Review

Advances in the biology, diagnosis and host–pathogen interactions of parvovirus B19

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Increased recognition of parvovirus B19 (B19), an erythrovirus, as a significant human pathogen that causes fetal loss and severe disease in immunocompromised patients has resulted in intensive efforts to understand the pathogenesis of B19-related disease, to improve diagnostic strategy that is deployed to detect B19 infection and blood-product contamination and, finally, to elucidate the nature of the cellular immune response that is elicited by the virus in diverse patient cohorts. It is becoming clear that at least three related erythrovirus strains (B19, A6/K71 and V9) are circulating in the general population and that viral entry into target cells is mediated by an expanding range of cellular receptors, including P antigen and β-integrins. Persistent infection by B19 is emerging as a contributory factor in autoimmune disease, a hypothesis that is constrained by the detection of B19 in the skin of apparently healthy individuals. B19 infection during pregnancy may account for thousands of incidences of fetal loss per annum in Europe, North America and beyond, yet there is currently only minimal screening of pregnant women to assess serological status, and thereby risk of infection, upon becoming pregnant. Whilst major advances in diagnosis of B19 infection have taken place, including standardization of serological and DNA-based detection methodologies, blood donations that are targeted at high-risk groups are only beginning to be screened for B19 IgG and DNA as a means of minimizing exposure of at-risk patients to the virus. It is now firmly established that a Th1-mediated cellular immune response is mounted in immunocompetent individuals, a finding that should contribute to the development of an effective vaccine to prevent B19 infection in selected high-risk groups, including sickle-cell anaemics.

Parvovirus B19

In 1975, Yvonne Cossart discovered what was to become known as human parvovirus B19 (B19) (Cossart *et al.*, 1975). B19 was first associated with disease in 1981, when it was linked to an aplastic crisis in a patient with sickle-cell disease. It has since been shown to cause erythema infectiosum (EI) (fifth disease of childhood), spontaneous abortion and some forms of acute arthritis (Anderson *et al.*, 1983; Kinney *et al.*, 1988; Woolf & Cohen, 1995).

B19 is a small, non-enveloped, ssDNA virus and, like all parvoviruses, the capsid proteins are arranged with icosahedral symmetry. B19 is 20–25 nm in diameter and has a genome of 5·6 kb (Clewley, 1984; Cotmore & Tattersall, 1984). The B19 capsid consists of an 83 kDa minor structural protein, VP1, and a 5 kDa major structural protein, VP2. VP2 makes up about 95 % of the total capsid, with VP1 accounting for the remaining 5 % (Ozawa *et al.*, 1987). The sequences of the two proteins are collinear, with VP2 being identical to the carboxyl-terminus of VP1; however, VP1 comprises an additional 227 aa domain that is unique to the amino-terminal (Fig. 1). To the left of these sequences on the B19 genome is the ORF for a non-structural protein, NS1, which encodes a protein product of 77 kDa. NS1 is a phosphoprotein with important regulatory functions, including control of transcription (Momoeda *et al.*, 1994a) and virus replication; it also plays a role in host-cell death (Ozawa *et al.*, 1988). It possesses DNA-binding properties (Raab *et al.*, 2002) and biochemical activities, such as ATPase, helicase and site-specific endonuclease activities, as well as nuclear localization signals (Li & Rhode, 1990; McCarty *et al.*, 1992; Jindal *et al.*, 1994; Brown & Young, 1997). B19 NS1 has also been shown to affect G1, but not G2, arrest in erythroid UT7/Epo-S1 cells (Morita *et al.*, 2003). B19 uses a single promoter, p6, which is capable of expressing structural and non-structural genes differentially (Blundell *et al.*, 1987;

Abbreviations: B19, parvovirus B19; EI, erythema infectiosum; EIA, enzyme immunoassay; FDA, US Food and Drug Administration; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; HSV, herpes simplex virus; IFA, immunofluorescent assay; IFN, interferon; IL, interleukin; IUFD, intrauterine fetal death; IVIG, intravenous immunoglobulin; NIHF, non-immune fetal hydrops; PBMCs, peripheral blood mononuclear cells; RA, rheumatoid arthritis; RBC, red blood cell; SI, stimulation index; TAC, transient aplastic crisis; Th cell, T helper cell; TNF, tumour necrosis factor; WHO, World Health Organization.
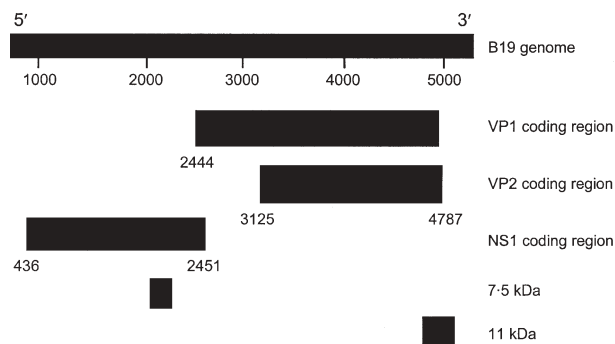
Fig. 1. Schematic representation of human parvovirus B19 genome and transcription map [redrawn with permission from Gray *et al.* (1998)].

Ozawa *et al.*, 1987). It has been demonstrated that NS1 interacts directly with the p6 promoter and with cellular transcription factors Sp1/Sp3 to affect transcriptional regulation (Raab *et al.*, 2002). Two other small polypeptides have been identified, one encoded by a region in the middle of the 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, with a predicted M_r of 11 kDa (St Amand *et al.*, 1991). Spliced transcripts of these two polypeptides have been found in infected cells, but their function has been hitherto unknown (Luo & Astell, 1993).

As a result of increased awareness of and screening for B19, a number of novel genotypes have been identified. Servant *et al.* (2002) suggested that B19 should be classified as a genotype 1 erythrovirus, with newly identified strains A6 (Nguyen *et al.*, 2002) and K71 (Hokynar *et al.*, 2002) classified as genotype 2 and erythrovirus V9 (Nguyen *et al.*, 1998) as the prototype of genotype 3. V9 has overall nucleotide sequence variation of about 12 % from B19 isolates, with the majority of sequence divergence occurring in the 5′ portion of the VP1 unique region; however, differences in sequence are not restricted to this area, but are scattered throughout the genome (Nguyen *et al.*, 1999; Heegaard *et al.*, 2001). K71 persists in human skin and has a nucleotide divergence of 10·8 % from B19 and 8·6 % from V9 (Hokynar *et al.*, 2002).

Infectivity, transmission and epidemiology

The only known host for B19 is humans. The virus replicates in human erythroid progenitor cells (late erythroid cell precursors and burst-forming erythroid progenitors) of the bone marrow and blood, inhibiting erythropoiesis (Mortimer *et al.*, 1983). Tropism of productive B19 infection is mainly due to the restrictive cellular distribution of the P blood group antigen globoside (Gb4) (Brown *et al.*, 1993, 1994), which is found most commonly on cells of the erythroid lineage, but also on platelets, tissues from the heart, liver, lung, kidney and endothelium and on synovium (Cooling *et al.*, 1995; Jordan & DeLoia, 1999). Individuals

who lack erythrocyte P antigens are very rare (1 in 200 000) and apparently cannot be infected by B19 (Brown *et al.*, 1994; Chipman *et al.*, 1996).

The limited tropism of B19 is not fully understood, as reduced capsid expression has been observed in non-permissive cells; however, intracellular factors that are found only in erythroid cells are assumed to be essential for optimal transcription and virus replication (Ozawa *et al.*, 1987; Kurpad *et al.*, 1999; Gallinella *et al.*, 2000). It has been shown that the level of P antigen expression on cells is not related directly to the efficiency of virus binding. In addition, some cell lines cannot be transduced by a B19 vector, despite P antigen expression and virus binding to the P antigen, thus indicating that a co-receptor is probably essential for virus entry into human cells (Weigel-Kelley *et al.*, 2001). Thus, recent evidence suggests that the presence of the P antigen alone is not sufficient to gain entry into cells (Weigel-Kelley *et al.*, 2001) and it has been suggested that multiple *β*-integrins may function as co-receptors for B19 cellular assimilation (Weigel-Kelley *et al.*, 2003).

B19 studies have been hampered by difficulties in propagating the virus *in vitro*. Recent studies have indicated that infection under hypoxic conditions [1 % (v/v) O_2] causes upregulation of B19 expression, which is associated with increased virus replication and production of infectious virions (Pillet *et al.*, 2002). Due to the cytotoxic nature of the non-structural protein NS1, no continuous cell line that propagates B19 has been established (Ozawa *et al.*, 1987; Momoeda *et al.*, 1994b).

B19 transmission occurs most commonly by personal contact via aerosol or respiratory secretions; however, contaminated blood products, such as clotting factor concentrates, are 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). B19 can be transmitted transplacentally from an infected mother to her fetus, which may lead to non-immune fetal hydrops (NIHF), spontaneous abortion or intrauterine fetal death (IUFD) (Clewley *et al.*, 1987; Miller *et al.*, 1998; Skjoldebrand-Sparre *et al.*, 2000). The P blood group antigen, which serves 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, levels of the P antigen are very high; they begin to decline in the second trimester and become undetectable by the mid-stages of trimester 3 (Jordan & DeLoia, 1999). This high level of globoside receptor on placental cells in early pregnancy may act as a pathway for B19 to be transmitted from the mother to the fetus, whereby the virus can then infect erythroid progenitor cells for replication. In support of this hypothesis, Wegner & Jordan (2002) have shown conclusively that ^{125}I-labelled VP2 capsid interaction with villous cytotrophoblast cells is mediated by P antigen.

Infection with B19 is very common and cases of infection have been reported all over the world in all seasons. Seroprevalence increases with age and, by adulthood,

>70 % of the adult population is seropositive (Kerr *et al.*, 1999). Children are the main source of transmission and outbreaks can persist for months in schools and day-care centres, due to the relatively large number of seronegative children and 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 & Adler, 1989; Valeur-Jensen *et al.*, 1999). Furthermore, infection by B19 during pregnancy can lead to spontaneous abortion or fetal anaemia. Consequently, the question must now be posed whether the B19 immune status of pregnant women should be determined routinely on initial presentation, to facilitate improved pregnancy outcome with respect to potential B19 infection.

Manifestations and clinical symptoms

B19 has been associated with an expanding range of clinical disorders since the discovery that it is the aetiological agent of EI. This is a mild childhood illness that is characterized by an erythematous rash that affects the face, trunk and limbs of the body. It is also associated with complications during pregnancy, acute arthropathy, disease in immunocompromised patients and transient aplastic crisis (TAC).

B19 infection and pregnancy

Exposure to and infection by B19 can lead to serious complications during pregnancy. Infection during pregnancy may result in fetal anaemia, spontaneous abortion and hydrops fetalis (Brown *et al.*, 1984; Kinney *et al.*, 1988; Heegaard & Hornsleth, 1995). About 30–40 % of women are non-immune and do not possess neutralizing antibodies to B19 and, therefore, are susceptible to infection by this virus. A vertical transmission rate of 33 % has been reported by the Public Health Laboratory Service in the UK (PHLS, 1990) and others have reported similar rates (Brown *et al.*, 1984; Hall *et al.*, 1990), although a recent study reported a transmission rate of 51 % (Yaegashi, 2000). There are over four million births in Europe per annum (Eurostat, 1998) and, because 30 % of pregnant women are B19-seronegative, over 1 200 000 European women are therefore susceptible to B19 infection during pregnancy. Assuming a combined rate of infection and fetal loss of 0·2 % (Levy *et al.*, 1997; Miller *et al.*, 1998; Wattre *et al.*, 1998), it can be estimated that approximately 3000 pregnancies per annum may be lost. Analogous birth rates in the USA and Canada imply that a similar incidence of fetal death due to B19 infection can be expected for these countries. These calculations are based solely on live births and, as the number of actual pregnancies is much higher, the above estimate is conservative. Pregnant women are most susceptible to B19 infection during epidemics and also when exposed to infected children in the home (Valeur-Jensen *et al.*, 1999). During outbreaks, transmission rates of 25 % in schools and 50 % at home have been reported (Anderson *et al.*, 1990). Most pregnant women are asymptomatic, but some do experience symptoms, such as exanthema and arthralgia (Komischke *et al.*, 1997). As these symptoms are commonly associated with pregnancy, acute B19 infection can often be overlooked; however, routine screening for symptoms of B19 infection or seroconversion would overcome this problem.

Fetal death usually occurs 4–6 weeks post-infection, 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 observed that fetal loss was confined 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 fetal loss as a consequence of intrauterine B19 infection is highest in, but not restricted to, the first 20 weeks of gestation (Hall *et al.*, 1990; PHLS, 1990). The critical time of infection has since been narrowed down to the 16th week of gestation (Yaegashi *et al.*, 1999). Most cases of fetal loss due to B19 infection have been reported in the second trimester (Enders & Biber, 1990; Torok, 1990; Wattre *et al.*, 1998). This susceptibility could be attributed, at least in part, to the relative immaturity of the fetal immune response at this stage. More important, though, is the tropism that B19 has for erythroid progenitor cells (Yaegashi, 2000) and the fact that in the second trimester of pregnancy, the life-span of fetal red blood cells (RBCs) is shortened and RBC mass increases three- to fourfold during this period of gestation (Rodis *et al.*, 1988). B19 replication within erythroid progenitor cells leads to apoptosis, which ultimately results in inhibition of erythropoiesis (Morey *et al.*, 1993). Erythroblastopenia can then occur as a consequence of B19 replication, causing severe fetal anaemia.

Anaemia is an underlying factor in the development of hydrops. Fetal hydrops was first associated with B19 in 1984 (Brown *et al.*, 1984). Since then, 10–20 % of NIHF cases 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 that are associated with fetal hydrops and caused by B19 have been reported most commonly in the second trimester and, to a lesser extent (unquantified as yet), in the third trimester of pregnancy (Sanghi *et al.*, 1997). When cases of IUFD that occurred 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, ten cases of IUFD were reported, which 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 were asymptomatic (Morey *et al.*, 1993).

Until recently, third-trimester fetal loss or IUFD caused by acute B19 infection had not been widely reported. However,

of 93 IUFD cases that were examined, 7·5 % had B19 DNA in placental tissue in the absence of fetal hydrops (Skjoldebrand-Sparre *et al.*, 2000). Unusually, none of the infected pregnant women in this study showed any clinical symptoms of B19 infection. B19-associated IUFD in the final stages of gestation may have been overlooked previously, due to inadequate diagnostic procedures and the difference in clinical features of third-trimester B19 infection. The most striking observation in these IUFD cases was the lack of fetal hydrops and the fact that many of the cases had either delayed or absent B19 IgG responses. Histopathological examination of the fetuses revealed no major abnormalities. Similar reports of non-hydropic, third-trimester IUFD that was associated with B19 infection have been published (Tolfvenstam *et al.*, 2001a). Here, it was revealed by PCR analysis of fetal or placental tissues that 15 % of IUFD cases were attributable to B19 infection. This study also observed delayed B19-specific antibody responses, as the mothers involved had no serological evidence of an acute B19 infection. However, follow-up studies showed evidence of seroconversion within 6 months. Tissue samples showed no signs of virus inclusions and immunochemistry analysis revealed no evidence of B19 proteins (Tolfvenstam *et al.*, 2001a). Although the concept of B19-induced, third-trimester fetal loss has proved somewhat controversial (Crowley *et al.*, 2001; Sebire, 2001), it further illustrates the requirements for awareness of B19 pathogenesis and diagnostic B19 PCR screening during pregnancy. Furthermore, Nunoue *et al.* (2002) suggested strongly that prospective studies to evaluate the relationship between time of infection and IUFD, with and without signs of fetal hydrops, are necessary. In fact, B19 PCR may be the most sensitive way of diagnosing intrauterine B19 infection, especially as >50 % of infected fetuses test negative for B19 IgM (Dieck *et al.*, 1999).

Administration of high-titre intravenous immunoglobulin (IVIG) has been shown to be successful in treating fetal hydrops in some cases (Selbing *et al.*, 1995; Alger, 1997). For cases of fetal infection, intrauterine blood transfusions may be beneficial (Schwarz *et al.*, 1988a; Hansmann *et al.*, 1989), 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). A study by Wattre *et al.* (1998) reported two cases where intrauterine blood transfusions led to the cessation of symptoms and the birth of normal babies. In a separate study, 38 cases of B19-associated fetal hydrops were reported, 12 of which received intrauterine blood transfusion. Although three of these fetuses 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, suggesting that treatment is not always necessary (Pryde *et al.*, 1992).

B19 infection during pregnancy is not a significant cause of birth defects; however, at least one incidence of congenital cardiomyopathy has been linked to B19 infection (Barton *et al.*, 1997).

Arthropathy

Like the rubella virus (Lee, 1960), B19 infection has also been linked to arthritis and arthralgias, most commonly in adults, but also in children (Reid *et al.*, 1985). On average, 50 % of adult cases of EI have associated joint manifestations that may persist for up to 1 month (Cassinotti *et al.*, 1995). B19 arthritis is usually symmetrical, affecting mainly the small joints of the hands, wrists and knees (Reid *et al.*, 1985). It is more common in females than males, with an estimated 60 % of women with symptomatic disease that manifests in arthropathy (White *et al.*, 1985; Woolf *et al.*, 1989). Symptoms generally subside within 3 weeks without any damage to the joints (Woolf *et al.*, 1991), but about 20 % of affected women suffer persistent or recurring arthropathy. About 75 % of these patients have an associated rash and <20 % have the typical 'slapped cheeks' facial exanthem. B19 has been proposed as the causative agent of arthritic conditions that exhibit similar symptomologies to those found in rheumatoid arthritis (RA), juvenile RA and erosive poly-arthritis, as recent B19 infection and high levels of B19 antibodies have been evident in many of these patients (White *et al.*, 1985; Nocton *et al.*, 1993; Mimori *et al.*, 1994; Tyndall *et al.*, 1994). It has been suggested that B19-associated arthritis is related to certain human leukocyte antigen (HLA) haplotypes of patients, with individuals of either HLA DR4 or B27 being most susceptible (Klouda *et al.*, 1986; Jawad, 1993); however, it is unclear how B19 causes symptoms that are associated with arthritis. Analogous to the appearance of exanthema in EI, arthritis usually occurs after development of B19-specific antibodies. This suggests that symptoms may be due to formation of immune complexes. Despite the fact that the P antigen is expressed on synovium, it has been shown that synovial membrane cells are non-permissive to B19 (Miki & Chantler, 1992; Cooling *et al.*, 1995). Normal human synovial fibroblasts have been shown to exhibit increased invasiveness following exposure to B19 viraemic serum, as judged by the acquired ability to degrade reconstituted cartilage matrix (Ray *et al.*, 2001). B19 may gain entry to cells that possess the B19 receptor but are not actively dividing, resulting in the production of excessive cytotoxic NS1 (Ozawa *et al.*, 1988). The B19 NS1 protein causes the secretion of proinflammatory cytokines, which could cause the inflammation and cell damage that are seen in patients with B19-associated arthritis and other inflammatory and autoimmune disorders that have been linked to B19 infections (Moffatt *et al.*, 1996; Mitchell, 2002). In one study, antibodies that were specific for the non-structural protein NS1 were found in patients with persistent B19-associated arthropathy, but not in serum from individuals with evidence of past infection without complications (von Poblotzki *et al.*, 1995a), thus suggesting an altered host response in the former cohort. However, others have disputed this, reporting similar NS1 antibody reactivity in

patients with 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.*, 2002a). von Landenberg *et al.* (2003) further suggested that B19 may be involved directly in the induction of autoimmune reactions that are mediated, at least in part, by anti-phospholipid antibodies, because of the prevalence of these antibodies in persistently B19-infected individuals.

There is significant evidence of B19 DNA persistence in bone marrow, peripheral blood and synovial tissues of patients with chronic, B19-associated arthropathy (Foto *et al.*, 1993; Musiani *et al.*, 1995; Nikkari *et al.*, 1995). However, it has also been shown that although B19 DNA persisted in the synovium tissue of 28 % of children who presented with chronic arthritis, an even higher proportion (48 %) of seropositive, immunocompetent volunteers had B19 DNA in their synovium tissues. These results imply that B19 DNA in synovium tissue may not be associated directly with symptoms of chronic arthropathy. None of the individuals tested had evidence of B19 DNA in their synovial fluid, bone marrow or blood and all were positive for B19 IgG antibodies (Söderlund *et al.*, 1997). Nonetheless, a recent report further enhanced the correlation between B19 infection and rheumatic childhood disease (Lehmann *et al.*, 2003). This work clearly elucidated 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 concluded that the rate of persistent B19 infection in these patients is significantly higher than in age-matched controls.

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).

Chronic B19 infection in the immunocompromised host

A host with a compromised immune system is particularly at risk of B19 infection, including people with AIDS, cancer patients who are receiving chemotherapy and transplant patients on immunosuppressive drugs (Young, 1996). Many are unable to produce neutralizing antibodies to clear the virus and this can lead to persistent infection, resulting in anaemia (Kurtzman *et al.*, 1989a; Young, 1996). In one case study, an AIDS patient developed severe anaemia as a result of chronic pure red-cell aplasia that was caused by B19 infection (Koduri *et al.*, 1997). Despite remission following IVIG transfusions, the patient suffered several recurrences of severe anaemia. In another report, sera obtained from transplant patients who were receiving bone-marrow grafts ($n = 27$) were analysed by PCR (Schleuning *et al.*, 1999). Of the cohort tested, 15 % were B19 DNA-positive and many of these patients also developed a reticular rash. Although

development of a rash during B19 infection is thought to be mediated by formation of immune complexes, these patients did not exhibit any signs of a B19-specific antibody response. Therefore, it was hypothesized that the rash was a consequence of a direct virus effect on the skin; this finding is supported by the fact that B19 DNA has been found previously in a skin biopsy from a male patient with B19-associated fever, rash and polyarthritis (Nikkari *et al.*, 1996). Persistant B19 infection results in chronic suppression of erythropoiesis with chronic anaemia. A report by Graeve *et al.* (1989) described how four children who were undergoing cancer chemotherapy treatment were infected by B19, resulting in chronic bone-marrow suppression. Schleuning *et al.* (1999) also reported that one of the transplant patients subsequently died from heart failure and B19 DNA was detected in the myocardium, but not in peripheral blood, indicating that heart failure was a consequence of B19 infection [heart failure has been recognized as a feature of B19 infection in the past (Chia & Jackson, 1996)]. Another of the transplant patients investigated was found to have developed hepatitis (Schleuning *et al.*, 1999), which was also attributed to B19 infection (Yoto *et al.*, 1996). As these transplant patients were subjected to strict decontamination procedures, including isolation in single rooms with positive airflow and decontaminated food, it is thus unlikely they contacted B19 via respiratory secretions. Platelet concentrates were screened for B19 prior to administration and were therefore not a likely source of transmission. However, as B19 DNA in clotting and immunoglobulin concentrates is known to cause infection, this may have been the route of transmission for the virus (Saldanha & Minor, 1996).

Many immunocompromised patients with chronic anaemia respond positively to IVIG therapy; however, individuals may suffer from recurrent relapses of aplasia (Koduri *et al.*, 1997, 1999; Moudgil *et al.*, 1997). In addition, administration of IVIG may not always be effective, as infection may persist despite treatment, particularly in transplant patients who are heavily immunosuppressed (Moudgil *et al.*, 1997; Schleuning *et al.*, 1999; Lui *et al.*, 2001). To date, no data are available on the actual protective level of B19 IgG, although levels of >6 IU ml^{-1} are thought to be protective (Searle *et al.*, 1997). As patients fail to mount an antibody response, serological diagnosis is futile and detection of B19 infection is therefore usually achieved by B19 DNA detection via a PCR assay.

Transient aplastic crisis (TAC)

B19-associated TAC may occur in individuals who exhibit underlying chronic haemolytic disorders, such as hereditary spherocytosis (Beland *et al.*, 1997). In 1981, it was discovered that B19 caused TAC in children with sickle-cell anaemia (Serjeant *et al.*, 1981) and it is now clear from subsequent studies that almost 70 % (118/177) of B19 infections in this cohort resulted in TAC (Serjeant *et al.*, 2001).

Vaccine

No specific therapy is required for B19 infection in immunocompetent individuals. Symptoms of arthropathy can be treated with non-steroidal, anti-inflammatory drugs.

Humoral immune response

B19 viraemia occurs 1 week after exposure and usually lasts about 5 days, with virus titres peaking on the first 2 days. B19-specific IgM antibodies are detected late in the viraemic stage (at about day 10 or 12) and can persist for up to 5 months (Anderson *et al.*, 1985; Schwarz *et al.*, 1988b; Yaegashi *et al.*, 1989) but, in some patients, can last even longer (Musiani *et al.*, 1995). Specific IgG antibodies are detectable about 15 days post-infection, remain high for several months and persist long-term. IgA antibodies are detectable for a short period following the onset of clinical symptoms (Erdman *et al.*, 1991). Development of the antibody response corresponds to virus clearance and also, in the vast majority of cases of B19 infection in immunocompetent individuals, protection from disease (Anderson *et al.*, 1985). A study of children with sickle-cell disease showed that those who had had one episode of B19-associated TAC did not suffer a second episode (Serjeant *et al.*, 1993).

Historically, the B19 VP1 protein, and in particular the VP1-unique region, was thought to be the immunodominant antigen and its incorporation into serological assays was thought to be essential (Rayment *et al.*, 1990). However, it is now clear that this observation, which was based on the absence of antibodies to linear epitopes of VP2 when screened by Western blot, was somewhat erroneous. It has now been established conclusively that antibodies against capsid VP2 are maintained, even when B19 IgG directed against the VP1-unique region is lost (Kerr *et al.*, 1999; Manaresi *et al.*, 1999; Corcoran *et al.*, 2000).

Specific anti-virus antibody is considered to be 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.*, 1990). Antibodies against linear epitopes of VP2 and, to some extent, VP1 disappear abruptly after B19 infection, whereas IgG reactivity against conformational epitopes of both VP1 and VP2 persists (Söderlund *et al.*, 1995; Kerr *et al.*, 1999). Persistent infections that are associated with chronic anaemia where the immune response to B19 has failed to produce neutralizing antibodies or they have been at very low levels have been observed (Kurtzman *et al.*, 1987, 1988; Coulombel *et al.*, 1989).

Diagnosis of B19 infection – choice of antigen

Accurate laboratory diagnosis of recent B19 infection or past exposure relies on screening plasma specimens for either specific antibody reactivity against virus capsid proteins that are expressed in eukaryotic expression systems (e.g. the baculovirus expression system) or for B19 DNA by using PCR. Immunoassays that only incorporate *Escherichia coli*-expressed B19 antigens, which have undergone denaturation as part of the manufacturing process, will produce false-negative results, due to the absence of conformational epitopes (Jordan, 2000). A unique advantage of the eukaryotic baculovirus expression system is its ability to direct the post-translational protein folding that is necessary for the production of soluble, conformationally intact VP2 capsid proteins (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 that retains conformational epitopes that are present in the native virion (Brown *et al.*, 1990; Kerr *et al.*, 1999).

A number of authors have stated that co-expression of VP1 and VP2 in eukaryotic expression systems results in the formation of empty capsids that are antigenically analogous to native B19 virions. Furthermore, it has been hypothesized that such co-capsids contain conformational epitopes that are essential for accurate detection of infection (Kajigaya *et al.*, 1989, 1991; Franssila *et al.*, 2001; Ballou *et al.*, 2003). To date, there are no data to prove that co-capsids are actually present in such preparations and it cannot be excluded that separate, conformationally intact entities that are comprised of either VP1 or VP2 are in fact present.

More recent evidence from a number of authors now suggests that B19 NS1 IgG and IgM detection also plays a significant role in diagnosis of acute infection, thereby supplementing the role played by B19 capsid antigens as diagnostic antigens (Ennis *et al.*, 2001; Heegaard *et al.*, 2002a).

B19 IgM immunodetection

Acute B19 infections are confirmed by B19-specific IgM reactivity, whereas past infections are detected by IgG reactivity (Anderson *et al.*, 1985). In most situations, IgM antibodies appear 7–10 days post-infection and are directed against linear and conformational epitopes of VP1 and VP2 (Palmer *et al.*, 1996; Manaresi *et al.*, 2001). It has been reported that IgM against conformational epitopes on VP1 and VP2 and against linear epitopes on VP1 appears at the same time post-infection and at the same frequency. However, it was also revealed that IgM reactivity against the minor capsid protein, VP1, may persist somewhat longer post-infection (Palmer *et al.*, 1996; Manaresi *et al.*, 2001). If IgM responses against conformational VP1 persist when other B19-specific IgM antibodies are absent, then diagnostic techniques that incorporate conformational VP1 may not be the most suitable markers of acute B19 infection. However, another study observed 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 disparity in IgM reactivity against native (conformationally intact) and linearized antigens for both VP1 and VP2.

Presently, there is no International Standard preparation for B19 IgM and only one B19 IgM diagnostic test that has been

cleared by the US Food and Drug Administration (FDA) is available. It is a mμ-capture enzyme immunoassay (EIA) that utilizes B19 recombinant VP2 capsids for the detection of specific IgM in human serum or plasma. This immunoassay has 89·1 % sensitivity and 99·4 % specificity (Doyle *et al.*, 2000) and is used widely for the diagnosis of recent B19 infection (Jordan, 2000; Mitchell *et al.*, 2001; Vuorinen *et al.*, 2002). Furthermore, validated alteration of the immunoassay cut-off, based on receiver operating characteristic analysis, facilitates improved immunoassay sensitivity, which may have a utility in detection of lower levels of B19-specific IgM in immunocompromised individuals and young children (Doyle *et al.*, 2000). No evidence of cross-reactivity with other viral infections, such as rubella, mumps, varicella-zoster virus, cytomegalovirus, herpes simplex virus-1 (HSV-1) and HSV-2, is apparent when this immunoassay is used in clinical settings. Previous studies have reported cross-reactivity with rubella in several commercial B19 IgM assays (Sloots & 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 false-positive rate of 5 % was reported when specimens from healthy volunteers were analysed with a range of commercially available B19 IgM immunoassays, probably due to cross-reactivity and lack of specificity in these immunoassays (Tolfvenstam *et al.*, 1996).

Detection of B19 NS1 IgM has received little attention as a marker of recent infection by 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 are important for detection.

B19 IgG immunodetection

Development of B19 IgG antibodies coincides with a decline in the IgM response. IgG reactivity against conformational epitopes of VP1 and VP2 persists post-infection; however, for both capsid proteins, reactivity against linear epitopes declines post-infection [abruptly against VP2, but more slowly against VP1 (Söderlund *et al.*, 1995; Kaikkonen *et al.*, 1999; Kerr *et al.*, 1999; Manaresi *et al.*, 1999)]. Antibody reactivity against linear VP2 epitopes usually disappears within 6 months of B19 infection (Söderlund *et al.*, 1995). This initial reactivity against linearized VP2 appears to be directed predominantly against a heptapeptide (amino acids 344–350) that was identified by analysis of acute-phase sera (Kaikkonen *et al.*, 1999).

Although the antibody response wanes against linear epitopes on B19 capsid proteins, it persists against conformational epitopes of both capsid proteins. The only FDA-cleared B19 IgG immunoassay that is available as a marker of past infection is a microplate immunoassay that utilizes capsid VP2 to detect B19 and erythrovirus V9 IgG

(Heegaard *et al.*, 2002b; A. Garbarg-Chenon, personal communication). This baculovirus-based immunoassay has been compared to another commercially available, *E. coli*-based VP1 immunoassay for detection of B19 antibodies in the sera of pregnant women (Jordan, 2000). A number of equivocal results were obtained by using the B19 VP1 immunoassay. To verify results, samples were tested by using a commercially available VP1 immunofluorescent assay (IFA). This assay examines seroreactivity against conformationally intact VP1. Although this assay may be somewhat subjective, as it primarily measures the degree of specimen fluorescence, when used in parallel with the VP2 IgG immunoassay, it can confirm B19 reactivity. Results from the VP1 IFA were similar to those obtained by the baculovirus VP2 EIA and were in accordance with the fact that many of the samples that were found to be equivocal by the *E. coli* VP1 EIA had clinical histories of B19 exposure. Availability of a B19 IgG International Standard (2nd International Standard 2003; code 01/602; 77 IU per ampoule) should further assist in accurate confirmation of past B19 infection by standardizing B19 IgG determination from different laboratories that use a variety of test systems (Ferguson *et al.*, 1997; Searle *et al.*, 1997).

Recently, the importance of antibodies against the B19 non-structural protein NS1 has been investigated, with a view to improving diagnosis of B19 infection. The presence of B19 NS1 IgG was thought to be associated primarily with persistent B19 infection (von Poblotzki *et al.*, 1995a, 1995b); however, several groups have subsequently found no significant difference between the level of NS1 IgG in control patients with past infection and those with chronic B19 infection (Searle *et al.*, 1998; Venturoli *et al.*, 1998; Jones *et al.*, 1999). Mapping of B-cell epitopes on NS1 identified three antigenic regions (amino acids 191–206, 271–286 and 371–386) that were equally reactive with sera from healthy individuals with past B19 infection and patients who were infected persistently by B19 (Tolfvenstam *et al.*, 2000). More recently, by using an *E. coli* expression system, NS1 IgG reactivity has been shown to be most prevalent in serum, following recent infection in pregnant women (61 %) (Hemauer *et al.*, 2000). These findings are supported by the work of Mitchell *et al.* (2001), who examined NS1 IgG reactivity in sera from individuals who were either infected by B19, had been exposed to B19 but were not infected, were suffering from a rash illness or chronic arthropathy, or were healthy controls. NS1 IgG reactivity was predominant in recently infected specimens and when follow-up samples from these individuals were analysed, the level of NS1-specific IgG reactivity had declined. In addition, there was no evidence of a connection between NS1 IgG antibodies and the development of arthropathy (Mitchell *et al.*, 2001). The NS1-specific IgG response wanes post-infection as the virus is cleared from the body; therefore, NS1 IgG reactivity may have some value as a marker of recent infection, in conjunction with the detection of IgG against linear epitopes on VP2 (Ennis *et al.*, 2001). This study demonstrated that 69 % of children who had been infected recently by B19 were NS1

IgG-seropositive (Ennis *et al.*, 2001). Heegaard *et al.* (2002a) also observed a seroprevalence of 60 % B19 NS1 IgG in recently infected individuals (<6 weeks post-infection) and suggested that NS1 IgG detection may significantly improve immunoassay sensitivity.

It is now clear that B19 IgM and IgG detection is optimal in immunoassays that utilize VP2 capsids for antibody detection. Antibody (IgG/M) detection of B19 NS1 protein may assist in the confirmation of recent B19 infection, when used in combination with VP2 capsid-based immunoassays. Erythrovirus V9 antibody detection is also feasible, by using immunoassays that are based on B19 VP2 capsids.

Cell-mediated immunity

Cell-mediated immunity to B19 has not been studied extensively; this is due primarily to the fact that the humoral response was thought to be most important in combatting B19 infection. Indeed, initial attempts to demonstrate specific T-cell proliferative responses to B19 were unsuccessful (Kurtzman *et al.*, 1989a) and, for some time, this work supported the prevailing theory that neutralizing antibody production was the major mechanism of immunity in B19. In 1996, *ex vivo* B19-specific CD4+ T-cell responses were first detected against *E. coli*-expressed VP1, VP2 and NS1 antigens (von Poblotzki *et al.*, 1996). T-cell responses of 16 individuals were analysed (ten seropositive and six seronegative blood donors), none of whom had any evidence of acute infection. The majority (90 %) of seropositive donors who were stimulated *ex vivo* by VP2 displayed specific T-cell responses, with 80 % displaying VP1-specific responses. There was no significant difference in T-cell proliferation for NS1 between seropositive and seronegative individuals. Upon inclusion of mAbs that were specific for class I and class II HLA, it was found that HLA class II-specific antibodies inhibited T-cell proliferation, thus indicating that the effector T-cell population of B19 are CD4+ cells. Subsequent peripheral blood mononuclear cell (PBMC) depletion of either CD4+ or CD8+ T cells and stimulation of the remaining population confirmed this observation.

More recently, significant *ex vivo* T-cell reactivity was observed in PBMCs of recently and remotely infected individuals by using a B19 candidate vaccine (Franssila *et al.*, 2001) and also the B19 recombinant proteins, VP1 and VP2 (Corcoran *et al.*, 2000). T cells from recently infected individuals responded strongly to the B19 capsids, giving a mean T-cell stimulation index (SI) of 36 (Franssila *et al.*, 2001). Blood donors with past infections gave comparable rates of T-cell stimulation. Seronegative individuals had SI 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) saw no difference in T-cell responses to NS1 in seronegative and seropositive individuals, significant responses to this antigen have been reported in 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 healthy individuals with past B19 infection, except for two individuals who were also NS1 IgG-seropositive.

Cellular immune response to an epitope of NS1 that is recognized specifically by CD8+ T cells was investigated recently by using major histocompatibility complex tetrameric complex binding (Tolfvenstam *et al.*, 2001b). The response of 21 individuals to this epitope was examined in healthy volunteers and human immunodeficiency virus (HIV)-1 infected adults and children. Sixteen of the volunteers were HLA-matched (HLA B35) and six were mismatched. Sixty-three per cent of matched individuals displayed specific CD8+ T-cell responses. Seventy-two per cent of matched individuals in the same cohort exhibited specific T-cell responses by using an interferon γ (IFN-γ) ELISpot assay. 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 combatting B19 infection (Tolfvenstam *et al.*, 2001b). B19-specific T-cell responses may now represent a novel method for confirming past B19 infection.

Recent evidence shows the importance of evaluating T-cell responses in understanding the nature of B19 infection. Chen *et al.* (2001) have identified an AIDS patient with persistent B19 infection who showed an initial remission of B19 infection. This remission was evident despite the lack of a specific antibody response, thus indicating a role for cellular immunity in combatting B19 infection. NS1-reactive lymphocytes have been detected in two B19-seronegative individuals who were exposed to the virus, indicating a possible subclinical B19 infection or perhaps a loss of antibodies against capsid proteins (Mitchell *et al.*, 2001). The importance of cellular immunity in B19 was further emphasized in a report by Tolfvenstam *et al.* (2001b). Here, investigations of B19-specific CD8+ T-cell responses identified two healthy adults and two HIV-1-infected patients who were seronegative for B19, with specific 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.

Significant T-cell transcriptional activation has been reported in a patient with acute B19 infection, causing increased levels of interleukin (IL) 1β, IL6 and IFN-γ mRNA (Wagner *et al.*, 1995). A subsequent study that analysed the sera of patients who were infected acutely by B19 showed that although IL1β, IL6, IFN-γ and tumour necrosis factor α (TNF-α) were secreted during the acute phase of infection, increased levels of both IFN-γ and TNF-α persisted and were detectable 2–37 months later, during a follow-up study (Kerr *et al.*, 2001). It has also been suggested that cytokine genetic polymorphisms may, in some way, affect the development of symptoms during B19 infection. To date, the transforming growth factor β (TGFβ) allele has been associated with skin rash at acute infection and the IFN-γ allele has been associated with NS1 antibody development (Kerr *et al.*,

2003). 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 T helper (Th1) cytokine IFN-γ, but not of IL2, was impaired when compared to convalescent adults (Corcoran *et al.*, 2000). In addition, *ex vivo* production of IFN-γ and IL2 that was observed in B19-seropositive pregnant women was lower than observed previously for healthy, non-pregnant individuals, suggesting a possible dimunition of the maternal anti-virus immune response that may subsequently increase the risk of fetal B19 infection (Corcoran *et al.*, 2003). Expression of the non-structural protein NS1 causes the production of increased levels of the inflammatory cytokine IL6 in a number of cell lines, including hematopoietic cell lines and human umbilical vein endothelial cells (Moffatt *et al.*, 1996). IL6 is known to be involved in synovial cell proliferation and, in addition, high levels of IL6, along with other inflammatory cytokines, have been found in inflamed joints of patients with RA, which would suggest an association between IL6 production and the joint manifestations that are observed with B19 infection (Bataille *et al.*, 1995). IL6 involvement in RA is supported by the fact that antibodies against IL6 cause inhibition of RA manifestations (Bataille *et al.*, 1995). As well as increased IL6 production, high levels of IFN-γ, TNF-α and IL8 have been detected in the sera of infants with B19-associated acute myocarditis (Nigro *et al.*, 2000). IL2 production at the maternal–fetal interface in women who seroconverted to B19 during pregnancy is thought to determine the outcome of the pregnancy, with high levels of IL2 on the fetal side being associated with pregnancies that result in a poor outcome (Jordan *et al.*, 2001).

B19 vaccine

Ballou *et al.* (2003) have shown recently that a recombinant vaccine (MEDI-491; Medimmune) that is comprised of B19 VP1 and VP2 capsid proteins could elicit neutralizing antibody titres in volunteer adults ($n = 24$). Sera from immunized individuals were also shown to be capable of inhibiting B19 replication *in vitro*. The efficacy of this formulation in preventing infection by B19 remains to be established; nonetheless, it is an encouraging and welcome advance in the fight against this ubiquitous pathogen.

PCR detection of B19 DNA

Many clinical laboratories now complement B19 antibody screening with diagnostic PCR and it is well-established that B19 PCR improves the sensitivity of detection of B19 infection (Skjoldebrand-Sparre *et al.*, 2000; Manaresi *et al.*, 2002). However, caution must be exercized with regard to the deployment of B19 PCR for a number of reasons: (i) the high viraemia that is associated with B19 infection, along with resultant amplicon load, may cause PCR false positivity – particularly when nested PCR is used for B19 detection; (ii) B19 DNA detection may not always be indicative of an acute infection; (iii) many PCR assays use in-house primer pairs of undefined sensitivity of detection; (iv) 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); and, finally, (v) many extraction methods are suitable for DNA purification from serum or plasma only and not from solid tissue (e.g. placenta or fetal tissue). Notwithstanding these caveats, some of which are discussed in more detail below, B19 PCR is an important tool in the technologist's armoury for detection of B19 infection.

During acute infection with B19, viral titres can reach $\sim 10^{12}$ genome equivalents ml^{-1} (Prowse *et al.*, 1997). In the immunocompetent host, virus DNA is detectable for at least 1 month post-infection (Erdman *et al.*, 1991). In chronic B19 infection, virus DNA can persist in the host without the presence of B19 IgM or IgG (Kurtzman *et al.*, 1988; Frickhofen *et al.*, 1990). However, it has also been shown that B19 DNA can persist in healthy, immunocompetent individuals at low levels for long periods (Cassinotti *et al.*, 1993; Kerr *et al.*, 1995b; Musiani *et al.*, 1995; Cassinotti & Siegl, 2000). Thus, B19 DNA, detected by qualitative PCR analysis, is not always indicative of recent infection. Cassinotti & Siegl (2000) used quantitative PCR to follow the amount of specific B19 DNA in an immunocompetent patient, expressed as genome equivalents ml^{-1}, from the time of acute B19 infection until convalescence. A series of samples was taken over a 1 year period and was analysed by using a real-time fluorogenic PCR assay. During the viraemic stage of B19 infection, viral load reached levels of $8 \cdot 8 \times 10^9$ genome equivalents (ml blood)$^{-1}$. At this stage, the patient was positive for specific IgM and negative for IgG reactivity. At week 164, viral load had declined to 95 genome equivalents ml^{-1}, IgM reactivity was lost and conformational IgG reactivity was strong. Specimens taken after this time-point were undetectable for B19 DNA. Thus, whilst the actual amount of circulating B19 DNA that was 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 impact negatively on PCR as a diagnostic tool in differentiating between recent or chronic B19 infection in a situation where a qualitative PCR assay of unspecified sensitivity of detection was employed. However, with the introduction of the World Health Organization (WHO) International Standard for Parvovirus B19 DNA (NIBSC 99/800), PCR assay standardization has become possible (Saldanha *et al.*, 2002). Using the WHO standard, a compatible PCR-ELISA that can detect levels as low as $1 \cdot 6 \times 10^3$ IU B19 DNA ml^{-1} was established (Daly *et al.*, 2002) and, by using real-time PCR technology, a sensitivity of detection of $15 \cdot 4$ IU ml^{-1} (10 Baxter units ml^{-1}) (Aberham *et al.*, 2001) was reached. Müller *et al.* (2002) and Thomas *et al.* (2003) have also described standardized B19 PCR assay systems. These standardized methods could be used not only in a diagnostic setting, but also for rapid screening of plasma mini-pools and blood products, thereby leading to determination of the amount of B19 DNA present and improved product safety.

PCR may also be used in screening for the newly isolated erythrovirus V9 (Nguyen *et al.*, 1998, 1999). A nested PCR assay that is capable of accurately detecting V9 and B19 DNA simultaneously, comprising a primary round of amplification by using a pair of consensus primers and a subsequent round of amplification by using separate primers for B19 and V9, has been developed (Heegaard *et al.*, 2001). By using this PCR assay, clinical samples, including 100 B19 IgM-positive specimens and untreated plasma pools that represented 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 facts that this V9 isolate is an emerging virus and that this erythrovirus may actually be more divergent than thought previously (Heegaard *et al.*, 2001). Thus, PCR could be used as a diagnostic tool for the identification of possible new erythrovirus isolates in cases where patients are negative for B19 DNA, but have displayed clinical symptoms of B19 infection.

B19 and blood-product safety

B19 can be transmitted through blood transfusions and plasma-derived products (Prowse *et al.*, 1997; Santagostino *et al.*, 1997). Screening of blood donations for the presence of B19 DNA is not routine (Blümel *et al.*, 2002), despite the fact that this virus is highly resilient and, like the hepatitis A virus, can withstand denaturation, even at high temperatures (Santagostino *et al.*, 1994). In fact, B19 can withstand processes that involve solvent/detergent treatment, lyophilization 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 (Blümel *et al.*, 2002). The most recent determination of B19 prevalence is 1 in 625 blood donations ($n = 16\,859$; range, 10^2 to $>10^7$ IU B19 DNA ml^{-1}) (Thomas *et al.*, 2003). Previously, B19 had been estimated to be present in 1 : 16 000 transfusions, based on the mean incidence of B19 infection in a non-epidemic period (320 cases per 100 000 population) and the fact that viraemia lasts for about 7 days (Prowse *et al.*, 1997). During epidemics, the incidence of viraemia in donations is greatly increased, with levels as high as 1 : 3790 reported in Ireland (O'Neill & Coyle, 1992) and 1 : 167 in Japan (Yoto *et al.*, 1995).

The infectious level of B19 in blood products has yet to be established with certainty and is likely to depend on the level of B19 IgG that is co-present in the product, in addition to recipient immune status. As part of a phase IV study, a group of 100 healthy volunteers who were seronegative for B19 were given 1 unit of plasma that had been solvent/detergent-treated (Davenport *et al.*, 2000). Of the volunteers who were screened subsequently for incidences of B19 infection, 18 % had seroconverted over the subsequent 3 months. Three of the ten batches of plasma that were used in the study were found retrospectively to contain high levels of B19 DNA ($>10^7$ genome equivalents ml^{-1}) and these batches coincided with the plasma that was administered to the volunteers who seroconverted. Interestingly, batches with low amounts of B19 ($<10^4$ genome equivalents ml^{-1}) did not cause B19 seroconversion. Upon discovery of these facts, the company that was involved in manufacturing this plasma (VITEX, Watertown, MA, USA) voluntarily recalled batches that were associated with B19 transmission and reviewed their screening process. Presently, plasma lots that contain 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 plasma pools ($n = 30$) that were similar to those utilized in the study of Davenport *et al.* (2000) and found B19 IgG levels in the range of $64 \cdot 7 \pm 17 \cdot 5$ IU ml^{-1}. Thus, it is possible that this level of B19 IgG may be capable of preventing recipient B19 infection when transfused with plasma that is contaminated by low levels of B19 ($<10^4$ genome equivalents ml^{-1}). Blümel *et al.* (2002) have identified two incidences of B19 transmission by separate lots of clotting factor concentrates and have shown that B19 levels of $8 \cdot 6 \times 10^6$ genome equivalents ml^{-1} (volume, 180 ml) and 4×10^3 genome equivalents ml^{-1} (volume, 966 ml) were responsible for seroconversion.

B19 seroprevalence is higher among haemophiliacs than the general population, presumably due to the fact that products that are derived from pooled plasma are more likely to contain infectious B19 DNA. This was shown conclusively when B19 seroprevalence in a haemophiliac population was compared with that of normal healthy individuals and it was discovered that haemophilic children who were treated with virally inactivated clotting factor had an increased level of B19 seroprevalence (92 %) (Eis-Hübinger *et al.*, 1996). Normally, in the general population, a continuous increase in B19 seroreactivity is observed with age, with a seroprevalence for adults over 60 that reaches about 72 %. Interestingly, the haemophilic population that was treated pre-1984 with non-inactivated clotting factor concentrates had an increased seroprevalence of 98 % (Williams *et al.*, 1990; Eis-Hübinger *et al.*, 1996).

Despite the fact that B19 infection can be transmitted via contaminated blood products, there are presently no strict regulatory prerequisites governing B19 contamination of pooled plasma or blood products prior to product release. However, it should be acknowledged that many manufacturers now perform B19 PCR on plasma mini-pools, in order to eliminate high B19 viral load plasma (Aberham *et al.*, 2001). 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). However, the level of B19 DNA does not necessarily correlate to the rate of infectivity, as has been demonstrated previously for the canine parvovirus; in this case, heat treatment was found to reduce canine parvovirus infectivity by >100-fold, despite the fact that the PCR assay titre was unaltered (Hart *et al.*, 1994).

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 mini-pool 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; Knöll *et al.*, 2002; Müller *et al.*, 2002; Thomas *et al.*, 2003), should alleviate problems caused by ambiguity between results from laboratories that use various methods of measuring and expressing B19 DNA levels and help to determine the infectious dose for B19.

It is highly significant that, in the Netherlands, a recommendation has been made that individual donor screening for B19 IgG to identify individual donors with continually high antibody levels (at $t = 0$ and 6 months) should be initiated (Health Council for the Netherlands, 2002; Groeneveld & van der Noordaa, 2003). Selected individuals who maintain high B19 IgG levels will subsequently form a panel of plasma or blood-product donors for high-risk recipients, such as immunocompromised individuals, thereby minimizing the risk of B19 transmission from acutely infected, although asymptomatic, donors. It is our view that the introduction of such a screening algorithm sets the standard for blood-product safety in the future, specifically with respect to minimizing the risk of B19 transmission.

Assays that are based on exploitation of the P antigen receptor of B19, known as receptor-mediated haemagglutination, have been proposed as a cheap way to screen plasma and to apparently detect whole virus; however, assay sensitivity is quite low, especially when compared to that of PCR (Cohen & Bates, 1995; Sato *et al.*, 1995; Wakamatsu *et al.*, 1999). A novel immunoassay that is capable of detecting $1-2 \times 10^6$ genome equivalents of B19 antigen (whole virus), in the presence or absence of B19 IgG/M, is currently under development (O'Keeffe *et al.*, 2003) and should find an application in either mini-pool or diagnostic screening, as an adjunct to PCR screening.

Acknowledgements

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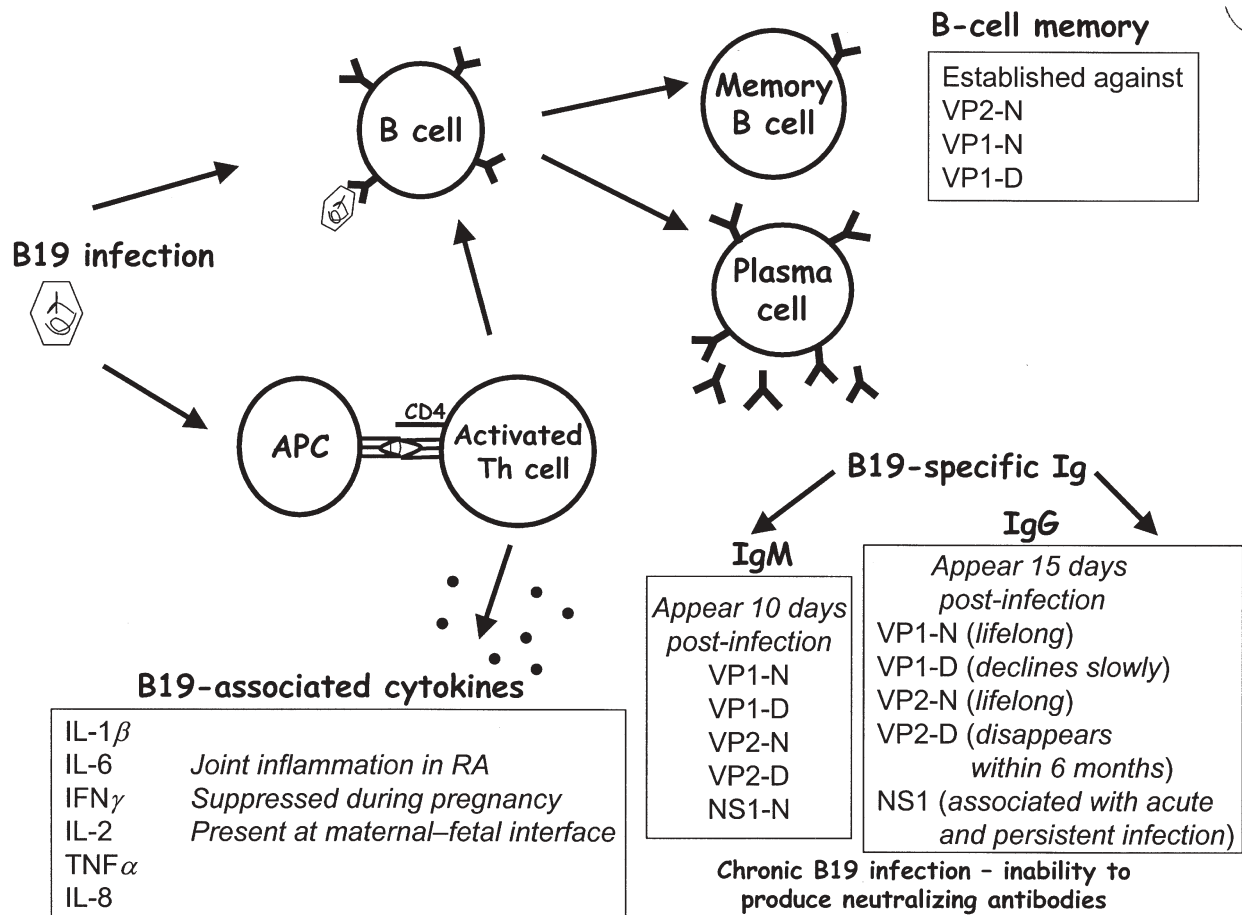
Fig. 2. Schematic depiction of T- and B-cell response to parvovirus B19 infection. Upon B19 infection, B cells divide to produce plasma cells and memory cells. Plasma cells secrete IgM antibodies that are specific for both conformational (N) and linear (D) epitopes of B19, which are detectable approximately 7 days post-infection. B19-specific IgG is detectable about 15 days post-infection and is directed initially against both linear and conformational epitopes of the capsid proteins (VP1 and VP2) and, to a lesser extent, against NS1, but declines against linear epitopes of the proteins in a time-dependent manner. Infection by B19 most likely confers lifelong protection on the host, due to the development of memory B cells that are specific for conformational regions of the B19 capsid and also linear regions of the VP1 protein (Corcoran *et al.*, 2004). Antigen-presenting cells (APC) process the virus and display B19 peptides on their surface to Th cells. These Th cells then secrete cytokines that play a role in mediating anti-virus immunity and may also be associated with pathogenesis of B19 infection (e.g. IL6 is associated with RA).

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