Human Parvovirus B19: Molecular Virology, Clinical Features, Prevalence, Diagnosis and Control

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Parvovirus B19-introduction

Parvovirus B19 (B19) is an erythrovirus and recent studies have classified B19 as a genotype 1 erythrovirus with genotypes 2 (erythrovirus K71 or A6) and 3 (erythrovirus V9) also present in the human population. B19 is a significant human pathogen which can cause foetal hydrops and foetal death if maternal infection, followed by transplacental foetal infection, occurs during pregnancy. The virus is also transmitted by inter-personal contact and potentially via blood product administration. Symptoms of B19 infection include malaise, rash and anthralgia. Significantly, maternal B19 infection during pregnancy can be asymptomatic and so careful monitoring of at-risk pregnancies is recommended. Both antibody- and cell-mediated immunity play an important role in the anti-viral response and effective diagnostic test systems, for both B19 antibody and DNA detection, are now available. B19-induced foetal hydrops can be effectively treated by intrauterine blood transfusion; however, no vaccine is available to prevent infection at present.

Molecular virology

Human parvovirus B19 (B19) was first identified in 1975 by Yvonne Cossart (Cossart et al., 1975). The virus was first associated with disease in 1981 when it was

\textsuperscript{☆}This chapter is dedicated to Ben.
linked to an aplastic crisis in a patient with sickle-cell disease. Subsequently, B19 has since been shown to be the causative agent of erythema infectiosum (EI) (Fifth disease of childhood), spontaneous abortion and some forms of acute arthritis (Anderson et al., 1983; Kinney et al., 1988; Woolf and Cohen, 1995). B19 is approximately 20 nm in diameter, has a genome of 5.6 kb (Clewley, 1984; Cotmore and Tattersall, 1984) and is a small, non-enveloped, single-stranded DNA virus. Like all parvoviruses, the constituent capsid proteins (VP1 and VP2) are arranged with icosahedral symmetry. The B19 capsid consists of an 83 kDa low-abundance structural protein, VP1, and a 58 kDa major structural protein, VP2. VP2 makes up about 95% of total capsid structure with VP1 accounting for the remaining 5% (Ozawa et al., 1987). The sequences of the two proteins are co-linear and the entire VP2 sequence is identical to the carboxyl-terminus of VP1. However, VP1 comprises an additional 227 amino acids unique to the amino-terminal, the so-called VP1 unique region (VP1u). To the left of these sequences on the B19 genome is the open-reading frame for a non-structural protein, NS1 which encodes a 77 kDa protein. NS1 is a phosphoprotein with important regulatory functions including transcriptional control (Momoeda et al., 1994a, b), virus replication and also plays a role in host cell death (Ozawa et al., 1988). NS1 also exhibits DNA-binding properties (Raab et al., 2002), and a multitude of enzymatic functions including ATPase, helicase and site-specific endonuclease activity, as well as containing nuclear localisation signals (Li and Rhode, 1990; McCarthy et al., 1992; Jindal et al., 1994; Brown and Young, 1998). It has been demonstrated that B19 NS1 also effects G(1), but not G(2), arrest in erythroid UT7/Epo-S1 cells (Morita et al., 2003).

A single promoter, p6, is employed by B19 which is capable of differentially expressing both structural and non-structural genes (Blundell et al., 1987; Ozawa et al., 1987). The NS1 protein interacts directly with the p6 promoter and with cellular transcription factors Sp1/Sp3 to effect transcriptional regulation (Raab et al., 2002). Two additional smaller polypeptides (p7.5 and p11) have been identified, one encoded by a region near the centre of the B19 genome with a predicted $M_r$ of 7.5 kDa and the other which is encoded at the extreme right-hand end of the genome of predicted $M_r = 11$ kDa (St Amand et al., 1991). Spliced transcripts of both proteins have been detected in infected cells but specific functions have not, as yet, been assigned to either protein (Luo, 1993).

As a result of the increased interest in, and molecular detection of, parvovirus B19 a number of new erythrovirus genotypes have been identified. Servant et al. (2002) has suggested that B19 be classified as a genotype 1 erythrovirus with newly identified strains A6 (Nguyen et al., 2002) and K71 (Hokynar et al., 2002) classified as genotypes 2 and erythrovirus V9 (Nguyen et al., 1998) as the prototype genotype 3. Based on inter-genotype phylogenetic relationships between NS1 and VP1u regions, respectively, erythrovirus genotype 3 (erythrovirus variant V9) has been shown to be the most prevalent erythrovirus in Ghana and appears to divide into two sub-types (Candotti et al., 2004).

B19 has only been shown to infect humans and replicates in human erythroid progenitor cells (late erythroid cell precursors and burst-forming erythroid pro-
genitors (BFU-E)) of the bone marrow and blood, resulting in an inhibition of erythropoiesis (Mortimer et al., 1983). Brown et al. (1993, 1994) elegantly demonstrated that the restrictive tropism of productive B19 infection is primarily due to the P blood group antigen, globoside (Gb4). This molecule is a significant cellular receptor for B19 and is most often found on cells of the erythroid lineage but also on platelets and tissues from the heart, kidney, lung, liver, endothelium and on synovia (Cooling et al., 1995; Jordan and DeLoia, 1999). Individuals lacking erythrocyte P antigens are very rare (1 in 200,000) and exhibit much diminished susceptibility to B19 infection (Brown et al., 1994; Chipman et al., 1996). However, the limited tropism of B19 is not fully understood as low-level capsid expression has actually been observed in non-permissive cells; nonetheless, intracellular factors unique to erythroid cells are thought to be essential for optimal transcription and viral replication (Ozawa et al., 1987; Kurpad et al., 1999; Gallinella et al., 2000).

Weigel-Kelley et al. (2001) have demonstrated that the P antigen-expression level on cell surfaces is not directly related to the efficiency of viral binding. In addition, despite P antigen expression and viral adherence to the P antigen, some cell lines could not be transduced with a B19 vector thereby indicating that a co-receptor is likely to be necessary for B19 entry into human cells. Thus, the presence of P antigen alone is not sufficient for B19 to gain entry into cells (Weigel-Kelley et al., 2001) and it has been suggested that multiple β-integrins may additionally function as co-receptors for B19 cellular uptake (Weigel-Kelley et al., 2003).

In vitro studies of B19 infectivity have been greatly hampered by difficulties in propagating the virus in vitro. Indeed, no continuous cell line propagating B19 has been established, due to the cytotoxic nature of the non-structural protein, NS1 (Ozawa et al., 1987; Momoeda et al., 1994). However, recent studies have shown that infection under hypoxic conditions (1%(v/v) O2) causes an upregulation of B19 expression which is associated with increased viral replication and of infectious virion production (Pillet et al., 2004). It has been demonstrated that hypoxia-inducible factor-1 (HIF-1), a key transcription factor involved in the cellular response to reduced oxygenation, binds an HIF-binding site (HBS) present in the B19 promoter region (Pillet et al., 2004). In a parallel work, Caillet-Fauquet et al. (2004) have shown that human plasma containing known amounts of B19 DNA (virus) could infect the human erythroid cell line KU812F and that under low oxygen pressure, higher yields of infectious B19 progeny virus and increased viral transcription were observed. These authors also demonstrated that anti-B19 IgG reduced B19 infectivity and suggest that this system represents a promising model to study B19 infectivity and infectivity control methodologies.

Transmission of B19 infection most often occurs by personal contact via aerosol or respiratory secretions, however contaminated blood products such as clotting factor concentrates are also a source of iatrogenic transmission (Anderson et al., 1985; Lyon et al., 1989; Williams et al., 1990; Santagostino et al., 1994; Erdman et al., 1997). Significantly, B19 can also be transmitted transplacentally from an infected mother to the foetus, on occasion leading to non-immune foetal hydrops (NIHF), spontaneous abortion or intrauterine foetal death (IUFD) (Clewley et al.,
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1987; Miller et al., 1998; Skjoldebrand-Sparre et al., 2000). A range of vertical transmission rates for maternal–foetal infection of 7–33% have been reported (Enders and Biber, 1990; Hall et al., 1990; Yaegashi, 2000). The P blood group antigen, which acts as a receptor for B19, has been detected on cells of the villous trophoblast of placental tissues in varying amounts during the course of pregnancy. In the first trimester, P antigen levels are elevated, begin to decline in the second trimester and become undetectable by the mid-stages of trimester 3 (Jordan and DeLoia, 1999). It has been proposed that this high level of globoside receptor on placental cells in early pregnancy may act as a pathway for maternal–foetus B19 transmission whereby the virus can then infect erythroid precursor cells for replication. Indeed, Wegner and Jordan (2002) have conclusively shown that I\(^{125}\)-labelled B19 VP2 capsids interact with villous cytotrophoblast cells via the P antigen. Moreover, in a detailed review of viral transmission at the uterine–placental interface, Pereira et al. (2005) have shown that cytotrophoblasts infected with human cytomegalovirus exhibit altered differentiation patterns and suggest that such aberrant behaviour may contribute to foetal growth restriction.

Clinical features and prevalence

B19 has been associated with an ever-expanding range of clinical disorders since the discovery that it is the aetiologic agent of EI, a facial and occasionally body-wide red rash. It is also associated with complications during pregnancy, acute arthropathy, severe disease in immunocompromised patients and transient aplastic crisis.

General clinical features and chronic infection

Infection with B19 occurs worldwide and cases of infection have been reported in all seasons. Seroprevalence increases with age and by adulthood at least 70% of the adult population are B19 IgG seropositive (Cohen and Buckley, 1988). B19 outbreaks can persist for months in schools as seronegative children represent a significant reservoir for B19 infection. Recently infected children therefore are the main source of transmission in day-care centres due to the relatively large number of seronegative children and the close contact of children within this environment (Tuckerman et al., 1986; Grilli et al., 1989). The annual seroconversion rate among women of childbearing age has been estimated to be 1.5% during endemic periods and 13% during epidemics (Koch and Adler, 1989; Valeur-Jensen et al., 1999).

B19 infection has also been linked to arthritis and arthralgias, most commonly in adults but also in children (Reid et al., 1985) as has been documented for the rubella virus (Lee, 1962). On average, 50% of adult cases reported with EI have associated joint symptomologies, which may last for up to 1 month (Cassinotti, 1995). B19 arthritis is usually symmetrical and affects mainly the small joints of the hands, wrists and knees (Reid et al., 1985). It is more common in females and an estimated 60% of women with symptomatic disease exhibit signs of arthropathy.
Symptoms generally diminish within 3 weeks without any permanent joint damage (Woolf et al., 1991), however about 20% of affected women suffer a persistent or recurrent arthropathy. About 75% of these patients have an associated rash and less than 20% have the typical ‘slapped cheeks’ facial exanthem. It has been hypothesised that B19-associated arthritis may be related to certain patient human leucocyte antigen (HLA) haplotypes, with individuals classified as either HLA DR4 or B27 exhibited increased susceptibility (Klouda et al., 1986; Jawad, 1993). At present, it is unclear how B19 produces symptoms associated with arthritis. Like the appearance of exanthema in EI, arthritis usually occurs after the development of B19-specific antibodies which suggests that symptoms may be due to immune-complex formation. Although the P antigen is expressed on synovium, it has been shown that synovial membrane cells are non-permissive to B19 (Miki and Chantler, 1992; Cooling et al., 1995). B19 may gain entry to B19 receptor positive cells, that are not actively dividing, resulting in the production of excessive, cytotoxic NS1 (Ozawa et al., 1988). The presence of the NS1 protein induces the expression of pro-inflammatory cytokines, which could cause the inflammation and cell damage seen in patients with B19-associated arthritis and other inflammatory and autoimmune disorders which have been associated with B19 infection (Moffatt et al., 1996; Mitchell, 2002). The precise significance of antibodies against the non-structural protein is unclear. Antibodies specific for NS1 were found in individuals manifesting persistent B19-associated arthropathy but not in convalescent serum (von Poblotzki et al., 1995a) thus suggesting a differential host response in these cohorts. However, similar NS1 antibody reactivity in patients with either chronic or acute B19-associated arthropathy (Mitchell et al., 2001) and recently infected healthy individuals (Searle et al., 1998; Ennis et al., 2001; Mitchell, et al., 2001; Heegaard et al., 2002b). Von Landenberg et al. (2003) have suggested that B19 may be directly involved in the induction of autoimmune reactions mediated, at least in part, by anti-phospholipid antibodies because of the prevalence of these antibodies in persistently B19-infected individuals.

Chronic infection with B19 may be of significance in pregnancy because, it can be speculated that, the altered maternal immune status may facilitate re-activation of viral infection (Bültmann et al., 2005). However, the clinical significance of chronic B19 infection is often unclear. Chronic infection may be due to the fact that B19 DNA persists in bone marrow, peripheral blood and synovial tissues of patients with chronic B19-associated arthropathy (Foto et al., 1993; Musiani et al., 1995; Toivanen, 1995). However, it has also been shown that although B19 DNA persisted in the synovium tissue of 28% of children presenting with chronic arthritis, an even higher proportion (48%) of seropositive immunocompetent volunteers had B19 DNA in their synovium tissues. These data suggest that B19 DNA in synovial tissue may be indirectly associated with symptoms of chronic arthropathy. None of the individuals tested had evidence of B19 DNA in synovial fluid, bone marrow or blood and all were positive for B19 IgG antibodies (Søderlund et al., 1997). Nonetheless, a recent report further enhances the correlation
between B19 infection and rheumatic childhood disease (Lehmann et al., 2003). This work clearly elucidates a significant difference in serum and/or synovial fluid-derived B19 DNA ($P < 0.0001$) between control (9/124; 7%) and patient (26/74; 35%) specimens and concludes that the rate of persistent B19 infection in these patients is significantly higher than in age-matched controls. However, the recent finding of B19 DNA in 64% (14/22) of control skin biopsies compared to 50% (18/36) of chronic urticaria patients confirms that caution should be exercised in drawing conclusions regarding B19 involvement in skin disorders, and possibly in other B19-associated clinical disorders (Vuorinen et al., 2002).

**B19 infection and pregnancy**

Exposure to, and infection with B19 can lead to serious complications during pregnancy (Fig. 1). Infection during pregnancy may result in foetal anaemia, spontaneous abortion and hydrops foetalis (Brown et al., 1984; Kinney et al., 1988; Heegaard and Hornsleth, 1995). Seroprevalence studies have shown that approximately 30–40% of women are not immune, therefore do not possess B19 antibodies, and are consequently at risk of B19 infection. A vertical transmission rate of 33% has been reported by the United Kingdom Public Health Laboratory Service (PHLS, 1990) and similar rates have been reported in other studies (Brown et al., 1984; Hall et al., 1990), although a recent report has disclosed a transmission rate of 51% (Yaegashi, 2000). On the basis of 4 million births occurring in Europe per annum (Eurostat, 1998), an average B19 seronegativity of 30% among pregnant women and a seroconversion rate for women of child-bearing age of 0.1–1%, it can be estimated, that up to 14,000 women will seroconvert during pregnancy. About 20% of B19 infections in pregnancy are thought to result in foetal loss, thus implying that 2800 incidences of foetal death will occur each year, in Europe, as a consequence of B19 infection (Levy et al., 1997; Miller et al., 1998; Wattre et al., 1998). This figure is based solely on live births and as the number of actual pregnancies is much higher, the above estimate is conservative. Based on overall birth rates, similar incidences of foetal death due to B19 infection can be predicted for the US and Canada.

Pregnant women are most susceptible to B19 infection during epidemics and also when exposed to recently infected children in the home (Valeur-Jensen et al., 1999). During outbreaks, transmission rates of 25% in the school and 50% in the home have been reported (Anderson et al., 1990). It is somewhat unfortunate that most pregnant women acutely infected with B19 remain asymptomatic (Fig. 1), however some do experience symptoms such as exanthema and arthralgia (Komischke et al., 1997; Enders et al., 2004). As these symptoms are commonly associated with pregnancy, acute B19 infection can often be overlooked, however routine analysis of maternal immune status with respect to B19 infection would overcome this problem. Although open to debate, we propose that pregnant women should be carefully monitored during pregnancy for acute B19 infection due to the high level of seroconversion in young women and because infection with
B19 during pregnancy can lead to spontaneous abortion or foetal anaemia. Given the availability of standardised and reliable diagnostic test systems, such screening should be relatively straightforward to implement.

Foetal death usually occurs 4–6 weeks postinfection but has been reported up to 12 weeks after B19 symptomatic infection (Hedrick, 1996). A study of 427 pregnant women with B19 infection in the UK found that foetal loss was limited to the first 20 weeks of gestation (Miller et al., 1998). This is supported by figures released in the UK and other studies which reported that foetal loss as a consequence of intrauterine B19 infection is highest in, but not restricted to, the first 20 weeks of gestation (PHLS, 1990; Hall, 1990). The outcome of one of the largest prospective studies of foetal complications as a result of serologically confirmed maternal B19 infection ($n = 1018$ individuals) was published in 2004 and provides some of the most important information to those involved in the management of

Fig. 1 Parvovirus B19 infection in pregnancy—possible outcomes. Exposure to B19 during pregnancy may result in either symptomatic or asymptomatic infection. Symptomatic infection can generally be identified by B19 serological or virological analyses. In the case of asymptomatic maternal infection, ultrasound and foetal haemoglobin determination will be required to diagnose foetal hydrops, in addition to B19 DNA detection. Recent evidence indicates that intrauterine foetal transfusion may be an important tool in the successful treatment of severe foetal hydrops (Enders et al., 2004).
B19-infected maternal infection (Enders et al., 2004). Over a 5-year period (1993–1998), B19 infection was serologically confirmed in 1018 pregnant women, 73% of whom presented without the classical symptoms of B19 infection such as rash and arthropathy. The incidences of hydrops foetalis and foetal death were 3.9% (40/1018) and 6.3% (64/1018), respectively with all foetal deaths occurring prior to 20 weeks gestation (11%; 64/579). The risk of foetal hydrops was highest when infection occurred during gestation weeks 13–20 (7.1%; 23/322). Intrauterine foetal transfusions were administered in 13/23 cases of severe foetal hydrops and a survival rate of 85% (11/13) was observed. Strikingly, no foetal survival was evident in the remaining 10/23 cases of severe foetal hydrops where intrauterine transfusion was not administered. Thus one of the major findings of this study has been that prompt intervention, via intrauterine transfusion following confirmation of severe foetal hydrops, can contribute to a reduction in the rate of foetal death (Fig. 1) (Enders et al., 2004).

Anaemia is, therefore, a key underlying factor in the development of hydrops. Foetal hydrops was initially associated with B19 infection in 1984 (Brown et al., 1984). Since then, 10–20% of cases of NIHF have been reported to be B19 associated (Yaegashi et al., 1994; Jordan, 1996), and in a study of B19 infection in Japanese women during pregnancy, the risk of hydrops was determined to be about 10% (Yaegashi et al., 1999). NIHF usually occurs 2–4 weeks after maternal B19 infection (Komischke et al., 1997). Cases of IUFD associated with foetal hydrops and caused by B19 have been most commonly reported in the second trimester and to a lesser extent in the third trimester of pregnancy (Sanghi et al., 1997). When cases of IUFD occurring during an 18-month period in the UK were examined it was discovered that 11 deaths were caused by B19 in the second trimester, and of these only three were hydropic (Wright et al., 1996). In a separate study over a 16-year period, 10 cases of IUFD were reported which were presented in gestational weeks 15–29. Of those cases, 90% of the fetuses were hydropic, 30–40% had associated heart failure and three of the maternal infections had been asymptomatic (Morey et al., 1992).

The critical time of infection has since been narrowed down to the 16th week of gestation (Yaegashi et al., 1999) with most cases of foetal loss due to B19 infection reported in the second trimester (Enders and Biber, 1990; Torok, 1990; Wattre et al., 1998). This susceptibility can be attributed, at least in part, to the relative immaturity of the foetal immune response at this stage. More importantly though, is the tropism B19 has for erythroid progenitor cells (Yaegashi, 2000) and the fact that in the second trimester of pregnancy the lifespan of foetal red blood cells (RBCs) is shortened and the RBC mass increases 3- to 4-fold during this period of gestation (Rodis et al., 1988). During an infection, B19 replication occurs within these cells which undergo apoptosis resulting in an inhibition of erythropoiesis (Morey et al., 1993). As B19 replicates, cell lysis occurs causing erythroblastopenia and therefore severe foetal anaemia, which may be fatal to the foetus. Indeed, Norbeck et al. (2004) have recently demonstrated that VP2 protein alone, or component peptides, have the potential to inhibit haematopoiesis both in and \textit{ex vivo}
and suggest its potential use for the treatment of diseases such as polycytemia vera. However, direct inhibition of haematopoiesis by B19 VP2 may also be associated with the pathogenicity of B19 infection towards the foetus via disruption of red blood cell maturation.

Third-trimester foetal loss or IUFD caused by an acute B19 infection had not been widely reported until recently. Skjoldebrand-Sparre et al. (2000) reported that of 93 cases of IUFD examined, 7.5% had B19 DNA in the placental tissue in the absence of foetal hydrops. None of the infected pregnant women in this study showed any clinical symptoms of B19, which again reinforces the proposal of routine screening for B19 exposure during pregnancy. Skjoldebrand-Sparre et al. suggested that, in the past, B19-associated IUFD in the final stages of gestation may have been overlooked due to inadequate diagnostic procedures and also the difference in clinical features of third-trimester B19 infection. However, one of the most unusual observations in these cases of IUFD was the lack of foetal hydrops and the fact that many of the cases (5/7) had either delayed or absent B19 IgG responses. Histopathological examination of the foetuses revealed no major abnormalities. Another report of non-hydropic third-trimester IUFD associated with B19 infection has been published (Tolfvenstam et al., 2001a). Here, it was revealed by PCR analysis, of the foetal or placental tissues, that 15% of IUFD was attributable to B19 infection. This study also found delayed B19-specific antibody responses, as the mothers involved had no serological evidence of an acute B19 infection. However, follow-up studies detected B19 antibody seroconversion within 6 months. Tissue samples exhibited no signs of viral inclusions and immunohistochemical analysis revealed no evidence of B19 proteins (Tolfvenstam et al., 2001a).

Although the concept of B19-induced third-trimester foetal loss data has proved somewhat controversial (Crowley et al., 2001; Sebire, 2001), it undoubtedly further illustrates the requirements for awareness of B19 pathogenesis and diagnostic B19 quantitative PCR screening during pregnancy. Furthermore, Nunoue et al. (2002) strongly suggest that prospective studies to evaluate the relationship between time of infection and IUFD, with and without signs of foetal hydrops, are necessary. In fact, B19 PCR may be the most sensitive way of diagnosing intrauterine B19 infection especially since more than 50% of infected foetuses test negative for B19 IgM (Dieck et al., 1999).

Although follow-up of foetal status is generally recommended to take place for up to 3 months following diagnosis of maternal infection, Nyman et al. (2005) have reported a case of IUFD occurring 5 months after primary diagnosis of infection and associated with prolonged B19 viraemia and the presence of serological markers. These authors have suggested that revision of current follow-up criteria may be necessary if extended viraemia is a more common occurrence than heretofore thought.

Rodriguez et al. (2002, 2005) have undertaken the analysis of NIFH in both stillborn and livebirth autopsy scenarios. Following the evaluation of 840 stillborn autopsies (Rodriguez et al., 2002), 51 cases of NIFH were observed which represented 6.07% of all stillbirths. Congenital infection, caused either by bacterial or
viral infection was identified in 17 of the 51 cases of NIFH of which B19 was uniquely identified in four cases (7.8%). In fact, B19 infection was the fifth most prevalent of a total of 23 individually classified NIFH aetiologies by these authors. Subsequently, Rodriguez et al. (2005) have also studied the aetiology of NIFH in liveborn infants \( n = 429, \) 14-year period) who died soon after birth. Although a similar incidence of NIFH was observed to the previous study (7.45%; 32/429), the aetiology of B19-associated NIFH, in an individual with systemic infection and myocarditis, was only 3.1% (1/32 cases). Given this lower incidence of livebirth with B19-associated NIFH, these observations support the view that the effects of B19 infection during pregnancy are more likely to result in either \textit{in utero} foetal death or the complete resolution of infection, with, or without, the administration of appropriate therapies.

An unusual case of foetal demise, caused by B19 infection, has been described by Marton et al. (2005). Here, despite the successful administration of multiple foetal blood transfusions to prevent anaemia, foetal hydrops worsened and cardiac enlargement was observed upon foetal echocardiographic analysis which resulted in foetal death after delivery at week 31 of gestation. Subsequent autopsy found evidence of liver fibrosis. In addition, cardiomegaly and cardiac failure was caused by B19 infection and B19-infected cardiac myocytes, as shown immunohistochemically, were found to have undergone apoptosis. Thus, therapeutic intervention is not always successful.

Although rare, B19 infection occurs during twin pregnancies and while it appears that both twins are equally susceptible to infection, individual foetus survival does occur (Wolff et al., 1999; Graesslin et al., 2005). Graesslin et al. have reported an occurrence of one foetal death at week 13 with foetal hydrops evident in the second twin. Following confirmation of B19 infection via B19 DNA detection in amniotic fluid and maternal B19 IgG reactivity, \textit{in utero} blood transfusion raised foetal haemoglobin levels from 3.9 to 5.9 g/dl and, apart from transient myocarditis, the twin survived and was born normally without any negative outcome.

While much focus has rightly been placed on foetal survival following B19 infection, maternal health may not go unaffected. Bültmann et al. (2005) have recently described how peripartum cardiomyopathy (PPCM), a rare disorder whereby left ventricular dysfunction, develops in late pregnancy or early puerperium, may be associated with virus-associated inflammatory responses in myocytes. These authors studied 26 cases of PPCM and analysed endomyocardiac biopsies for a range of viral genomes and identified B19 \( n = 4 \), HHV6 \( n = 2 \), CMV \( n = 1 \) and EBV \( n = 1 \) DNA in eight patients (30.7%). Borderline myocarditis was evident in all cases and one B19 infected patient also exhibited dilated cardiomyopathy with inflammation. Although a similar occurrence of viral DNA was detected in control patients (30.3%), no inflammatory responses were evident. Consequently, Bültmann et al. (2005) have hypothesised that changes to immune function during pregnancy may facilitate reactivation of latent viral infection, or potentiate the effects of a recent infection, resulting in inflammatory cardiomyopa-
thy associated with viral infection. In any case, this study clearly shows the re-
quirement for virological assessment of PPCM cases.

To date, there is very little evidence to suggest that maternal B19 infections
increase the risk of congenital anomalies and generally B19 is considered to be
embryocidal rather than teratogenic. However, there have been case reports of
central nervous system, eye and craniofacial anomalies (Markenson et al. 1998;
Levy et al., 1997).

**Immunity to B19 infection**

*Antibody-mediated immune response*

Upon exposure of immunocompetent individuals to B19, high-titre B19 viraemia
usually occurs within 1 week and lasts about 5 days with virus titres peaking on the
first 2 days. B19-specific IgM antibodies are detected late in the viraemic stage, on
about day 10–12 and can persist for up to 5 months postinfection (Anderson et al.,
1985; Schwarz et al., 1988; Yaegashi et al., 1989), but in some patients can last even
longer (Musiani et al., 1995). B19-specific IgG antibodies appear about 15 days
postinfection, remain elevated for many months and generally persist long term
(years). Antibodies against linear epitopes of the B19 VP2 protein and to some
extent VP1, disappear abruptly after B19 infection whereas IgG reactivity against
conformational epitopes of both VP1 and VP2 persist (Soderlund et al., 1995; Kerr
et al., 1999). B19 IgA antibodies are detectable for a short period following the
onset of clinical symptoms to B19 (Erdman et al., 1991) but have attracted little
attention as diagnostic markers of infection. The development of the B19-specific
antibody response corresponds with viral clearance and also, in the vast majority of
cases of B19 infection in immunocompetent individuals, protection from subse-
quent infection (Anderson et al., 1985)—although absolute confirmation of pro-
tective and neutralising B19-specific antibodies has yet to be established. However,
Serjeant et al. (2001) have shown that in children with sickle cell disease, only one
episode of B19-associated transient aplastic crisis (TAC) is ever detected, thereby
strongly suggesting an absence of re-infection.

The B19 VP1 protein, and in particular the VP1u, was thought to be the
immunodominant antigen. Moreover, its incorporation into serological assays was
thought essential (Rayment et al., 1990). However, it is now clear that this ob-
ervation, which was based on the absence of antibodies to linear epitopes within
the VP2 protein, when screened by Western blot, is erroneous. It has now been
conclusively established that antibodies against capsid VP2 are maintained even
when B19 IgG directed against the VP1u is lost (Kerr et al., 1999; Manaresi et al.,
1999; Corcoran et al., 2000). Although, the key role of cellular immunity against
B19 infection is emerging (see the section on Cellular immunity), specific anti-viral
antibody is considered to represent a significant mechanism of immune protection,
based on the circumstantial evidence that high-dose immunoglobulin therapy is
sometimes beneficial in infected patients (Kurtzman et al., 1989b; Schwarz et al.,
Additionally, persistent infections associated with chronic anaemia have been observed where the immune response to B19 has failed to produce neutralising antibodies or they have been at very low levels (Kurtzman et al., 1987, 1988; Coulombel et al., 1989).

**Cellular immunity**

Cellular immunity to parvovirus B19 has not been studied as comprehensively as the humoral response, predominantly due to the fact that antibodies were thought to be the most important response in combatting B19 infection. Initial attempts to demonstrate specific T-cell proliferative responses to B19 were unsuccessful (Kurtzman et al., 1989a) which for some time supported the prevailing theory that neutralising antibody production alone conferred immunity to B19. *Ex vivo* B19-specific CD4+ T-cells responses were first detected in 1996 against *Escherichia coli*-expressed VP1, VP2 and NS1 antigens (von Poblotzki et al., 1996). T cell responses of 16 individuals were analysed *ex vivo* (10 seropositive and six seronegative blood donors), none of whom had evidence of acute infection. Of the seropositive cohort stimulated with VP2, 90% displayed specific T-cell responses and 80% exhibited VP1-specific responses. There was no significant difference in T-cell proliferation for NS1 between seropositive and seronegative individuals. Upon inclusion of HLA class I and II-specific monoclonal antibodies it was determined that HLA class II-specific antibodies inhibited T-cell proliferation, indicating that the effector T-cell population are CD4+ cells. Subsequent peripheral blood mononuclear cells (PBMC) depletion of either CD4+ or CD8+ T cells and stimulation of the remaining population confirmed this observation.

Subsequent studies showed significant *ex vivo* T-cell reactivity in PBMC of recently and remotely infected individuals using a B19 candidate vaccine (Franssila et al., 2001) and also B19 recombinant proteins, VP1 and VP2 (Corcoran et al., 2000). Recently infected individuals displayed very strong T-cell stimulation responses to the B19 capsids exhibiting average T-cell stimulation indices (S.I.) of 36 (Franssila et al., 2001). Blood donors with past infections gave comparable rates of T-cell stimulation. Seronegative individuals had S.I. values of about 3.3 and this study also showed that the responding population of T cells were CD4+ . Although von Poblotzki et al. (1996) found no difference in T-cell responses to NS1 in seronegative and seropositive individuals, significant responses to this antigen have been reported in both recently infected individuals and patients who developed chronic arthropathy following B19 infection (Mitchell et al., 2001). T-cell responses to NS1 were not seen in the group of healthy individuals with past B19 infection except for two individuals who notably, were also NS1 IgG seropositive.

The cellular immune response to a 15-mer epitope of NS1 that is specifically recognised by cytotoxic CD8+ T cells was investigated using major histocompatibility complex (MHC) tetrameric complex binding (Tolfvenstam et al., 2001b). The response of 21 individuals to this epitope was examined in healthy volunteers and HIV-1-infected adults and children. Sixteen of the volunteers were HLA matched
(HLA B35) and 6 were mismatched; 63% of matched individuals displayed specific CD8+ T-cell responses; 72% of matched individuals in the same cohort exhibited specific T-cell responses causing the production of interferon-γ (IFN-γ). The level of B19-specific CD8+ T-cells was similar among healthy and HIV-infected individuals. The results presented in this report showed the important cellular role of cytotoxic T cells in combating B19 infection (Tolfvenstam et al., 2001b). Moreover, B19-specific T-cell responses may now represent a novel method for confirming past B19 infection.

Using a combination of ex vivo analytical approaches (e.g. IFN-γ ELISpot and ⁵¹Cr-release assays following T-cell stimulation with peptides essentially representing the entire B19 proteome), Norbeck et al. (2005) have demonstrated that CD8+ T-cell responses are induced, and maintained for up to 2 years, following B19 infection of immunocompetent female individuals (n = 5). Moreover, these authors have also identified a number of HLA restricted CD8+ T-cell epitopes, most of which are located within the B19 NS1 protein. These workers further showed that while all individuals exhibited ex vivo IFN-γ responses to NS1 peptides, only 2/5 individuals were responsive to VP2-derived peptides and none to VP1u peptides. Norbeck et al. concluded that while CD4+-mediated immunity is directed towards the B19 structural proteins, it appears that CD8+ cytotoxic immunity is primarily directed against epitopes located within NS1 protein and postulated that B19 may represent a model organism to explore temporally extended viral–host interactions.

The importance of evaluating T-cell responses in understanding the nature of B19 infection was demonstrated recently by Chen et al. (2001). An AIDS patient with persistent B19 infection was identified. An initial remission of B19 infection in the patient was evident despite a lack of a specific antibody response, thus indicating a role for cellular immunity in combatting B19 infection. In addition to this case, NS1-specific lymphocytes have been detected in two B19 seronegative individuals who were exposed to B19, indicating a possible sub-clinical B19 infection or perhaps a loss of antibodies against capsid proteins (Mitchell et al., 2001). Investigations by Tolfvenstam (Tolfvenstam et al., 2001b) identified two healthy immunocompetent adults and two HIV-1-infected patients, seronegative for B19, with specific CD8+ T-cell responses against B19 by either IFN-γ ELISpot or tetramer-binding studies, thus implying the presence of a cellular response in the absence of a humoral response.

The production of a number of specific cytokines has thus far been associated with B19 infection. Significant T-cell transcriptional activation has been reported in a patient with acute B19 infection, causing increased levels of interleukin (IL)-1β, IL-6 and IFN-γ messenger RNA (mRNA) (Wagner et al., 1995). In a study of recently infected children it was shown that although strong T-cell proliferative responses were evident to both capsid proteins, production of the Th1 cytokine, IFN-γ, but not IL-2, was impaired when compared to convalescent adults (Corcoran et al., 2000). Corcoran et al. also demonstrated that ex vivo T-cell responses from B19 seropositive compared to seronegative individuals pregnant individuals (n = 149) exhibited significantly higher IFN-γ levels for following VP1 (268 + 36...
versus $103 + 19 \text{ pg/ml}; p = 0.003$) and VP2 ($242 + 42 \text{ versus } 91 + 16 \text{ pg/ml; } p = 0.01$) antigen stimulation. Significantly higher levels of IL-2 were also observed in B19 seropositive individuals following both VP1 ($p = 0.0003$) and VP2 ($p = 0.0005$) stimulation (Corcoran et al., 2003). However this ex vivo production of IFN-$\gamma$ and IL-2 observed in B19 seropositive pregnant women was lower than previously observed for healthy non-pregnant individuals suggesting a possible diminution of the maternal anti-viral immune response, which may subsequently increase the risk of foetal B19 infection.

The effect of maternal immune status, during pregnancy, on B19 infection has been further clarified by the work of Franssila et al. (2005) whereby weaker T-cell proliferative responses and also specific cytokine secretions (IFN-$\gamma$ and IL-10) were detected in recently infected pregnant women ($n = 3$) compared to control and recently infected non-pregnant women. In fact, there was no evidence of IL-10 production in the B19-infected pregnant individuals. Interestingly, one of the patients with no symptoms of infection displayed stronger IFN-$\gamma$ responses against VP1/2 co-capsids than against VP2 capsids only, when compared to the other two pregnant and B19-infected (symptomatic) individuals. Foetal loss occurred only in the case of asymptomatic B19 infection and it could be hypothesised that the greater reactivity to B19 VP1 epitopes, demonstrated by elevated ex vivo production of the Th1 cytokine, IFN-$\gamma$, may in some way be associated to this observation. Overall, the relatively weaker T cell responses to B19 antigens during pregnancy, observed by Franssila et al. (2005) is in accordance with the data and proposal of Corcoran et al. (2003) that pregnancy may contribute to the pathogenicity of B19 infection through attenuation of the anti-viral immune response.

Expression of the non-structural protein, NS1, causes the production of increased levels of the inflammatory cytokine IL-6 in a number of cell lines including hematopoietic cell lines and human umbilical vein endothelial cells (Moffatt et al., 1996). IL-6 is known to be involved in synovial cell proliferation and in addition, high levels of IL-6 along with other inflammatory cytokines have been found in inflamed joints of patients with rheumatoid arthritis (RA), which would suggest an association between IL-6 production and the joint manifestations observed with B19 infection (Bataille et al., 1995). In addition to IL-6 production, high levels of IFN-$\gamma$, TNF$\alpha$ and IL-8 have been detected in the serum of infants with B19-associated acute myocarditis (Nigro et al., 2000). There has been some evidence to suggest that IL-2 production at the maternal–foetal interface of women who seroconvert during pregnancy will determine the outcome of the gestation. There was a trend towards more CD3$^+$ T cells and IL-2 secretion on the foetal side from pregnancies with poor outcome, whereas IL-2 on the maternal side within the intervillous space was associated with a favourable prognosis (Jordan et al., 2001).

**Diagnosis of B19 infection**

Extremely accurate laboratory diagnosis of recent B19 infection or past exposure relies on testing (i) serum or plasma specimens for either specific antibody reactivity...
against viral capsid proteins, VP2 or VP1, expressed in eukaryotic expression systems (e.g. baculovirus-expression system) by ELISA or (ii) for B19 DNA in maternal or foetal tissues (e.g. amniotic fluid or blood) by qualitative or quantitative PCR (Fig. 2). It is important to note that B19-specific immunoassays incorporating E. coli-expressed B19 proteins only, which have undergone denaturation as part of a manufacturing process, will produce false-negative results due to the absence of conformational epitopes (Jordan, 2000) and the use of such systems should be avoided in the interests of patient care. A specific advantage of the eukaryotic baculovirus-expression system is its ability to enable the post-translational protein folding necessary for the generation of soluble VP2 capsids (Fig. 3) (Brown et al., 1990; Kerr et al., 1995a). Unlike B19 VP2, VP1 does not appear to form soluble capsid structures, however, VP1 has been produced as a 'conformationally intact' protein which has been shown to retain conformational epitopes present in the native virion (Brown et al., 1990; Kerr et al., 1999). Co-expression of VP1 and VP2 in eukaryotic expression systems has been proposed to result in the formation of empty capsids, which are antigenically indistinguishable from native B19 virions.

**Fig. 2** Detailed serological and virological screening algorithm for the diagnosis of parvovirus B19 infection in pregnancy. Women who are exposed to parvovirus B19 should be assessed to determine if they are susceptible to infection or have an acute infection. If a woman tests negative for parvovirus B19 IgM reactivity and positive for B19 IgG, the woman is immune and can be reassured that she will not develop infection. If the woman is B19 IgG and IgM negative, the woman is not immune and could develop infection. B19 PCR analysis will confirm maternal status. If the woman is positive for B19 IgM and either positive or negative for B19 IgG reactivity, she has been recently infected and there is a risk of a transplacental infection. B19 PCR analysis of the amniotic fluid will confirm transmission to the foetus.
Furthermore, it has been hypothesised that such co-capsids contain conformational epitopes essential for accurate detection of infection (Kajigaya et al., 1989, 1991; Franssila et al., 2001; Ballou et al., 2003).

Evidence has recently emerged that B19 NS1 IgG and IgM detection may also contribute to the diagnosis of acute B19 infection, thereby supplementing the detection of B19-specific antibodies, reactive towards B19 capsid antigens, as diagnostic markers of B19 exposure (Ennis et al., 2001; Heegaard et al., 2002b).

Detection of b19 IgM

Acute B19 infections can be detected and confirmed by the presence of B19-specific IgM reactivity while past infections are detected by IgG reactivity (Anderson et al., 1985) (Fig. 2). Generally, IgM antibodies appear 7–10 days postinfection and are directed against linear and conformational epitopes of VP1 and VP2 (Palmer et al., 1996; Manaresi et al., 2001).

Only one B19 IgM diagnostic test is available that has been approved by the United States Food and Drug Administration (FDA) for the detection of B19-specific IgM as a marker of recent infection in pregnancy. This test system is a μ-capture enzyme immunoassay (EIA) that utilises highly purified recombinant B19 VP2 capsids (Fig. 3) for the detection of B19-specific IgM in either human serum or plasma. The immunoassay has a sensitivity of 89.1% and a specificity of 99.4% (Doyle et al., 2000) and is extensively used for the diagnosis of recent B19 infection (Jordan, 2000; Mitchell et al., 2001; Vuorinen et al., 2002). Validated alteration of the immunoassay cutoff, based on receiver operating characteristic (ROC) analysis can accommodate superior immunoassay sensitivity (>89.1%) which may have a utility in the detection of lower levels of B19-specific IgM in pregnancy, immunocompromised individuals and young children (Doyle et al., 2000). Of special significance is the observation that no evidence of cross-reactivity with antibodies specific for other pregnancy-related viral infections (e.g. rubella, mumps, varicella zoster virus, cytomegalovirus and herpes simplex virus-1 (HSV-1)) and HSV-2 is apparent when this immunoassay is used in clinical settings. Although at present there is no international standard preparation for B19 IgM, the widespread utilisation of this immunoassay system means that inter-laboratory results can be compared with confidence. It should be stated that previous studies have reported cross-reactivity with rubella in several commercial B19 IgM assays (Sloots and Devine, 1996; Tolfvenstam et al., 1996) and as the symptoms of rubella infection are similar to those of B19 infection this was a cause for concern, particularly in the diagnosis of infection in pregnant women. A 5% false-positive rate was reported when specimens from healthy volunteers were analysed with a range of commercially available B19 IgM immunoassays, probably due to cross-reactivity and the lack of specificity in these immunoassays (Tolfvenstam et al., 1996). However, as of 2005 many of the systems employed in these studies are no longer available.

Beersma et al. (2005) have shown that B19 DNA levels in sera exhibit significant correlation with the presence of B19 VP2-reactive IgM (Pearson coefficient,
Moreover, these authors have demonstrated that in sera with B19 DNA loads greater than $10^6$ IU/ml, B19 IgM reactivity (as defined by specimen/assay cutoff ratio) always exceeds 3.0. Thus, it is clear that the presence of B19 VP2 IgM antibodies in sera is predictive for the presence of B19 DNA. Beersma et al. also evaluated differential antibody reactivity between B19 VP1 and VP2 antigens and suggest that because only low levels of B19 DNA were present in the small number
of sera (4/212) which were B19 VP1-only IgM reactive that B19 VP2 IgM is sufficient to detect highly B19 viraemic sera. These observations represent extremely useful information in a clinical context as they represent the first data correlating viral load with B19 IgM antibody levels.

Some reports have suggested that IgM directed against conformational epitopes on VP1 and VP2 proteins, and against linear epitopes on VP1, are produced at the same time postinfection and with identical frequencies. However, it has also been suggested that IgM reactivity against the minor capsid protein, VP1, may persist somewhat longer postinfection (Palmer et al., 1996; Manaresi et al., 2001). Thus, should IgM responses against conformational VP1 persist at a time when other B19-specific IgM antibodies are absent, then diagnostic techniques incorporating conformational VP1 may not be the most suitable marker of acute B19 infection. However, other work could elucidate no difference in IgM reactivity against conformational epitopes of the capsid proteins in diagnosing B19 infection (Kerr et al., 1999). Furthermore, these authors observed no differences in IgM reactivity against native (conformationally intact) antigens and linearised antigens for either VP1 or VP2.

Although detection of B19 NS1 IgM has received little attention as a marker of recent infection with parvovirus B19, Ennis et al. (2001) observed that 27.5% (11/40) of specimens that were B19 VP2 IgM positive also contained B19 NS1 IgM when tested by ELISA. Interestingly, when these samples were analysed by Western blot there was no evidence of NS1 IgM reactivity which indicates that conformational epitopes of the NS1 protein may be important for detection.

Detection of B19 IgG

After exposure to B19, the appearance of B19 IgG antibodies coincides with diminishing IgM antibody response, and B19 IgG reactivity against conformational epitopes of VP1 and VP2 persists postinfection (Fig. 2). However, for both capsid proteins, reactivity against linear epitopes declines postinfection, abruptly against VP2 but more slowly against VP1 (Soderlund et al., 1995; Kaikkonen et al., 1999; Kerr et al., 1999; Manaresi et al., 1999)—an observation which has significant consequences for diagnosis. Antibody reactivity against linear VP2 epitopes, predominantly directed against a heptapeptide (amino acids 344–350) identified by analysis of acute-phase sera (Kaikkonen et al., 1999), usually disappears within 6 months of B19 infection (Soderlund et al., 1995). Thus detection of B19-specific IgG, directed against linear epitopes of VP2, may assist in timing B19 exposure to within a 6-month period.

Although the antibody response wanes against linear epitopes on B19 capsid proteins it persists against conformational epitopes of both capsid proteins. Only one FDA approved B19 IgG immunoassay is available to detect B19 IgG as a marker of past infection (Corcoran et al., 2000). This microplate immunoassay which utilises capsid VP2 to detect B19 and erythrovirus V9 (genotype 3) IgG (Heegaard et al., 2002a; Candotti et al., 2004; Corcoran et al., 2005b) (Fig. 3).
studies undertaken to fulfill FDA approval criteria, inter-assay reproducibility data for this B19 IgG-specific immunoassay was determined by blinded analysis of a panel of unreactive, weakly reactive and reactive B19 IgG specimens \((n = 16)\) across three separate manufactured lots of the immunoassay, at three test sites, over a 3-day period. Consequently, each specimen was assayed three times per day (in duplicate) per lot, on three different days, at each of the three laboratories \((n = 81\) assays per specimen). Total inter-assay reproducibility was excellent, and % coefficient of variation \((%CV)\), ranged from 11.2 to 21.8\%CV or 15.6 to 26.8\%CV when expressed in terms of either immunoassay index (specimen/cutoff ratio) or OD values, respectively. Linear regression analysis of inter-site data confirmed the high reproducibility and robustness associated with the B19 IgG-specific immunoassay and correlation coefficients of 0.99 were observed for both index and OD values, respectively, for both inter-site (site 1 versus 2, site 2 versus 3 and 1 versus 3) and all inter-lot comparative scenarios. Comparative studies of this baculovirus-based immunoassay to another commercially available *E. coli*-based VP1 immunoassay for the detection of B19 antibodies in pregnant women have been undertaken and confirmed the accuracy of diagnosis, and absence of equivocal results, of the VP2 capsid immunoassay system (Jordan, 2000). The availability of a B19 IgG international standard (2nd International Standard 2003; code 01/602; 77 IU/ampoule) further contributes to the accurate confirmation of past B19 infection by standardising B19 IgG determination from different laboratories using different test systems (Ferguson et al., 1997; Searle et al., 1997).

Candotti et al. (2004) have recently presented controversial data which suggested that enzyme immunoassays utilising B19 VP2 capsids derived from genotype 1 did not detect a subset of erythrovirus genotype 3 (V9)-derived IgG. These findings have been disputed (Corcoran et al., 2005b) and work is currently underway to resolve this significant issue. Initial data (not shown) from analysis of blinded specimens suggest that B19 VP2 capsids can indeed detect all V9-derived IgG, a finding which confirms the diagnostic utility of this validated immunoassay system and that VP1 presence is not necessary to diagnose B19 infection.

The importance of NS1-specific IgG merits consideration with view to improved diagnosis of acute B19 infection. Although the presence of B19 NS1 IgG was originally proposed to be associated with persistent B19 infection (von Poblotzki et al., 1995a, b), it now appears to be the case that no significant difference between the level of NS1 IgG in control patients with past infection and those with chronic B19 infection is evident (Searle et al., 1998; Venturoli et al., 1998; Jones et al., 1999). In addition, Tolfvenstam et al. (2000) have mapped B-cell epitopes on NS1 and identified three antigenic regions (amino acids 191–206, 271–286, 371–386), which exhibited equal reactivity towards antibodies from healthy individuals with past B19 infection and B19-persistently infected patients. Hemauer et al. (2000) showed that NS1 IgG reactivity was most prevalent in serum following recent infection in pregnant women (61%) which, in turn, was supported by the work of Mitchell et al. (2001). Mitchell et al. examined NS1 IgG reactivity in sera from a range of cohorts (i.e. individuals (i) infected with B19, (ii) who had been
exposed to B19 but were not infected, (iii) who were suffering from a rash illness, chronic arthropathy or (iv) were healthy controls) and observed that NS1 IgG reactivity was predominant in recently infected specimens. Moreover, when subsequent specimens from these individuals were analysed, the level of NS1-specific IgG reactivity had declined. In addition there was no evidence of a correlation between NS1-specific IgG and the onset or development of arthropathy (Mitchell et al., 2001). Ennis et al. (2001) demonstrated that 69% of children recently infected with B19 were NS1 IgG seropositive and Heegaard et al. (2002b) also observed a seroprevalence of 60% B19 NS1 IgG in recently infected individuals (<6 weeks postinfection) and suggest that NS1 IgG detection may significantly improve immunoassay sensitivity. Thus as the NS1-specific IgG response diminishes, as the virus is cleared, NS1 IgG reactivity may contribute to accurate diagnosis as a marker of recent infection, in parallel with the detection of IgG against linear epitopes on VP2 (Ennis et al., 2001).

In summary, detection of B19-specific IgM indicates recent infection with B19 and B19 IgG detection confirms past exposure. Detection is optimal in immunoassays utilising VP2 capsids for antibody detection. Antibody detection against B19 NS1 protein may contribute to the confirmation of recent B19 infection but only when in association with standardised VP2 capsid-based immunoassays. Genotype 3 (erythrovirus V9) antibody detection is also feasible using immunoassays based on B19 VP2 capsids.

**B19 DNA detection by PCR**

Without doubt, the most sensitive method of diagnosis of B19 infection in pregnancy is the detection of B19 viral DNA in maternal–foetal blood or tissue by PCR. Many clinical laboratories now provide parallel B19 antibody screening and diagnostic PCR which greatly improves the sensitivity of detection of B19 infection (Skjoldebrand-Sparre et al., 2000; Manaresi et al., 2002). However, caution must be exercised with regard to the deployment of B19 PCR for a number of reasons: (1) the high viral titres associated with B19 infection may cause cross-contamination of samples and hence cause PCR false positivity. This is particularly problematic when nested PCR is used for B19 detection. (2) Low levels of B19 DNA may remain in the host long after infection thus, B19 DNA detection may not always be indicative of an acute infection. B19 viral titres can reach greater than $10^{12}$ genome equivalents per ml (ge/ml) (Prowse et al., 1997) during the stage of acute infection. In healthy, immunocompetent individuals viral DNA is detectable for at least 1 month postinfection (Erdman et al., 1991) but has also been shown to persist at low levels for long periods (Cassinotti et al., 1993; Kerr et al., 1995b; Musiani et al., 1995; Cassinotti and Siegl, 2000). In cases of chronic B19 infection, viral DNA can persist in the host without the presence of B19-specific IgM or IgG (Kurtzman et al., 1988; Frickhofen et al., 1990). Thus, B19 DNA detected by qualitative PCR analysis is not always indicative of recent infection. In fact, quantitative PCR was used by Cassinotti and Siegl (2000) to determine the amount of B19 viral DNA in
an immunocompetent patient from the time of acute B19 infection until convalescence. Over a 1-year period a series of blood samples were taken and analysed using a real-time PCR analysis. The B19 viral titre reached levels of $8.8 \times 10^9$ ge/ml of blood during the viremic stage of infection. At this point the patient was positive for B19-specific IgM but had no evidence of IgG reactivity. By week 164 the viral load had declined to 95 ge/ml, IgM reactivity was lost and IgG reactivity against B19 capsid proteins was strong. Subsequent specimens taken had no detectable B19 DNA. Thus, while the actual amount of circulating B19 DNA present following B19 infection diminished dramatically after the first few weeks of infection, it persisted for some time before being cleared from the host despite the development of circulating B19 IgG. This slow rate of B19 DNA clearance from an immunocompetent host could have a negative impact on PCR as a diagnostic tool in differentiating between recent and chronic B19 infection in a situation where a qualitative PCR assay, of unspecified sensitivity of detection is employed. (3) Many PCR assays are developed in-house and employ primer pairs of undefined sensitivity of detection. (4) Many extraction methods are suitable for DNA purification from serum or plasma only and not from solid tissue (e.g. placenta or foetal tissue). (5) Finally, false negativity may be observed with respect to non-B19 strains (e.g. erythrovirus V9, K71 or A6) due to minor sequence differences (Hokynar et al., 2002; Nguyen et al., 2002; [Servant et al., 2002]. An in-house nested PCR assay capable of accurately detecting V9 and B19 DNA simultaneously has been developed (Heegaard et al., 2001), which comprises of a primary round of amplification using a pair of consensus primers and a subsequent round of amplification using separate primers for B19 and V9. Using this PCR assay, clinical samples, including 100 B19 IgM-positive specimens and untreated plasma pools representing 100,000 blood donor units from the Danish population, were screened for both V9 and B19 DNA. None of the specimens analysed were positive for V9 DNA, which may be due to the fact that this V9 erythrovirus isolate is an emerging virus and may actually be more divergent than previously thought (Heegaard et al., 2001). PCR analysis has also revealed another B19 viral genotype, K71, which persists in human skin and has a nucleotide divergence of 10.8% from B19 and 8.6% from V9 (Hokynar et al., 2002). Many of the aforementioned issues associated with ‘home-brew’ PCR tests to detect the parvovirus B19 in clinical specimens can be overcome with the use of commercially available test systems. Analysis of two commercially available validated quantitative B19 PCR systems, the LightCycler-Parvovirus B19 quantification kit (Roche Diagnostics; http://www.roche-diagnostics.com/) and the RealArt Parvo B19 LC PCR (Artus; http://www.artus-biotech2.com) was performed by Hokynar et al. (2004) to examine their ability to detect, quantify and also differentiate between genotypes. The study revealed that although the Roche system was capable of detecting genotype 1 DNA at high sensitivity, it proved unsuitable for genotype 2, and to some extent, genotype 3 DNA detection. Conversely, the quantitative PCR system manufactured by Artus proved equally efficacious with respect to genotype detection, although again, high-sensitivity genotype 3 DNA detection was somewhat problematic. Schneider et al.
(2004) and Liefeldt et al. (2005) have also recently reported high-sensitivity strategies for quantitative detection and differentiation of all three erythrovirus genotypes.

Notwithstanding these limitations, B19 PCR is an effective technique used to detect B19 infection. In addition, with the introduction of the WHO International Standard for Parvovirus B19 DNA (NIBSC 99/800), PCR assay standardisation has become possible whereby B19 DNA units are quoted in International Units/ml (IU/ml) (Saldanha et al., 2002). Using the WHO standard, a number of compatible B19 PCR assay systems have been established (Daly et al., 2001; Müller et al., 2002; Thomas et al., 2003) and, using real-time PCR technology, a sensitivity of detection of 15.4 IU/ml (10 Baxter-Units/ml) was reached (Aberham et al., 2001).

**Alternative detection methods**

In cases of foetal infection and also in the immunocompromised host, when no B19-specific antibodies are present, patient histology can be used to assist with the confirmation of B19 infection in foetal tissue, whereby characteristic B19 inclusion bodies can be visualised either by DNA hybridisation or by antigen-detection techniques (van Elsacker-Niele, 1998). However, although these techniques are specific, sensitivity of detection remains problematic (van Elsacker-Niele and Kroes, 1999). Assays based on exploitation of the P antigen receptor of B19, known as receptor-mediated hemagglutination (RHA), have been proposed as a cheap way of screening plasma and apparently detect whole virus, however the assay sensitivity is low when compared to PCR and, more importantly, it has not been assessed in an obstetrics context (Cohen and Bates, 1995; Sato et al., 1995; Wakamatsu et al., 1999). In cases where patients are treated with intravenous immunoglobulin (IVIG) to treat chronic B19 infection, assessment of antigen-specific B cell memory allows one to discriminate IVIG- and individual-derived B19 IgG, which is important in determining the seroconversion status of the individual (Corcoran et al., 2005a).

Elevated maternal serum alpha fetoprotein (MSAFP) levels have been associated with foetal parvovirus B19 infection, probably due to damage to foetal liver cells, thus MSAFP levels could potentially serve as an indirect indicator of foetal infection (Carrington et al., 1987; Bernstein and Capeless, 1989). However, the sensitivity of this test is unknown and as several cases have reported severe foetal infection with normal levels of MSAFP (Saller et al., 1993; Johnson et al., 1994), the association between elevated MSAFP levels and B19 foetal infection is weak and, therefore, cannot be accepted as a reliable marker of infection.

The most reliable way to diagnose acute foetal infection is to detect B19 DNA in amniotic fluid or foetal serum by PCR or viral particles by electron microscopy. Clinical use of these tests however remains to be evaluated. Although B19 infection can be diagnosed through PCR analysis of amniotic fluid obtained by amniocentesis, invasive diagnosis of infection is not required for all suspected/confirmed maternal infections. It must be noted that viral particles are only present at
the viremic stage and the method used to detect these, an EIA, is generally insensitive. The presence of B19 IgM in foetal blood cannot be depended on to make a diagnosis of foetal infection as IgM appears in foetal circulation only after 22 gestational weeks (Rodis et al., 1988). Even beyond 22 weeks of foetal development IgM diagnosis can present misleading false-negative results (Pryde et al., 1992).

**Blood product safety and pregnancy**

Currently, there is no strategy for the best management of foetal hydrops caused by B19 infection during pregnancy but many cases are treated with intravascular transfusion. In a survey of maternal–foetal medicine specialists involving 539 cases of B19-induced hydrops, death occurred after intrauterine transfusion in 6% of cases, and in 30% of cases without intrauterine transfusion (Rodis et al., 1998). Treatment of B19 infection with transfused blood is not always effective and it is imperative that one is cognisant of the potential presence of high titre B19 virus in blood products (Prowse et al., 1997; Santagostino et al., 1997).

Screening of blood donations for the presence of B19 DNA is not routine (Blumel et al., 2002) despite the fact that this virus is highly resilient and can withstand denaturation even at high temperatures (Santagostino et al., 1994). In fact, B19 is thought to withstand processes involving solvent–detergent treatment, lyophilisation and temperatures of 100°C for 30 min, and despite these harsh virucidal processes, still have the capacity to contaminate factor VIII and factor IX concentrates (Santagostino et al., 1997). B19 contamination of such purified blood products is particularly problematic as, in the absence of B19 IgG, the infectious potential of B19 may be enhanced (Blumel et al., 2002). The most-recent determination of B19 prevalence is 1 in 837 blood donations (n = 5025, range $7.1 \times 10^4$ to $2.1 \times 10^{12}$ IU B19 DNA/ml) (Henriques et al., 2005) Previously, B19 levels had been estimated to be present in 1:16,000 transfusions based on the average incidence of B19 in a non-epidemic period (320 cases per 100,000 population) and the fact that viraemia lasts about 7 days (Prowse et al., 1997). During epidemics the incidence of viraemia in donations is greatly increased with levels reported as high as 1:167 in Japan (Yoto et al., 1995).

The infectious level of parvovirus B19 in blood products has yet to be established with certainty and is likely to depend on the amount of neutralising B19 IgG co-present in the product, in addition to recipient immune status. As part of a phase IV study, a group of 100 healthy volunteers, seronegative for B19, were given 1 unit of plasma that had been solvent/detergent treated (Davenport et al., 2000). Of the volunteers subsequently screened for incidences of B19 infection, 18% had seroconverted over the subsequent 3 months. Three of the 10 batches of plasma used in the study were retrospectively found to contain high levels of B19 ($>10^7$ ge/ml) and these batches coincided with the plasma administered to the volunteers who seroconverted. Interestingly, batches with low amounts of B19 ($<10^4$ ge/ml) did not cause B19 seroconversion. Presently, plasma lots containing high levels of B19 are eliminated from manufacturing batches of plasma. Thus, there is a level of
virus, as yet undetermined, that will not cause B19 infection. Notably, Daly et al. (2002) undertook a retrospective study of similar plasma pools \((n = 30)\) to those utilised in the study of Davenport et al. (2000) and found B19 IgG levels in the range 64.7 + 17.5 IU/ml. Thus it is possible that this level of B19 IgG may be capable of preventing recipient B19 infection when transfused with plasma contaminated with low levels of parvovirus B19 \(<10^4 \text{ ge/ml}\). Blumel et al., 2002 however, identified two incidences of B19 transmission by separate lots of clotting factor concentrates, one with \(8.6 \times 10^6 \text{ ge/ml}\) (volume: 180 ml) and the other shown to have \(4 \times 10^7 \text{ ge/ml}\) B19 DNA (volume: 966 ml), which were responsible for seroconversion.

Despite the fact that B19 infection can be transmitted via contaminated blood products, regulatory requirements relating to B19 contamination of pooled plasma or blood products prior to product release have only recently been implemented. The European Pharmacopoeia now stipulates that B19 DNA levels must be less than \(10^4 \text{ IU/ml}\) in plasma pools destined for anti-D IgG manufacture (European Pharmacopoeia, 2004). However, it should be acknowledged that many manufacturers now perform B19 PCR on plasma mini-pools to eliminate high B19 viral load plasma (Aberham et al., 2001) and many blood banks supply this upon request under the designation ‘Parvo-safe blood’. PCR screening of blood products has been shown to facilitate removal of 23 B19 PCR-positive donations from a plasma pool of 6000 resulting in a 10–100-fold decrease in viral load (Prowse et al., 1997).

Nonetheless, the issue of whether high-risk populations, such as pregnant women, immunocompromised patients and people with chronic anaemia, should undergo administration of any B19-containing products while the level of infectious B19 DNA is unknown, and minipool screening is not mandatory, must be addressed. The aforementioned availability of an international standard preparation of B19 DNA (Saldanha et al., 2002) in addition to a number of compatible and quantitative B19 PCR detection systems (Aberham et al., 2001; Daly et al., 2002; Knoll et al., 2002; Müller et al., 2002; Thomas et al., 2003), should alleviate problems caused by ambiguity between results from laboratories using various methods of measuring and expressing B19 DNA levels and help determine the infectious dose for parvovirus B19.

**Treatment and vaccination**

Parvovirus B19 infection is self-limiting in the immunocompetent host and, therefore, no specific therapy is required for such individuals. However, in cases where individuals suffer from arthritic complications, symptoms can be treated with non-steroidal anti-inflammatory drugs. The administration of high-titre IVIG has proven successful in the treatment of patients with chronic infection but this is expensive and remission may be temporary in the immunocompromised host (Kurtzman et al., 1989b; Fukushige et al., 1995; Lui et al., 2001). In addition, IVIG treatment does not work in all cases and no data is available on the actual pro-
A protective level of B19 IgG although levels greater than 61U/ml are thought to be protective (Searle et al., 1997). For cases of foetal infection, intrauterine blood transfusions may be beneficial especially in the case of hydrops but this procedure does involve additional risks to the outcome of pregnancy (Berry et al., 1992; Cameron et al., 1997; Bousquet et al., 2000; Enders et al., 2004).

If B19 infection occurs during pregnancy, the pregnancy should be allowed to proceed but carefully monitored. In cases of mild hydrops or with evidence of resolution of hydrops, foetuses should be closely monitored by ultrasound to detect any signs of hydrops, oedema, ascites and effusions (Morgan-Capner and Crowcroft, 2002). If hydrops worsens, a diagnostic cordocentesis and foetal blood transfusion should be considered. Currently, primary management of hydropic foetuses is cordocentesis to assess foetal haemoglobin and reticulocyte count, and intrauterine transfusion, if necessary (Markenson et al., 1998). A reticulocyte count in a foetal blood sample could provide evidence of bone marrow recovery. At present there is no reliable way to predict prognosis for individual foetuses and termination of pregnancy should not be recommended (Barrett et al., 1994). At delivery, examination of the cord blood for B19 IgM will reveal whether the virus has crossed the placenta and infected the foetus. The child should be carefully followed up for several weeks to check for any delayed sequelae.

The administration of high-titre IVIG has proven successful in treating foetal hydrops in some cases (Selbing et al., 1995; Alger, 1997). Alternatively, clinical symptoms of infection have been treated effectively by intrauterine blood transfusions (Schwarz et al., 1988; Hansmann et al., 1989). A study by Wattre reported two cases where intrauterine blood transfusions led to the cessation of symptoms and to the birth of normal babies (Wattre et al., 1998). In a separate study, 38 cases of B19-associated foetal hydrops were reported, 12 of whom received intrauterine blood transfusion. Although three of these foetuses subsequently died, the probability of death among fetuses that did not receive a blood transfusion was significantly higher (Fairley et al., 1995). In addition, spontaneous resolution of hydrops without intervention has been reported thus suggesting that treatment is not always necessary (Pryde et al., 1992).

Infection with B19 and rubella can be detrimental to the foetus if the mother is infected during pregnancy. However, primary infection with rubella in the first trimester of pregnancy is associated with a high risk of congenital abnormalities (Gibbs and Sweet, 1994; Pastuszak, 1994) unlike B19 infection, which is most likely to affect the foetus adversely during the second or third trimester (Tolfvenstam et al., 2001a). As both infections present with similar symptoms, it is essential to distinguish between the two infections to decide upon the appropriate course of action.

The major finding that intrauterine foetal transfusion to treat severe foetal hydrops was associated with 85% foetal survival and that no foetal survival was apparent in the absence of intrauterine transfusion (Enders et al., 2004) is perhaps the most compelling evidence that this therapeutic strategy should be given serious consideration should B19 infection occur during pregnancy.
As of 2005, there is no effective vaccine against B19 infection available for either seronegative pregnant women or for immunosuppressed individuals. A possible candidate vaccine comprising baculovirus-expressed B19 empty virus-like particles (VLP) is presently under evaluation which is the first B19 vaccine to reach human trials and is sponsored by MedImmune, Inc (Gaithersburg, MD) (Bansal, 1993). Ballou et al. (2003) have recently shown that the recombinant vaccine (MEDI-491; Medimmune), comprises B19 VP1 and VP2 capsid proteins, could elicit neutralising antibody titres in volunteer adults (n = 24). Sera from immunised individuals was also shown to be capable of inhibiting *in vitro* B19 replication. The efficacy of this formulation to prevent infection with parvovirus B19 remains to be established, nonetheless it is an encouraging and welcome advance in the fight against this insidious pathogen.

**Uncited References**

Bansal et al., 1993; Cassinotti et al., 1995; Markenson and Yancey, 1998; O'Keeffe et al., 2003; Wegner and Jordan, 2004; Wolff et al., 2005.

**Acknowledgements**

The authors wish to acknowledge both the European Union (Grant number: QLK2-CT-2001-00877) and the Irish Health Research Board for financial support (1998–2004). Biotrin Limited is also acknowledged for granting permission to include previously unpublished data.

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