype has been documented (Ekman et al., 2007).

The infection is widely diffused and is more commonly acquired during childhood, with the primary route of transmission via respiratory droplets. B19V is responsible for a broad range of clinical patterns, including fifth disease in children (Anderson et al., 1983), transient aplastic crisis in patients with haematological disorders (Chorba et al., 1986), non-immune hydrops fetalis in pregnant women (Brown et al., 1984), persistent anaemia in immunocompromised patients (Broliden et al., 1998; Eid et al., 2013), arthropathy and inflammation of various other tissues (Adamson-Small et al., 2014).

B19V shows a selective, but not exclusive, tropism for erythroid progenitor cells in the bone marrow, which may largely explain some clinical manifestations and, following a primary infection, can reach and establish lifelong persistence in several tissues (Norja et al., 2006; Soderlund-Venermo et al., 2002). Currently, the pathological potential of persistent virus on the cellular signalling pathways is still unclear. In non-erythroid tissues, the infection is, usually, abortive, and the virus is presumed to exert its pathological role through indirect mechanisms, such as induction of inflammatory and autoimmune processes (Adamson-Small et al., 2014) or through virus-induced apoptosis mediated by the non-structural NS1 and 11 kDa proteins (Moffatt et al., 1998; Poole et al., 2004; Chen et al., 2010a), or by phospholipase activity of the VP1-unique region (VPu) of the B19V minor capsid protein (Lu et al., 2006).

2. Target cells and outcome of infection

The primary targets of B19V are human erythroid progenitor cells (EPCs) in the bone marrow, particularly, during the stages of burst forming unit-erythroid (BFU-E) to colony forming unit-erythroid (CFU-E), which are fully permissive for viral replication (Young et al., 1984; Srivastava and Lu, 1988). The tropism of the virus for the erythroid lineage cells is determined by the presence, on the cell surface, of primary receptor known as glycolipid globoside or as blood group P antigen (Brown et al., 1993), and of a secondary receptor (co-receptor) still not well defined, which is necessary for virus entry into the cell (Leisi et al., 2013, 2016a, 2016b). The first interaction of viral shell with the P antigen leads to the externalization of VP1u, which then can interact with co-receptors allowing the virus to enter by endocytic pathway.

Following internalization of the viral particles, the viral ssDNA genome is translocated to the nucleus and converted to double stranded DNA (dsDNA) replicative form by cellular DNA repair enzymes. The ssDNA conversion in dsDNA is necessary to start viral transcription and replication. After DNA replication via a rolling hairpin mechanism, the expression pattern initially characterized by synthesis of non-structural protein (NS1) involved in viral DNA replication, shifts in the prevalent expression of structural proteins (Bua et al., 2016) (Fig. 2).

It has been demonstrated that activation of erythropoietin (Epo) pathway and a hypoxic condition like that present in bone marrow are also required to create intracellular environment permissive for viral replication (Luo and Qiu, 2015).

Apart from erythroid progenitor cells in the bone marrow, B19V may infect many other cell types such as endothelial or connective tissue cells, which are not permissive for viral replication. In this case the virus enters the cells using probably different kind of coreceptor(s), not yet identified or Antibody Dependent Enhancement (ADE) mechanism via c1q receptor described for infection of endothelial cells (von Kietzell et al., 2014) and lymphocytes B from tonsil tissue and lymphocytes B (Pyoria et al., 2017). In the non-permissive cells, the virus does not replicate. Studies of B19V infection in heterogeneous population of erythroid progenitor cells demonstrated, that depending on cell type, on cell differentiation stage and on cell cycle phase, there can be several levels of intracellular restriction to interfere with different steps of viral replication: (1) the synthesis of second DNA strand and start of macromolecular synthesis; (2) the replication of viral genome; (3) the switch of expression profile from early to late pattern (4); the assembly of viral particles; (5) the release of infectious virus.

Little is known about B19V infection in secondary, non-permissive target cells. There are relatively few studies, which describe the pattern

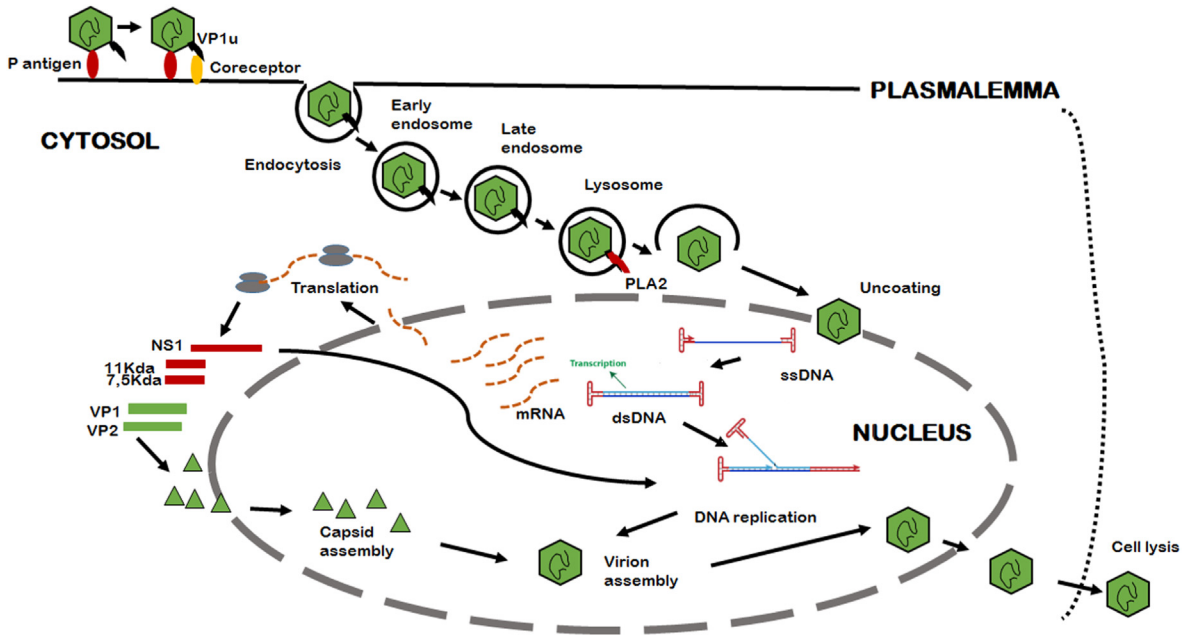

Fig. 2. B19V productive infection.
B19V binds to receptor and coreceptor on the target cell and enter the cell by endocytosis. In the lysosome the activation of phospholipase A2 (PLA2) (pH 4.0) occurs. The activated PLA2 destroys the integrity of the lysosomal membrane, and the virions are released into the cytosol. The virions are transported towards the nucleus and target on the karyotheca with the help of the VP1 nuclear localization signal (NLS). The viral genome expression and replication begins and the assemble of virions after trafficking through the nuclear pore complex (NPC). The matured virions finally move through the NPC and are released from the cells.

of genome expression and replication and virus-induced alterations within these cells, and the pathological consequences of abortive infection. Recent studies on different viruses, including B19V, have demonstrated, that even in the absence of viral replication, the infected cells can undergo some changes induced by viral proteins or by interaction of viruses with cell surface receptors. Viral particles or viral molecules can stimulate production of different cytokines and factors that induce cell modifications as well as activate innate immunity (Delaloye et al., 2009; Ichinohe et al., 2010; Muruve et al., 2008), as also observed for B19V (Zakrzewska et al., 2019; Arvia et al., 2020, 2021). It is supposed, that changes in specific cellular environment or co-infection with other viruses could contribute to modulate B19V expression (Guan et al., 2009; Pozzuto et al., 2011; Bock et al., 2014).

3. Pathogenesis of B19V- associated diseases

B19V infection is associated with a broad spectrum of clinical conditions, which often depend on haematological and immunological status of the patient. Virus enters the organism via the respiratory tract, reaches the bloodstream and then the bone marrow where infects erythroid progenitor cells which are permissive for virus replication. The apoptosis and depletion of infected cells leads to erythropoiesis arrest, to erythroid aplasia, and to appearance of giant erythroblasts, typical of B19V infection. The replication and release of virus from bone marrow leads to a viremia, which can reach extremely elevated levels (10^{12} virus/mL), and to systemic diffusion of the virus.

In healthy subjects with normal haematological and immunological status, the block of erythropoiesis caused by B19V infection is usually transient and asymptomatic and resolves spontaneously following the immune response which neutralize and clean the virus from the organism (Musiani et al., 1995; Lindblom et al., 2005). In contrast, in patients with underlying haematological disorders (decreased production or reduced lifespan of erythrocytes) such as α- and β-thalassemia, hereditary spherocytosis, sickle cell disease or chronic autoimmune haemolytic anaemia (Serjeant et al., 1993), B19V can lead to self-limited, severe anaemia known as transient aplastic crisis (TAC). In patients with congenital or acquired immunological disorders due to HIV infection (Koduri, 2000), chemotherapeutic (Lindblom et al., 2008) or immunosuppressive treatments (Eid et al., 2013; Ohrmalm et al., 2013), incapable to neutralize and clean infection, the virus can persist. The prolonged viral replication and progressive depletion of erythroid progenitors can lead to persistent anaemia of different severity.

Erythema infectiosum, also known as fifth disease, a typical manifestation of B19V infection in children (Chorba et al., 1986; Anderson et al., 1984) and arthropathies, more frequent in adult woman (Reid et al., 1985; White et al., 1985), are due to immune complexes deposition in the skin or in the joins respectively. In fact, the onset of both manifestations coincides with appearance of B19V-specific antibodies in the serum. Finally, B19V infection during pregnancy may lead to hydrops fetalis and intrauterine foetal death (Ornoy and Ergaz, 2017).

During systemic infection, B19V can reach different organs and can infect different kind of cells, which are not permissive for virus replication. Little is known about B19V abortive infection in the secondary targets as well as about virus-induced alterations within these cells and its clinical consequences. In this case, the pathogenic mechanism is probable due to cytotoxic or apoptotic effect induced by viral proteins, or to inflammatory response stimulated by viral proteins, NS1 but also by the VP1u associated phospholipase A2 (PLA2) (Adamson-Small et al., 2014; Lu et al., 2006; Thammasri et al., 2013; Fu et al., 2002).

In addition to the diseases for which the etiological role of the virus has been fully demonstrated, there are some clinical conditions, including hepatitis (Hillingso et al., 1998; Sokal et al., 1998), myocarditis (Verdonschot et al., 2016; Rigopoulos et al., 2019) or autoimmune diseases (Kerr and Cunniffe, 2000) presumably, but not certainly, associated with B19V infection [Table 1].

In the following sections we describe the recent findings which give us novel view about the B19V pathogenetic role in some diseases.

4. B19V and systemic sclerosis

Several reports suggest that B19V could play a role in the aetiology of some autoimmune rheumatologic diseases such as rheumatoid arthritis

Table 1
Clinical manifestations of parvovirus B19 infection.

Category	Frequent	Sporadic
Hematological	Transient anemia Aplastic crisis Chronic anemia Chronic Pure Red Cell Aplasia	Bone marrow necrosis and fat embolism Myelodisplastic syndrome Thrombocytopenia Granulocytopenia Pancytopenia Idiopathic Trombocytopenic purpura Hemophagocytic Lymphohistiocytosis
Specific tissue/organ disease	Erythema infectiosum Mono or poly-Arthitis Chronic arthralgias	Petecchial purpura Henoch-Schonlein purpura Papuplar-purpuric glove-and-socks syndrome Acute Myocarditis/pericarditis Chronic inflammatory myocarditis Myositis Hepatitis Glomerulonephritis Meningitis Encephalitis Peripheral neuropathy Thyroiditis and thyroid neoplasia (?)
Systemic	Aspecific febrile illness	Chronic fatigue syndrome Vasculitis Scleroderma Lupus erythematosus
Infection in Pregnancy	Intrauterine infection (30–50%) Fetal anemia Fetal hydrops Fetal death	Mirror syndrome Meconium peritonitis Fetal malformations Congenital anemia Neurodevelopmental delay (?)

A compilation of diseases and clinical manifestations associated to parvovirus infection. Frequent clinical manifestations are typical of B19V infection, although the presentation and course of disease can vary depending on the physiological and immune status of the individuals. Sporadic clinical manifestations include diseases that have been more rarely associated to B19V infection, occurring at a low frequency and without any predictable concurrent factor. The list of sporadic clinical associations is not exhaustive.

(RA), Juvenile idiopathic arthritis (JIA), systemic lupus erythematosus (SLA) or systemic sclerosis (SSc) (Kerr, 2016).

The possible mechanisms involved in B19V-associated autoimmunity include molecular mimicry (Lunardi et al., 1998; Thomas et al., 2006), B19V-induced apoptosis with T lymphocytes activation by self-antigens (Thammasri et al., 2013; Levine et al., 1999), and B19V VP1u-associated phospholipase A2 activity (Zadori et al., 2001; Dorsch et al., 2002), which is required for infectivity but may contribute to the inflammatory processes. In particular, the production of leukotrienes and prostaglandins, induced by B19V, may also lead to the generation of unnatural cleavage products from cellular phospholipids that may induce anti-phospholipid antibodies in combination with a distinct genetic background (Kerr, 2016; Von Landenberg et al., 2003; Lehmann et al., 2003; Tzang et al., 2007; Chen et al., 2010b).

The systemic sclerosis is a complex autoimmune chronic disease characterized by early endothelial damage and immunological abnormalities with progressive fibrosis in multiple organs, including skin, heart, vasculature and lungs. Although its aetiology remains poorly understood, the main traits of SSc are injury to the endothelial cells, overproduction of extracellular matrix proteins, and aberrant activation of both immune and non-immune effector cells (Denton and Khanna, 2017). As for many other immune-mediated diseases, the most accepted hypothesis is that the combination of a predisposing genetic background and a triggering factor or event may cause a break of tolerance toward self-antigens with persistent activation of the immune system (Benfaremo et al., 2022). The endothelial dysfunction plays a pivotal role in the initiation and perpetuation of vasculopathy associated with SSc (Benfaremo et al., 2022). The subsequent fibroblast activation causes an excessive extracellular matrix (ECM) deposition and an unrestrained tissue fibrosis, the main process leading to end-organ failure (Distler and Cozzio, 2016).

The hypothesis that B19V can contribute to the onset and/or progression of SSc is supported by some epidemiological and experimental data which demonstrated that, compared to healthy subjects, the SSc patients showed higher prevalence of B19V viremia (Ferri et al., 1999a) and/or higher rate of B19V persistence in the bone marrow and/or in the skin (Ferri et al., 1999b; Ohtsuka and Yamazaki, 2004; Zakrzewska et al., 2009). They also presented with higher prevalence of anti-B19V NS1 antibodies, proposed as a marker of persistent infection (Kerr and Cunniffe, 2000; von Poblotzki et al., 1995). Subsequently, it has been demonstrated that B19V DNA can persist in SSc dermal fibroblasts propagated *in vitro* (Ferri et al., 2002). Moreover, in fibroblasts and in perivascular inflammatory cells of the skin from SSc patients, B19V DNA and TNF-α mRNAs has been detected and the degree of B19V mRNA expression correlated with endothelial cell degeneration and inflammation, suggesting a causal role of B19V in the propagation of the endothelial cell dysfunction (Magro et al., 2004).

In vitro studies provided evidence that B19V can infect the target cells of SSc such as dermal fibroblasts (Arvia et al., 2020; Zakrzewska et al., 2005), endothelial cells from various tissues (von Kietzell et al., 2014; Zakrzewska et al., 2005) and different cell types belonging to the heterogeneous group of bone marrow-derived circulating angiogenic cells (CACs) (Schmidt-Lucke et al., 2015), essential for vascular regeneration. Infection of all these cells is abortive, characterized by expression of viral genome but not by its replication or by production of viral particles. However, little is known about the B19V-driven functional alterations of these of cells. The question to answer is: could such alterations contribute to inflammation, fibroblast dysfunction, or defective endothelial cell homeostasis, the hallmarks of SSc? Some recent *in vitro* investigations tried to respond this question.

Regarding B19V-induced fibroblast modifications, the study of Arvia and collaborators (Arvia et al., 2020) showed that B19V infection can activate normal dermal fibroblasts, increasing their ability of both migration and invasiveness, and expression of mRNA of different profibrotic genes (α-SMA, EDN-1, IL-6, TGF-β1 receptors 1 and 2, Col1α2), some genes associated with inflammasome platform (AIM2, IFI16, IL-1β, CASP-1) and genes for some metalloproteases (MMP 2, 9 and 12). Since it has been previously demonstrated that MMP12 overexpressed by SSc fibroblasts is able to induce impaired human microvascular vein endothelial cells (HMVECs) proliferation, invasion, and capillary morphogenesis (D'Alessio et al., 2004; Serrati et al., 2006), B19V infection of normal human dermal fibroblasts (NHDFs) could contribute to the defective angiogenic process, a pathological hallmark of SSc, responsible for the capillary loss and the subsequent ischemic organ injury (Arvia et al., 2020). The demonstration of the ability of B19V to activate dermal fibroblasts suggests that the virus may have a role in the pathogenesis of the fibrotic lesions.

There is emerging evidence that excessive accumulation of senescent cells is associated with some chronic diseases and suggests a pathogenic role of cellular senescence in the fibrotic process, such as that occurring in ageing or in SSc (Schafer et al., 2018). SSc-associated fibrosis shares some pathological features with other age-related fibrotic diseases, such as idiopathic pulmonary fibrosis. Among the most important traits there are: epithelial and endothelial injury, immune dysregulation and fibroblasts activation with increased deposition of extracellular matrix (Herzog et al., 2014). Furthermore, several studies have shown that advanced age is associated with a higher incidence and more severe course of disease and with increased mortality of SSc patients (Strickland et al., 2013), suggesting the association of SSc with aging. Moreover, a higher SA-β-gal expression in SSc skin than in healthy controls was demonstrated and the SA-β-gal expression was observed in sclerotic skin lesions of SSc patients, while it was not detected in non-sclerotic lesions.

Likewise, cultured fibroblasts from SSc patients showed higher SA-β-gal expression than control fibroblasts. The study of Dumit and collaborators (Dumit et al., 2014), employing a proteomic approach, identified numerous age-dependent differences between normal and scleroderma fibroblasts, including the accumulation of SA-β-gal, and showed the that latter displayed evidence indicative of cellular senescence.

Our recent investigation showed that B19V *in vitro* infected cells develop typical senescence features like that observed in SSc dermal fibroblasts: enlarged and flat-shaped morphology, SA-β-gal activity and SASP-like phenotype characterized by mRNA expression and release of some pro-inflammatory cytokines, along with activation of the transcription nuclear factor NFk_B. Moreover, as demonstrated by comet assay, a subpopulation of fibroblasts from B19V-infected cultures showed a significantly higher level of DNA strand breaks and oxidative damage compared with mock-infected cells suggesting, that B19V can induce DNA damage in infected fibroblast cultures. An increased level and nuclear localization of ϒH2AX, a hallmark of DNA damage response, were also observed (Arvia et al., 2021). B19V-induced senescence and production of SASP-like factors in normal dermal fibroblasts could represent a new pathogenic mechanism of non-productive B19V infection, which may have a role in the fibrotic process.

B19V infection was associated with impaired endothelial regeneration through induction of apoptosis and dysregulated trafficking of infected CACs. These observations indicate that B19V infection can result in dysfunctional endogenous vascular repair (Schmidt-Lucke et al., 2015, 2018; Zobel et al., 2019). B19V-induced apoptosis of CACs, through the viral proteins NS1, VP1u and 11 kDa, was due to downregulation of BIRC3 (cellular inhibitor of apoptosis-2 (cIAP2), a potent suppressor of apoptotic cell death activation of caspases-8 and -10 (Zobel et al., 2019) while the enhanced migration of CACs was associated with increased expression of surface CXCR4 (Schmidt-Lucke et al., 2018).

Lastly, it is known that abnormal innate immune response plays an important role in the pathogenesis of SSc (O'Reilly, 2014). Circulating mononuclear cells from patients with scleroderma, compared to that from healthy subjects, produce higher levels of cytokines and molecules involved in inflammation and fibrosis (Dantas et al., 2018; Duan et al., 2008). Moreover, different studies suggested the role of the inflammasome in the development of fibrosis (Gasse et al., 2007; Zhang et al., 2019). It has been demonstrated that, at least 40 genes involved in the inflammasome pathway and NLRP3-mediated secretion of IL-1β and IL-18 are upregulated in scleroderma fibroblasts (Artlett et al., 2011). Many viruses can activate inflammasomes (Delaloye et al., 2009; Ichinohe et al., 2010; Muruve et al., 2008), and it was hypothesized that persistent infection and chronic inflammasome activation is involved in the pathogenesis of SSc (Artlett et al., 2011). *In vitro* studies demonstrated that B19V abortive infection induces NLRP3-dependent caspase-1 activation and caspase-1 mediated IL-1β secretion in monocytic PMA-differentiated THP-1 cells. In addition, cultured monocytes obtained from scleroderma patients showed increased TNF-α production, NLRP3 expression and caspase-1 activation following B19V infection (Zakrzewska et al., 2019).

Altogether, these recent findings on B19V-induced functional alterations of SSc target cells may give us novel insights into the in-depth mechanisms responsible for inflammation, fibroblast dysfunction and defective endothelial cell homeostasis in systemic sclerosis. We can hypothesize that the abortive infection and fibroblast alteration observed *in vitro* may also occur in vivo either as result of primary B19V infection in adult patients or as result of expression of persistent virus, acquired in childhood. In fact, it has been speculated that changes in the specific cellular environment or co -infection with other viruses could induce expression of persistent B19V (Pozzuto et al., 2011; Bock et al., 2014).

5. Risk in pregnancy

A relevant property of B19V is its ability to cross the placental barrier and infect the fetus, mainly posing a risk to non-immune women of childbearing age (Puccetti et al., 2012; Bascietto et al., 2018; Xiong et al., 2019). Mechanism of transmission have not been studied in detail. The small dimensions of the virion may facilitate translocation through the trophoblast layer, but more specific mechanisms can be considered. The villous trophoblasts of the placenta express high levels of globoside in the first trimester, progressively decreasing (Jordan and DeLoia, 1999). Trophoblasts are not permissive to the virus but may bind viral capsid via the globoside receptor, hence their role in facilitating transcytosis of the virus to fetal circulation (Jordan and Butchko, 2002; Wegner and Jordan, 2004). On the other hand, endothelial placental cells can support viral replication contributing to placental and fetal damage (Pasquinelli et al., 2009). When in fetal circulation, the virus can infect erythroid progenitor cells, in the liver, or the bone marrow depending on the gestational age and can be detected in cells circulating in the vessels of several tissues as well as in the amniotic fluid (Bonvicini et al., 2011). The block in fetal erythropoiesis can be severe, mainly due to the physiologically expanded erythropoietic compartment combined with an immature immune response, and lead to fetal anemia, tissue hypoxia, possible development of nonimmune hydrops, or possible fetal death (Puccetti et al., 2012).

The natural course of fetal infection is affected by several factors. Firstly, the gestational stage plays a role since it determines the expression levels of globoside in the placenta, but also the pattern of erythropoiesis in the fetus. Secondly, the immune status of the mother; normally an intrauterine infection occurs as a primary infection. Clinical fetal manifestations vary from anemia, tissue hypoxia, and nonimmune hydrops to death (Bascietto et al., 2018; Xiong et al., 2019). Consequences are more severe in the first two trimester, while late intrauterine fetal death is a rarer event (Enders et al., 2004). Despite the relatively high risk and consequences, antenatal screening in women of childbearing age is not presently recommended by guidelines, and diagnostic testing is mainly advised in case of a suspected diagnosis. These statements appear mainly due to a lack of appreciation of the pathogenic potential of the virus coupled to the limited availability of prophylactic or therapeutics interventions.

6. Immune response

6.1. Innate immunity

The role of innate immunity in contrasting B19V infection has not been adequately investigated. In principle, infecting B19V virions might be recognized through PAMPs by cellular PRRs as other viruses do, and general symptoms related to cytokine production are typical of a prodromal phase of infection. Examination of cytokine responses to B19V infection shows that they are of the Th1 type, with IL-2, IL-12, and IL-15 being detected in acutely infected patients, correlating with the sustained CD8+T cell response. There is no imbalance of cytokine patterns in persistent infection, except for an elevated IFN-γ response (Isa et al., 2007). Detailed knowledge on the process, what component of the virus may be recognized and act as PAMPs and what cellular system may act as sensor is still open to investigation (Wu et al., 2016). The viral genome is a ssDNA, devoid of stimulatory sequences; however, its terminal regions are GC-rich and can be recognized by receptors such as TLR9, such as the CpG-ODN 2006 TLR9 ligand (Guo et al., 2010). This molecule selectively inhibits erythroid cells growth and induces the accumulation of cells in S and G (2)/M phases and consequent apoptosis, features that are similar to those observed in erythroid progenitors infected with B19V. Experiments in COS-7 cells transfected with expression vectors producing NS or VP proteins indicated that NS played a major role in inducing both short and long-term upregulation of defensins and TLR9, with some effects also played by VP2 protein (Hsu et al., 2011). Finally, B19V genome may have been evolutionary shaped by its interaction with cellular restriction factors, such as members of the APOBEC family (Poulain et al., 2020). Overall, further work is required to elucidate the interaction of B19V with the cellular innate recognition pathway and cellular antiviral restriction patterns.

6.2. Adaptive immunity: humoral response

Antibodies are the hallmark of the adaptive immune response to the virus and a critical diagnostic parameter (Gallinella, 2018). In naive individuals, B19V-specific antibodies are produced early after infection and are assumed to be able to neutralize viral infectivity, leading to a progressive clearance of infection. IgM antibodies are initially produced at 8–12 days post-infection and last for 3–6 months. The production of IgG antibodies follows IgM a few days later. During the following weeks and months, IgM antibodies wane to an undetectable level, whereas IgG antibodies prevail (Manaresi et al., 2002). IgA antibodies have also been detected and probably protect nasopharyngeal mucosa (Erdman et al., 1991). The antibody response is mainly directed against both structural proteins, and several epitopes have been identified on VP2 and VP1 (Soderlund et al., 1995a, 1995b). The capsid shell is known to present mainly conformational epitopes, and an atomic structure of fab binding to capsid has been resolved (Sun et al., 2019). Epitopes on the VP1+2 common region have been mapped in several regions dispersed on the capsid surface (Sato et al., 1991; Yoshimoto et al., 1991), and probably act by impairing binding to globoside and virion disassembly following internalization. Epitopes on the VP1u region are mainly linear (Saikawa et al., 1993; Anderson et al., 1995) and have been mapped close to N-terminus (Zuffi et al., 2001), corresponding to the coreceptor binding moiety, and probably act by blocking such specific interaction. In fact, most of the neutralizing epitopes of VP1 are localized in the VP1u or the VP1-VP2 junction region, eliciting a more efficient response than VP2 epitopes (Manaresi et al., 1999, 2001; Musiani et al., 2000). B19-specific B cell memory is well established and maintained against conformational epitopes of VP2 and linear epitopes of VP1 but not linear epitopes of VP2 (Corcoran et al., 2004). The development of a neutralizing activity is typical of a mature and effective immune response, while antibodies with incomplete neutralizing activity are typical of persistent infections. The presence of antibodies against NS1 has also been documented in about 30% of subjects with recent or chronic infection, although it is more associated to a pattern of past immunity and its role does not appear relevant (Venturoli et al., 1998; Heegaard et al., 2002).

6.3. Adaptive immunity: cellular response

B19V-specific cellular immunity is directed against both the capsid proteins VP1 and VP2 and the nonstructural protein NS1. T-cell-mediated immune responses to infection were first shown by measuring the proliferative responses of PBMC following in-vitro stimulation by recombinant VP1, VP2, and NS proteins. Results indicated the presence of B19V-specific cellular immunity directed against the capsid proteins VP1 and VP2, presented to $CD4^+$ T cells by HLA class II molecules (von Poblotzki et al., 1996). B19V-specific T helper cell proliferation was detected in infected patients, and B cells recognizing the VP1/2 capsids receive class II-restricted help from $CD4^+$ T cells. $CD8^+$ T cell responses were also observed in patients acutely infected with the virus, remaining sustained over months, even after viremia clears (Tolfvenstam et al., 2001). Ex vivo measurement of B19V-specific CD8+T cell responses confirmed that an HLA-B35 restricted peptide derived from the NS1 protein is highly immunogenic in B19V-seropositive donors (Norbeck et al., 2005). In contrast, persistently infected individuals show more cellular immune responses to VP1 and VP2 than to NS1 (Isa et al., 2005, 2006). The $CD8^+$ T cell response may thus play a prominent role in the control of productive infection (Franssila and Hedman, 2004; Franssila et al., 2005). Both the VP1/2 and VP2-only capsids stimulate T helper cells to release gamma interferon (IFN-γ) and IL-10, suggesting that VP2 provides the major target for B19V-specific T helper cells years after virus infection. Overall, B19V-specific T-cell responses are peculiar, combining characteristics typical of viruses capable of lytic infections as well as capable of establishing a persistent infection, with the requirement for continuous surveillance by the immune system.

6.4. Vaccine development

The development of a vaccine for B19V has been a long and still open issue. In principle, a vaccine would be useful and effective, as B19V is a virus adapted exclusively to the human host, transmitted by direct interpersonal contact, and effectively neutralized by the immune response. On the other hand, infection is in most cases subclinical; clinical consequences are mostly mild and self-limiting, or on the opposite with a tendency to chronicization even in the presence of an immune response, so the impact on global health of a vaccine might be questioned. On the opposite, a rationale for the development and introduction of a vaccine would mainly be to protect at-risk populations, such as patients with underlying hematological disorders or non-immune women of childbearing age to avoid intrauterine transmission (Penkert et al., 2020).

A vaccine against B19V is an attainable goal, and technically feasible (Kajigaya et al., 1989, 1991). Candidate vaccines composed of VP2 or VP2+VP1 proteins, produced in heterologous expression systems such as Baculovirus or yeasts, self-assembling in VLPs are highly effective as immunogens in animal experimental systems (Bansal et al., 1993). First-generation candidates were tested successfully in a phase 1clinical trial (Ballou et al., 2003), but proved to be too reactogenic in a phase 2a trial (Bernstein et al., 2011), so this line of development was actually discontinued. Attributing the observed adverse effects to the VP1 associated PLA2 enzymatic activity, recently developed vaccine candidates have been engineered by introducing an inactivating mutation in the region (Chandramouli et al., 2013). Vaccine candidates have a good immunogenicity and a potentially better safety profile but have not yet been tested further than in unrelated animal models (Penkert et al., 2017). On the whole, because of all limitations, research on a B19V vaccine is still in its initial phase and its implementation is not included among the WHO priorities (1).

7. Prevention and therapy

Most B19V infections are mild and self-limiting, but in some situations, they can lead to more severe presentations requiring clinical care. Typically, this occurs in patients with underlying hematological disorders or in patients with immune system deficits, while the sporadic occurrence of atypical presentations calls for inclusion of B19V in the diagnostic workout (Gallinella, 2018). In addition, the risk of infection in pregnancy with its possible consequences on the fetus is of major concern (Bonvicini et al., 2017a). Therefore, a prompt and accurate diagnosis of infection is required, and a comprehensive approach including prophylactic, therapeutic, and monitoring actions should be considered for future directions. Actually, this is a current field of research and progress in diagnostic and antiviral strategies can lead to a more appropriate and proactive attitude.

7.1. Screening and blood products' safety

The presence in the course of infection of a high-titer early viremic phase in absence of specific symptoms, as well as the delayed clearance of the virus from the bloodstream and possible occurrence of low-titer chronic infections, may pose a question regarding the risk of transmission of the virus through the use of blood, blood components, or blood products (Juhl and Hennig, 2018). The major elements playing a role in determining the clinical outcome of parenteral exposure to the virus, are the total amount of virus transfused to recipients and the immune status and competence of the receiving subjects. Reported experience indicate possible viral concentration levels necessary for the transmission of infection. In the absence of specific IgGs in both donor and recipient, a threshold level of about 1e7 International Units (IU) per mL seems necessary to obtain an infection, as determined by seroconversion and viremia in recipients (Brown et al., 2001). The presence of specific antibodies in the donated blood interferes with infectivity, and

the presence of previous immunity in recipients seems to be protective. Thus, given both the reported frequencies of high-titer viremic blood units and seroprevalence rates in the population, the probability of infection by exposure to single blood or blood component units is low, as also shown in large-scale donor-recipient studies (Kleinman et al., 2012).

A quite different situation is present for blood products, that are manufactured from large pools of donations. In these cases, the inclusion of high titer viremic units in the manufacturing pools can be expected with high probability, and dilution during the manufacturing process may not be sufficient to reduce viral low below a safety threshold level. Hence, screening of manufacturing pools to discard contamination above 1e4 IU/mL has been required firs as a voluntary standard and thereafter by regulatory bodies (Marano et al., 2015). In addition to screening and removal of high-titer contaminated donations, physical removal (Roth et al., 2020) or inactivation steps normally included in the downstream processing of plasma-derived products (Blumel et al., 2010; Hellstern and Solheim, 2011) have been shown effective in reducing infectivity of B19V, though its small dimensions and relatively high stability to heat and chemicals, further reducing the residual risk of transfusion-transmitted infections.

7.2. Treatment

Specific prophylactic and therapeutic options for B19V are limited. Occasionally, acute- or chronic phase symptomatology may require symptomatic treatments. Transfusions are required to treat the anemia in cases of transient aplastic crisis or prolonged anemia. In cases of arthralgias, nonsteroidal anti-inflammatory drugs (FANS) may exert beneficial effects. In cases of fetal infections, intrauterine transfusions are indicated when the hemoglobin concentration in the fetal circulation falls below a threshold level (Bonvicini et al., 2017a).

Administration of high doses of intravenous immunoglobulins (IVIG) is currently considered the only available option to neutralize the infectious virus in its viremic phase, and mainly finds indications to control infections in cases of an impaired immune system response such as in immunodeficient or immunosuppressed patients (Crabol et al., 2013). IVIG preparations, being prepared from large pools of donors representing the collective immune memory of a population, usually contain high levels of neutralizing anti-B19V antibodies and can be used with success to reduce the viral load (Modrof et al., 2008). The treatments are generally found to be beneficial, but high doses and repeated cycles are normally required, while passive immunization per se it is not sufficient to resolve infection in patients unless the development of own neutralizing immune response. On the whole, experience on IVIG is accumulating on empirical basis, and there is a lack of an extensive and controlled study of the efficacy of passive immunization therapeutic or prophylactic schemes. Given the latest technological developments, Human monoclonal antibodies can be considered a more effective option. In the past, a few antibodies have been developed (Gigler et al., 1999), but their therapeutic or prophylactic use has not been evaluated, so this option remain to be further investigated.

7.3. Antiviral drugs development

Given the absence of a vaccine, and the limited efficacy of symptomatic treatments including passive immunization options, active research in the development and refinement of specific antiviral strategies for B19V should be considered as a priority (Manaresi and Gallinella, 2019; Hu et al., 2022). So far, research on this field has lagged in comparison to other viruses, some factors being critically limiting in the search for compounds with antiviral activity against B19V. Besides the lack of animal models, the virus has a narrow cell tropism and depends largely on host cell factors for its replication. *In vitro*, the virus requires demanding cell culture conditions, in particular when using *in vitro* differentiated erythroid progenitor cells, while infections always show a restrictive pattern with relatively low productivity. Finally, infections are normally carried out by using native virus from viremic patients, thus limiting the possibility of genetic investigation and the identification of druggable targets. This last difficulty has been overcome by the development of B19V synthetic genomes and the possibility of generating infectious virus (Manaresi et al., 2017), and of further genetic manipulation (Reggiani et al., 2022). In addition, the production, purification and characterization of viral proteins up to molecular structural details, allow the development of rational drug design, or the setup of medium-to high-throughput *in vitro* assays for evaluation of target-specific inhibitory activity following screening of chemical libraries (Xu et al., 2019).

In recent years research has progressed based mainly on three directions: i) retargeting of known drugs for a possible activity against B19V; ii) serendipity testing of compounds with promising potential for a possible antiviral activity; iii) chemical library screening, from in silico to *in vitro*. Concerning the first approach, some compounds have indeed shown to possess inhibitory activity against B19V. Hydroxyurea is an antiproliferative drug used in the treatment of sickle-cell disease that through inhibition of ribonucleotide reductase also possesses antiviral properties. Experiments indicated that it also has inhibitory activity against B19V at concentrations normally attained in blood during maintenance therapy (Bonvicini et al., 2017b). Therefore, a dual beneficial effect can be attributed to hydroxyurea in this patient category, in agreement with the observed protective effect against the severe consequences of infection observed in a cohort of treated pediatric patients (Hankins et al., 2016). On a wider scope, the nucleotide analog Cidofovir (CDV) is a broad-range antiviral mostly active against dsDNA viruses that was also tested for an antiviral activity against B19V, a ssDNA virus which in its replicative form is a dsDNA molecule depending for its replication on cellular metabolism and replicative machinery. In *in vitro* system, CDV indeed showed an antiviral activity including B19V (Bonvicini et al., 2015). Although inhibition of replication could not be abrogated in target erythroid progenitor cells in a single round of replication, extended exposure to the compound in serial passages attained the scope (Bonvicini et al., 2016). The use of CDV is not favored because of its unfavorable toxicity profile, but in a single reported experience it proved to be effective in clearing a chronic infection in an immunosuppressed patient (Nair et al., 2020). To overcome general limits of CDV, Brincidofovir (BCV) has been developed as a lipid derivative of CDV with improved efficacy, bioavailability and safety profile. Although its use as a broad-range antiviral did not yield expected results in clinical trials, its testing on B19V confirmed an increased efficacy compared to BCV (Bua et al., 2019), also inhibiting replication in erythroid progenitor cells, thus opening the possibility of a therapeutic use of this molecule in selected cases.

Further research indicated a few possible lines of development in antivirals. Newly synthesized coumarin derivatives, which showed a modest but measurable inhibiting activity, offer possibilities as scaffolds for further design of molecules with antiviral activity (Conti et al., 2019). A first Identification of some flavonoid molecules, with direct inhibitory activity against NS1 protein, indicates a possible line of development for direct antiviral agents (Xu et al., 2019). This line of research, based on serendipity or screening of small chemical libraries, has been expanded through a high-throughput screening, leading to identification of a specific purine derivative inhibiting the NS1 endonuclease activity, therefore directly acting as a target-specific antiviral agent (Ning et al., 2021). Continuing research in the field is expected to provide better characterization of viral proteins at a structural level, detailed knowledge of the viral lifecycle and a precise understanding of virus-cell interactions. This would expand the set of relevant pharmacological targets and will offer novel opportunities for developing more efficient, targeted antiviral agents, which can be translated into available therapeutic options.

8. Final remarks

Parvovirus B19 infection is highly prevalent in the general population. Since the infection is usually asymptomatic or causes mild, self-

limiting diseases, the virus often remains clinically underestimated. However, it is important to remember that in some "at risk" patients, such as those with hematological or immune disorders, infection can have profoundly serious consequences. These are the patients who could benefit from vaccination or from specific antiviral therapy.

For many years the study of the pathogenic potential of B19V has been hampered by the lack of methodological tools, mostly by the lack of cell cultures to propagate the virus, to study viral replication and cellular alterations due to the infection. The improvement of the methods that allow the study of the virus-cell interactions at the molecular level, as well as the introduction of the new sensitive techniques for the detection of the virus in clinical samples have made it possible to demonstrate that B19V can infect many kinds of cells and can induce their alterations even if the cells are not permissive for viral replication. Therefore, the range of pathologies caused by B19V could be much wider than that currently known and could also include diseases of strong clinical relevance.

Currently, there is strong evidence that the virus could be involved in various pathologies involving endothelial and connective tissues, or autoimmune diseases. For this reason, further studies are necessary to understand the real significance of parvovirus B19 in the human diseases. Moreover, the study and development of vaccines and of new antiviral drugs to prevent and treat B19V infections in the patients at risk to develop severe diseases, is necessary.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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