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Parvovirus B19 Infection

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Human parvovirus B19, discovered in 1974, is a single-stranded DNA virus which causes erythema infectiosum, arthralgia, aplastic crisis in patients with red cell defects, chronic anaemia in immunocompromised patients, and fetal hydrops. Seroprevalence in developed countries is 2–10% in children less than 5 years, 40–60% in adults more than 20 years, and 85% or more in those over 70 years. The virus may be transmitted by the respiratory route and by transfusion of infected blood and blood products. After an incubation period of six to eight days, viraemia occurs, during which reticulocyte numbers fall dramatically resulting in a temporary drop in haemoglobin of 1 g/dl in a normal person. Clearance of viraemia is dependent on development of specific antibody to the B19 structural proteins, VP1 and VP2. The red cell receptor for the virus is blood group P antigen. Diagnosis in immunocompetent persons depends on detection of specific IgM in serum. Diagnosis in immunocompromised persons depends on detection of B19 antigen or DNA in serum. There is no specific treatment for B19 infection; however, human normal immunoglobulin may be used as a source of specific antibody in chronically infected persons. A recombinant parvovirus B19 vaccine is under development.

Human parvovirus B19 is the aetiological agent of the common rash illness, erythema infectiosum or "fifth disease," arthralgia, aplastic crisis in patients with red cell defects, chronic anaemia in immunocompromised patients, and fetal hydrops (1). B19 virus was discovered in 1974 during screening of healthy blood donors for hepatitis B (2). Seroprevalence studies revealed that B19 is a common infection, and it was linked in 1981 with aplastic crisis in patients with sickle cell disease (3), in 1983 with erythema infectiosum during a school outbreak (4), in 1984 with fetal hydrops (5), in 1985 with arthropathy (6, 7), and in 1987 with chronic anaemia in a patient with congenital immunodeficiency (8).

B19 has not been shown to infect animals, and the animal parvoviruses have not been shown to infect humans (9). Parvovirus B19 is the only known parvovirus to infect humans. Two other members of the autonomous parvoviruses, the faecal parvovirus (10) and the RA-1 virus (11), are reported as potential human pathogens but observations have not been confirmed. In this review, aspects of B19 infection will be considered under the headings Virological Aspects, Epidemiology, Diagnosis, Clinical Manifestations of Infections, Treatment, Prevention of Infections, and Future Prospects.

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Virological Aspects

Human parvovirus B19 is a member of the family *Parvoviridae* and is the only member of the recently created genus, *Erythrovirus* (12). B19 is autonomous, not requiring the presence of a helper virus, and until recently was classified in the genus *Parvovirus*.

DNA. The B19 genome consists of a single-stranded linear molecule of 5,596 nucleotides (nt), composed of an internal coding sequence of 4,830 nt, flanked by the terminal repeat sequences of 383 nt each (13). B19 DNA occurs in virions as both plus and minus strands in approximately equal numbers. The terminal repeat sequences fold to form hairpins which serve as primers for the synthesis of the complementary strand (14). Suspension culture of B19 in human erythroid bone marrow has allowed study of molecular events associated with B19 replication (15, 16). The current model of B19 replication is based on

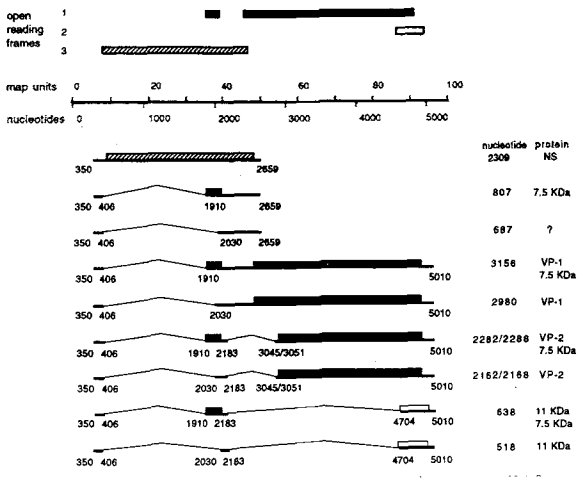

Figure 1: Parvovirus B19 transcription map (24).

a model proposed by Cavalier-Smith to explain eukaryotic chromosomal replication. The model makes several predictions which have been supported by studies of B19 and other parvoviruses (17). First, the model predicts the existence of a monomer duplex replicative intermediate form in which one end is covalently joined. Such molecules can be detected in infected cells. Second, the model predicts that the termini are the origins of replication. While no data directly support this conclusion for B19, experiments have shown that this is the case for the adeno-associated virus (17). Third, the model predicts that the terminal sequence should be inverted in the process of replication. This prediction was confirmed for B19 as DNA sequencing of the termini showed both "flip" and "flop" configurations. The model predicts that, because both terminal hairpins are identical, DNA replication can initiate at the 3' end of both strands and that equal amounts of molecules having the "flip" or "flop" configuration are generated. Therefore, the complementary strand is first synthesised to form monomer duplex DNA. This is then replicated to form dimer duplex DNA, which serves as the template for production of progeny virion DNA (15).

B19 isolates have been divided into various groups on the basis of restriction endonuclease digestion (18) and the single-stranded conformational polymorphism (SSCP) assay (19). Using restriction endonuclease digestion, Japanese workers have demonstrated that particular genotypes circulate within Japan and are associated with increases in prevalence of B19 infection (20, 21). In general, there has been no correlation between genotype and clinical disease presentation. However, one study showed that, of seven persistently infected persons, five had the same SSCP type with respect to the region studied: nucleotides 1,399–1,682, within the B19 nonstructural gene (22).

RNA. Transcription produces at least nine overlapping messenger RNA transcripts, all initiated from the P6 promoter at the extreme left side of the genome (23, 24). There are two sets of RNA transcripts encoding the structural proteins VP1 and VP2, and the 11 kDa and 7.5 kDa proteins, but only one RNA transcript encoding the nonstructural (NS1) protein (Figure 1) (24–26).

Nonstructural Proteins. The major nonstructural protein (NS1) was identified by immunoblot analysis of B19-infected tissues (27), has a molecular weight of 77 kDa, and is encoded by the left side of the genome, nucleotides 435 to 2,448 (28). Parvovirus nonstructural proteins are fairly homologous between different species consistent with their role in virus propagation (29, 30). The B19 NS1 protein contains a highly conserved 145 amino acid sequence located in the middle of the NS1 gene, nt 1,390–1,794 (28), which has significant homology with the nonstructural proteins of other parvoviruses, the T antigens of polyomavirus and SV40, and the E1 proteins of papillomavirus (31). This region contains two phosphorylation sites, an amidation site, and a nucleotide binding site. Mutation analysis of the nonstructural gene of the rat parvovirus H-1 has demonstrated that some of the multiple activities of the protein can be abolished by a single amino acid substitution from lysine to serine in the nucleotide binding site within this region of homology (32).

B19 NS1 has been localised to the nuclei of infected cells (33), and may account for cytotoxicity and cell death (34). It has been hypothesised that early in infection, a low concentration of B19 NS1 protein positively regulates viral DNA replication, while late in infection, high concentrations lead to cell death, lysis, and virus release. B19 NS1 cytotoxicity may also account for the high frequency of neutropenia and thrombocytopenia during B19 infection, since B19 does not replicate in myeloid precursors in vitro (15, 16, 35–37).

In transient transfection experiments, B19 NS1 protein was found to transactivate expression of genes under the control of the HIV-1 long terminal repeat (LTR) (38). This effect of the B19 NS1 protein was only apparent in the presence of the HIV-1 Tat protein, suggesting that HIV-1 gene expression could be affected by the B19 NS1 protein in dually infected cells. Immediate early proteins

from other DNA viruses such as herpesviruses have been shown to transactivate the HIV-1 LTR but the B19 NS1 protein seems unique in that it requires the presence of Tat. Some herpesviruses are considered to act as '"cofactors" in HIV expression, and possibly participate in the evolution of the disease (39, 40). B19 infection may play a similar role in haemopoietic tissue.

The biological functions of the 7.5 kDa and 11 kDa proteins are unknown. Nevertheless, the B19 genome is very small and it is logical that these proteins are employed for a specific purpose related to either the virus replication cycle or regulation of the host response (26).

Structural Proteins. Structural proteins VP1 and VP2 are encoded by the right side of the genome, by nucleotides 2,444–4,786 and 3,125–4,786, respectively (28). The major structural protein, VP2, has a molecular weight of 58 kDa. The minor capsid protein, VP1, is identical to VP2 but with an additional 226 amino acids at the amino-terminus, and has a molecular weight of 84 kDa. The sequence for the mRNA-encoding VP2 is entirely contained within that for VP1, a mechanism which increases the coding potential of the small B19 genome.

Virus Morphology. B19 virus is an icosahedron consisting of 60 copies of the capsid proteins with 96% VP2 and 4% VP1 (41). The capsid proteins are produced in this ratio due to the relative inefficiency of VP1 RNA translation. Using genetic engineering, the capsid proteins can be expressed in a variety of both mammalian (41, 42) and insect cell lines (43, 44), and have been shown to self-assemble as recombinant empty capsids in the absence of B19 DNA. VP1 is not required for capsid formation (41, 42). Although the ratio of VP1 to VP2 appears fixed in B19 virions, in baculovirus-produced empty capsids the ratio can be markedly altered by changing the relative concentrations of VP1 and VP2 recombinant viruses used for infection (45). Antisera specific to the unique amino-terminus of VP1 can immunoprecipitate both recombinant empty capsids and plasma-derived virions (45), suggesting that at least some of the unique minor capsid proteins are expressed on the virion surface. The VP1 unique region may therefore extend to the outside of the virion.

Cellular Receptor. B19 has a very narrow target cell range, being highly tropic for proliferating human erythroid cells in late S phase of the cell cycle. The cellular receptor for B19 is blood group P antigen (globoside; a tetrahexose ceramide) (46), and persons with the rare "p" phenotype, who do not have P antigen, are naturally resistant to B19 infection (47). B19-infected bone marrow shows an identical pattern of cellular expression of P antigen and B19 capsid proteins (48).

The P blood group, discovered by Landsteiner and Levine (49) in 1927, contains two common antigens, P_1 and P, and a third, P^k, that is much rarer. P antigen was subsequently identified as globoside (50). Red cells of persons with the blood group P_1 phenotype have both P and P_1 antigens; persons with the P_2 phenotype have P antigen alone. The rare persons with the $P_1{}^k$ and p phenotypes have no P antigen on their red cells; persons with the $P_1{}^k$ phenotype have both P_1 and P^k antigens (50).

The tissue distribution of P antigen is consistent with the known tropism of B19. The antigen is found on erythroblasts and megakaryoblasts (51); endothelial cells which may be target cells in transplacental transmission (5), the rash of fifth disease (52), and vasculitis (53); and on fetal myocardial cells (54) in which B19 has been demonstrated (55, 56).

Using monoclonal antibody-mediated haemagglutination-inhibition and mutational analysis, B19 virus attachment proteins have been localised to VP2 amino acids 57–77 (57, 58) and 345–365 (1).

In Vitro Infection. Erythroid progenitor cells from a number of different sources have been shown to support B19 replication. For example, human bone marrow (15, 35), fetal liver (59, 60), erythroid cells from a patient with erythroleukaemia (61), human umbilical cord blood (62, 63), and peripheral blood (64, 65). All these systems require erythropoietin to maintain the erythroid cells in rapid division. The main target cells for B19 infection are late erythroid progenitors (CFU-E) and erythroblasts (66), and immunophenotyping has identified susceptible cells from fetal liver as pronormoblasts, expressing CD36 and glycophorin A (67). B19 has also been propogated in two megakaryoblastoid cell lines, UT-7 (68) and MB-02 (69).

In erythroid progenitors, B19 infection causes a cytopathic effect, with characteristic light (16) and electron microscopic (EM) (37, 60) changes. Infected cultures show the presence of giant pronormoblasts or "lantern" cells (70). These are early erythroid cells, 25–32 μm in diameter, with cytoplasmic vacuolisation, immature chromatin, and large eosinophilic nuclear inclusion bodies.

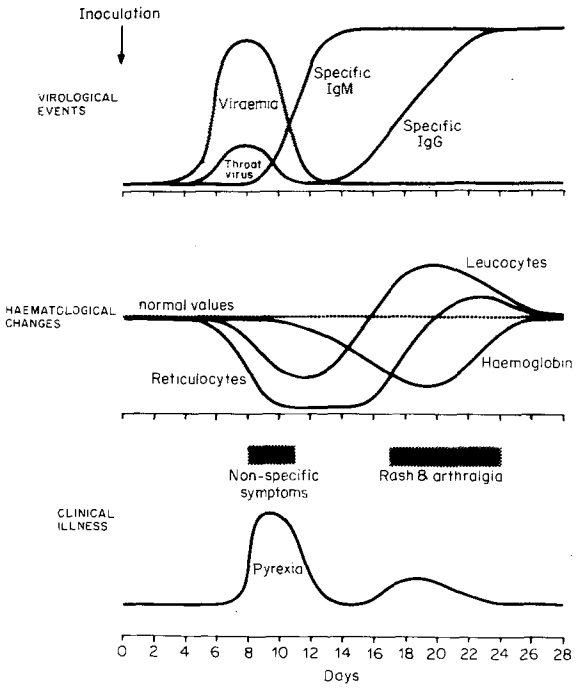

Figure 2: Virological, immunological, haematological, and clinical course of experimental B19 infection in a normal person (73).

It has previously been assumed that B19 infection results in cell death simply due to cell lysis (71); however, in vitro culture of fetal erythroid precursors has shown ultrastructural features typical of programmed cell death or apoptosis (72). The NS1 protein of B19 may have an important role to play in this process, consistent with its known cytotoxicity. Morey et al. (72) also demonstrated large crystalline arrays protruding from the surface of infected cells in the form of blebs, separation of which may represent a means of viral egress without membrane rupture and thus protection from host antibody.

Pathogenesis. The course of infection in humans has been characterised by two volunteer studies (73, 74). The time course of virological, immunological, haematological, and clinical findings are summarised diagrammatically in Figure 2. Following intranasal inoculation, viraemia appeared on day 6, accompanied by a mild illness with pyrexia, myalgia, pruritis, and excretion of virus from the respiratory tract. In the second week after inoculation, as the titre of viraemia falls, IgM appears and is detectable for two to three months. IgG is first detectable in the third week after inoculation and persists indefinitely. At the first detection of anti-B19 IgG, a fine pruritic maculopapular skin rash and arthralgia occurred, presumably on the basis of immune complex deposition (75); these symptoms lasted approximately four days. In these studies the prior presence of serum anti-B19 IgG was protective.

During viraemia, reticulocyte numbers fall to undetectable levels, recovering 7 to 10 days later in a normal person, resulting in a temporary drop in haemoglobin of 1 g/dl. Clinically nonsignificant lymphopenia, neutropenia, and thrombocytopenia occur 6–10 days after inoculation.

Immune Response. Both virus-specific IgM and IgG are produced following experimental (73) and natural (76) B19 infection. In transient aplastic crisis, serum anti-IgM may be present at the time of reticulocyte nadir and during the subsequent 10 days, however serum anti-B19 IgG does not appear until the time of recovery. IgM may persist in serum samples several months after exposure (77). IgA can also be detected and presumably plays a role in resistance to natural infection by the nasopharyngeal route (78).

In normal individuals, resolution of B19 infection is associated with specific antibody production, and sera from these persons can neutralise the inhibitory effect of B19 on erythrocyte colony formation in vitro (36). In view of this and since a proliferative cellular response to B19 has not been demonstrated (79), the humoral response to B19 would seem to be the crucial factor in disease resolution. Kurtzman et al. (79) demonstrated that in humans, the early antibody response to B19 infection consists of IgM and is almost entirely VP2 specific. As the response matures, IgG becomes the major antibody subclass and the primary viral protein detected on immunoblots is VP1, despite its much lower relative concentration in the virion. VP1 is also the major target specificity of pooled human immunoglobulin (79) used in the treatment of chronic infection. In persistently infected patients, including HIV-infected individuals who are able to generate high titres of B19-specific antibody, the switch from predominant IgM and VP2 reactivity to predominant IgG and VP1 reactivity does not occur. The avidity of anti-B19 IgG has been shown to increase with time after acute infection (80). Therefore, differentiation between high and low avidity anti-B19 IgG helps to discriminate between past and recent infection.

Several regions containing neutralising epitopes have been localised to linear sequences of B19, one region at the amino-terminus of VP2 at amino acids 38–87 (58) and six others distributed with-

in the carboxy terminal half of VP2 at amino acids 253–272, 309–330, 328–344, 359–382, 449–468, and 491–515 (81, 82). Neutralising epitopes are also found in the unique region of VP1 (45). An immunodominant region recognised by a panel of monoclonal antibodies to empty capsids was localised to amino acids 259–426 of VP2 (83).

Addition of VP1 to the capsid has two effects. It facilitates presentation of the spike region to the immune system and adds its own intrinsic neutralising determinants. Antisera raised to the unique region of VP1 neutralise virus infectivity and precipitate empty capsids and virions, suggesting that the unique region is expressed on the virus surface (45). Linear epitopes from the VP1 unique region, presented as fusion proteins or synthetic peptides, are far more efficient at eliciting a neutralising immune response than peptides from the common or VP2 protein sequence (84). Both anti-VP2 and anti-VP1 specificities are present in normal human convalescent antisera, and sera that predominate in either specificity effectively neutralise the virus. However, VP1 is the major antigen recognised on immunoblot by late convalescent phase antiserum or in commercial immunoglobulin preparations (79).

Only a limited number of linear epitopes have been detected, but the majority of B19 neutralising monoclonal antibodies do not recognise sequences within the capsid proteins and therefore presumably bind to conformationally determined epitopes (58). While VP2 contains neutralising epitopes, these are not presented to the immune system in empty capsids consisting only of VP2 (43). These findings suggest that the conformation of some VP2 determinants is altered by insertion of one or two VP1 molecules per 60 protein subunit capsid (85). A further alteration probably occurs with insertion of DNA into empty capsids containing both proteins, as many monoclonal antibodies raised to virions and screened by enzyme immunoassay (EIA) fail to recognise empty capsids consisting of VP1 and VP2 in the same test (1).

Persistent B19 infection is the result of failure to produce effective neutralising antibodies by the immunocompromised host. In these patients, a poor reaction on immunoblotting is a consistent finding, correlating with poor neutralising activity for the virus in erythroid colony assays (79). These results suggest that the linear epitopes detected by immunoblots are functionally very important, and that the clinical findings in persistent infection are analogous to animal data in which development of a neutralising antibody response is dependent on presentation of specific epitopes, particularly those in the amino-terminal region of VP1 (1). Perhaps because of the limited number of epitopes presented by B19 to the immune system, the congenital immunodeficiency states associated with persistent infection may be clinically subtle, with susceptibility largely restricted to parvovirus B19 (1).

Epidemiology

Parvovirus B19 is a common human infection. The seroprevalence in developed countries is 2–10% in children less than 5 years, 40–60% in adults more than 20 years, 60% in blood donors (86), and 85% or more in those over 70 years (87). Antibody acquisition occurs earlier in developing countries (75). However, there are areas of the world without B19 infection, and it has not yet been found among members of certain tribes (88, 89). In temperate climates, there is an increased prevalence from late winter to early summer (90, 91), and increased infection rates occur every four to five years (75). B19 is transmitted in the community by the respiratory route causing infection both sporadically and in outbreaks, which are easily apparent in schools (92). The case-to-case interval is 6 to 11 days and is independent of the type of disease. Nosocomial transmission has been described (93–96) but would seem to be infrequent. According to the Centers for Disease Control, Atlanta, USA, patients with transient aplastic crisis or persistent infection should be considered infectious and admitted to private rooms with standard source isolation procedures (i.e. efficient handwashing after patient contact, masks for close contact, gloves for contact with body secretions, and gowns when soiling is anticipated) (97). Transmission of B19 has also occurred among staff in medical laboratories working with native virus (98–100).

The virus can be found in serum, the incidence of which is estimated between 1:3,300 (101) and 1:50,000 (102), and infection has been transmitted by clotting factor concentrates (102–109), but not by intravenous IgG (101), red cell, white cell, and platelet- or plasma-containing single donor components.

Diagnosis

Serum anti-B19 IgM and IgG can be detected by

Table 1: Clinical manifestations of parvovirus B19 infection.

Syndrome	References
Asymptomatic B19 infection	(116)
Erythemia infectiosum	(75)
Arthralgia	(6, 7)
Aplastic crisis	(3)
Fetal anaemia and hydrops	(155)
Congenital red cell aplasia	(165)
Chronic bone marrow failure	(79)
Myocarditis	(56, 155, 184, 185)
Vasculitis	(53)
Glomerulonephritis	(212)
Congenital malformation*	(158, 159)
Neurological disease*	(186–189)
Kawasaki disease*	(193)
Chronic fatigue syndrome*	(22)
Systemic lupus erythemtatosus*	(202–204)
Kikuchi's disease*	(207)
Hepatic dysfunction*	(209, 210)
Conjunctivitis*	(211)

* Causality not confirmed.

IgM antibody capture radioimmunoassay (RIA) (86), IgM antibody capture EIA (110), and immunofluorescence using insect cells expressing B19 antigens (44). IgM detection is the best method for detection of recent infection in immunocompetent persons. In an antibody capture RIA (86), IgM antibody can be detected in over 90% of cases by the third day of transient aplastic crisis or at the time of rash in erythema infectiosum, and remains detectable for two to three months following infection. IgG is usually present by the seventh day of illness and is probably present lifelong thereafter. As more than 50% of the population have anti-B19 IgG, its detection may not be useful for diagnosis of acute infection. However, Gray et al. (80) have shown that by including urea as a mild denaturing agent in a fluorescent antibody test, high and low avidity anti-B19 IgG may be discriminated, which helps to distinguish recent from past infection.

Due to the inability to grow B19 in standard cell culture systems, there has been a shortage of viral antigen for diagnostic assays. The expression of B19 capsid proteins as virion-like particles using transfected B19 genome into Chinese hamster ovary (CHO) cells (41), COS-7 cells (42), and the baculovirus expression system (43, 44) has therefore been employed, greatly improving the diagnostic possibilities. These recombinant antigens are relatively easy to mass produce and are noninfectious, and serological results obtained using them correlate well with those using native virus (43, 111). Baculovirus-expressed B19 proteins are now being commercially marketed in the form of EIA and fluorescent antibody tests.

The development of B19 monoclonal antibodies has led to the development of assays for B19 virus. Although these lack sensitivity, they may be used to localise the histological site of infection and to demonstrate other tissues permissive for B19 replication (112). Electron microscopy can also be used to detect B19 virus. For serum, immune-electron microscopy has approximately the same sensitivity as antigen assays. Within cells it may be difficult to recognise the virus due to the presence of similar-sized ribosomes.

Dot-blot and in situ hybridisation can be used to detect B19 DNA in serum and cells, respectively. The polymerase chain reaction (PCR) has greatly increased sensitivity of B19 DNA detection in both serum and tissues (19, 113–115).

Clinical Manifestations of Infection (Table 1)

Asymptomatic Infection. B19 infection is frequently asymptomatic. In one study, 25% of affected persons recalled no symptoms (116). In IgM-positive pregnant women who had been exposed to cases of B19 infection, fewer than half reported symptoms of rash or arthropathy (91, 117).

Erythema Infectiosum. Erythema infectiosum, or "fifth disease" is the major manifestation of B19 infection. Erythema infectiosum was well studied prior to the discovery of B19 (118–120), and is characterised by a nonspecific prodromal illness that often goes unnoticed but may cause fever, coryza, headache, nausea, and diarrhoea. The exanthem in classic cases of erythema infectiosum occurs in three stages. The first begins 18 days after acquisition of infection and is characterised by a "slapped cheek" eruption with relative circumoral pallor. The second stage occurs one to four days later with an erythematous maculopapular rash on the trunk and limbs, which may spread to involve large areas; toward the end of this stage there is central clearing of the rash to give the characteristic lacy or reticular pattern. The third stage is highly variable in duration, lasts one to three or more weeks, and is characterised by marked changes in rash intensity related to environmental factors such as sunlight and temperature (75).

Arthralgia. Joint symptoms associated with B19 infection occur in approximately 8% of children and up to 80% of adults, the majority being wom-

en (116, 119, 121, 122). Affected joints are painful, swollen, and stiff. B19 arthralgia may affect any joint but usually occurs symmetrically in the wrist, hand, knee, and ankle (6, 7, 116). Joint symptoms last one to three weeks, although in 20% of affected women arthropathy may persist for months to years. In those with prolonged symptoms there is no corresponding increase in the elevation or duration of the anti-B19 IgM response. The arthralgia may also occur without the rash. Approximately half of these patients meet the American Rheumatism Association (ARA) criteria (123) for the diagnosis of rheumatoid arthritis (124). In normal individuals, B19 virus is rapidly cleared by the development of a neutralising antibody response. However, the persistence of rash and arthropathy in some patients raises the possibility that persistent infection and an abnormal immune response to B19 sometimes occurs even in the normal patient.

Abnormal immune responses to B19 antigens have been demonstrated in patients who develop B19 arthropathy. Using unique VP1 peptides as antigen, multiple epitopes were recognised by the serum of individuals with asymptomatic B19 infection; however, patients with acute self-limiting B19 arthropathy and chronic B19 arthropathy lacked these antibodies (125).

B19 DNA has been found in the synovial fluid of a woman with serologically proven B19 infection (126) and in synovial fluid cells of a patient with "reactive arthritis" (127).

Rheumatoid Arthritis. Generally, a considerable number of patients with B19-induced arthritis develop a chronic rheumatoid arthritis-like polyarthritis (6, 7, 128). Most of these patients are women (6, 7) and rheumatoid factor may be present or may rise following B19 infection (128, 129). In one study, 19 of 153 patients attending an early synovitis clinic in the UK had evidence of recent B19 infection. These patients typically presented with sudden onset of symmetrical peripheral polyarthropathy of moderate severity (7), and in three patients the clinical illness fulfilled the ARA diagnostic criteria for rheumatoid arthritis (123). Sixty-six percent of these 19 patients were HLA-DR4 compared to 36% of controls (130). The rheumatoid arthritis and juvenile chronic arthritis associated haplotypes, DR4 and DRw11, are considered by some authors to be risk factors for chronicity of B19-induced arthritis (129–132).

Cohen et al. (129) found a significantly increased level of B19 seropositivity in rheumatoid arthritis patients; 36 of 39 rheumatoid arthritis patients were positive compared with 25 of 37 inflammatory arthritis patients and 42 of 69 control patients. In 4 of 69 cases of early rheumatoid arthritis (129) and one case of juvenile chronic arthritis (131), serum anti-B19 IgM was detected.

Using PCR to detect B19 DNA, Saal et al. (113) tested the synovial tissue and peripheral blood leukocytes (PBL) of 20 patients with rheumatoid arthritis, 24 patients with other arthritides or osteoarthritis (nonrheumatoid arthritis), and 34 healthy blood donors. B19 DNA was demonstrated in the synovium of 75% of patients with rheumatoid arthritis but in only 16.7% of patients with nonrheumatoid arthritis. In autologous PBL, B19 DNA was found in approximately 15% of rheumatoid arthritis cases, nonrheumatoid arthritis cases, and healthy controls. All patients with B19 DNA in PBL alone or in both PBL and synovium were positive for serum anti-B19 IgG; in contrast, about 40% of patients with B19 DNA restricted to the synovium were negative for serum anti-B19 IgG. Foto et al. (133) detected B19 DNA by PCR in the bone marrow of four patients who developed chronic joint symptoms 24 to 42 months after acute B19 infection, compared with none of seven B19-seronegative and none of six B19-seropositive normal bone marrow donors. At presentation, all four patients tested positive for serum anti-B19 IgM and IgG. At the time of bone marrow aspiration, all four patients tested negative for serum anti-B19 IgM and positive for serum anti-B19 IgG. In the same study, three additional patients with acute B19 infection and acute but nonchronic joint symptoms had B19 DNA detected in bone marrow aspiration 2 to 18 months after infection; serologically, all three patients tested negative for serum anti-B19 IgM and positive for serum anti-B19 IgG at the time of bone marrow aspiration. These studies suggest that B19 arthropathy is associated with B19 persistence. The duration of persistence of B19 DNA after acute infection may be considerable. For example, Kerr et al. (22) demonstrated persistent infection in 7 of 53 persons (13.2%) for an average of 57 months after acute B19 infection. None of these were known to be immunosuppressed.

However, not all studies support an aetiological role for B19 in rheumatoid arthritis. Nikkari et al. (134) found serological evidence of recent parvoviral infection in 4 of 135 rheumatoid arthritis patients and 2 of 62 controls. No evidence for the presence of B19 DNA was detected in 18 samples of synovial fluid, 21 samples of synovial fluid

granulocytes, and 40 sera, all obtained from 65 patients with early rheumatoid arthritis. Hajeer et al. (135) measured serum anti-B19 IgG in 155 twin pairs discordant for rheumatoid arthritis to assess the association between exposure to B19 and susceptibility to rheumatoid arthritis. The authors concluded that overall, previous exposure to B19 did not explain disease susceptibility in either monozygotic or dizygotic twin pairs discordant for rheumatoid arthritis. Using PCR, Kerr et al. (114) found B19 DNA in the synovium of 10 of 26 rheumatoid arthritis patients and 9 of 26 osteoarthritis patients; all tissues were negative for B19 capsid proteins.

As synovial membrane cells are nonpermissive for B19 replication (136) and P antigen has not been found in synovium (114), the pathogenesis of B19-induced arthritis may involve immune complex deposition (7).

Aplastic Crisis. Transient aplastic crisis (TAC) was the first clinical entity associated with B19 virus (3), and is manifested by the abrupt cessation of erythropoiesis, reticulocytopenia, absence of erythroid precursors in the bone marrow, and sudden worsening of anaemia, usually occurring in patients with a defined haematological predisposition, such as hereditary spherocytosis (137), thalassaemia (138), red cell enzymopathies such as pyruvate-kinase deficiency (139, 140), and autoimmune haemolytic anaemia (141). TAC can also occur under conditions of erythroid stress, such as haemorrhage (142) and iron deficiency anaemia (143), and following kidney (144) or bone marrow (145) transplantation. In normal patients a drop in erythrocytes and reticulocytes occurs (73), and although acute anaemia has been described in normal patients (146–148), there is usually sufficient haempoietic reserve for the effect on the bone marrow to remain subclinical.

TAC usually presents with pallor, weakness, and lethargy, and patients may report nonspecific systemic symptoms one to seven days earlier (149). Although TAC is ultimately self-limiting, patients can be severely ill and fatalities have occurred (150). In contrast to patients with erythema infectiosum, TAC patients are often viraemic at the time of presentation, with blood virus concentrations as high as 10^{14} genome copies per ml (151). Thus symptoms and viraemia occur at the same time in TAC, and patients pose a risk of transmission to others (95); in erythema infectiosum, symptoms usually occur after the viraemia. TAC is a unique event in the patient's life, following which immunity is life-long.

Infection during Pregnancy. The pathogenesis of fetal damage appears to be similar to that leading to TAC in other conditions in which the red cell has a shortened life-span. During the second trimester there is a great increase in red cell mass. The immature immune system of the fetus may not be supported by transplacental transfer of maternal antibody of sufficient quantity or quality for protection until late in pregnancy (152). Consequently, the fetus may develop a persistent infection and anaemia leading to congestive heart failure, generalised oedema, and death. Although B19 DNA, antigen, and virions have been detected in a wide variety of fetal tissues, including lung, kidney, thymus, and heart (153), in most of these tissues the virus is located within the vascular lumen (154). In addition, there is evidence that the fetus may develop myocarditis (56, 155), compounding the severe anaemia and secondary heart failure. By the third trimester the fetus is able to mount a more effective immune response to the virus, which may account for the decrease in fetal loss at this stage of pregnancy.

Infected fetuses show evidence of a leukoerythroblastic reaction: large pale cells with eosinophilic inclusion bodies and margination of the nuclear chromatin. Inclusion-bearing erythroid cells may be distributed in the liver, lung, and other tissues, and are sufficiently characteristic to enable a provisional diagnosis of B19 infection on histological examination. About 50% of mothers report symptoms of rash or arthropathy occurring 4 to 13 weeks before fetal death, the remainder being asymptomatic (91).

Results from two prospective studies and other published reports indicate that fewer than 10% of maternal infections lead to fetal death (156, 157). In the study by the Public Health Laboratory Service (PHLS), UK (156), 186 pregnant women positive for serum anti-B19 IgM were followed up, 90% of whom became IgM positive at < 20 weeks gestation; 156 (84%) had normal deliveries and 30 (16%) suffered fetal loss. Fetal tissues from 14 of the fetuses were tested for B19 DNA, and those found to be positive were likely to have died from the infection. Six of the 14 were positive and two were equivocally positive. If this rate is applied to all 30 fetal deaths, then an upper limit estimate for B19-related fetal death among infected women would be 9.2%. It is therefore clear that most B19-infected pregnant women did not suffer fetal loss. Also, approximately 25% of normal infants born to IgM-positive mothers had evidence of fetal infection, for example, anti-B19 IgM in cord blood

or serum anti-B19 IgG at one year of life. Therefore, maternal infection does not usually involve the fetus. The risk of fetal death was less than 10% overall, but was increased in infection acquired in the first 20 weeks of gestation. These infants are still being followed up to detect any late sequelae.

Following a large outbreak of erythema infectiosum in Connecticut, USA, 39 pregnant women positive for serum anti-B19 IgM were followed up (157). No fetus was hydropic but two women, who were infected in the first trimester, had miscarriages. The overall fetal loss rate following B19 infection was estimated to be 5%.

Torok et al. (4th Parvovirus Workshop, 1991, Abstract 11-2) followed up 190 pregnant women with acute B19 infection along with susceptible and immune age-matched controls. The overall fetal loss rate was 5.9% in the infected group, compared with 2.9% in the susceptible group and 3.7% in the immune group. As in the PHLS study (156), there was an increased risk of fetal loss following B19 infection in the first 20 weeks of gestation.

Congenital Infection. There are two case reports of congenital malformation associated with B19 infection, both from the Netherlands. In the first case (158), maternal B19 infection was confirmed serologically at six weeks, and the pregnancy was electively terminated at 12 weeks for other reasons. Ocular abnormalities similar to those seen in rubella were observed. B19 DNA was detected in placental and fetal tissue, and there was no evidence of rubella. In the second case (159), a fetus with multiple structural defects was seen at prenatal ultrasound examination. After termination, a bilateral cleft lip, alveolus, and palate, micrognathia, and webbed joints were seen. B19 DNA was detected in fetal tissues by dot-blot hybridisation and PCR. Although sporadic reports have noted an association between B19 infection and genitourinary abnormalities (156, 157), causality has not been confirmed as the abnormalities are relatively common (1). In addition, studies have shown no evidence of congenital abnormalities following maternal B19 infection (156, 160–162).

Fetal B19 infection can persist after birth. An infant born with thrombocytopenia was noted to be B19 viraemic at birth (163). The viraemia spontaneously resolved and the platelets returned to normal without therapy. Two weeks later specific antibody developed. Koch et al. (164) followed up 19 infants born to B19-infected mothers. All were normal at birth; however, three had B19-specific IgM. Two of these were IgG and IgM negative at the age of 11 and 16 months, respectively, and one had B19 viraemia with a high IgM titre at the age of seven months. These results suggest that intrauterine infection may be frequent and may occasionally cause an asymptomatic postnatal infection.

Brown et al. (165) reported an association between B19 and congenital red cell aplasia in three children with congenital anaemia after intrauterine B19 infection. All fetuses developed hydrops fetalis that was treated with blood transfusion. After delivery the infants had hypogammaglobulinaemia. In all three cases, B19 DNA was detected in the bone marrow but not in the serum. All three patients were treated with immunoglobulin. One child died and B19 was found in various tissues at post mortem, including thymus, brain, heart, liver, and spleen. In the other two cases, virus could no longer be detected after therapy but the patients remained persistently anaemic, suggesting a "hit and run" mechanism for the anaemia rather than a cytotoxic effect. The authors hypothesised that infection during a critical stage of development, other host characteristics, or intrauterine blood transfusion may have induced tolerance to B19 and/or an autoimmune response to erythroid precursors. The cellular receptor for B19 is P antigen (46) and an anti-idiotype response to B19 might therefore be directed against erythroid precursors. Intrauterine blood transfusion may have allowed survival of congenitally infected fetuses which would otherwise have died in utero. Diamond-Blackfan anaemia (congenital red cell aplasia) has heterogeneous manifestations and is hereditary. Most affected children develop anaemia by the third month of life, and may have cellular and humoral immunodeficiencies (166). Persistent B19 infection should be suspected in infants with Diamond-Blackfan anaemia exposed to B19 during fetal life (165).

Chronic Bone Marrow Failure. Persistent B19 infection has been reported in various groups of immunocompromised patients including patients with congenital immunodeficiency syndromes, AIDS, lymproliferative disorders, and transplant patients, and results in low titre viraemia (10^6 genome copies/ml) (79), being manifested as pure red cell aplasia (PRCA). PRCA is characterised by chronic lysis of red cell precursors and gradual onset of severe, persistent anaemia.

Syndromes of congenital immunodeficiency predisposing to persistent B19 infection include

Nezelof's Syndrome, a combined B- and T-cell defect (79), common variable immunodeficiency, predominantly a CD4+ lymphopenia with normal immunoglobulin levels (167), and severe combined immunodeficiency (168). One report of two patients with possible congenital immunodeficiency illustrates this syndrome (169). This report describes a 24-year-old man with a ten-year history of chronic red cell aplasia, intermittent fever, rheumatic symptoms, and a need for regular blood transfusions. When B19 infection was diagnosed, he had a high titre of B19 DNA in his serum despite high levels of anti-B19 IgM and low levels of anti-B19 IgG. This man probably had persistent infection for ten years, and had an older brother who also had chronic red cell aplasia. The brother died before B19 infection was diagnosed, presumably of complications of chronic blood transfusion. When B19 was diagnosed, the patient was treated with commercial intravenous immunoglobulin and responded with a prompt reticulocytosis, an increase in haemoglobin, and a decrease in serum B19 DNA. Immunoglobulin therapy was discontinued after approximately four months and the patient remained B19 DNA negative over the next year. Although there was no history of increased susceptibility to infections, a diagnosis of immunodeficiency was supported by low serum immunoglobulins and generally deficient in vitro cellular responses to antigens and mitogens. Chronic B19 infection was the only manifestation of immunodeficiency in this man.

The prevalence and clinical significance of B19 infection in HIV-infected persons is unknown at present. Abkowitz et al. (170) found B19 DNA in only 1 of 191 (0.5%) HIV-infected homosexuals and 4 of 24 (17%) transfusion-dependent HIV-infected homosexuals. Naides et al. (172) found B19 DNA in 9 of 14 (64%) HIV-infected individuals treated with dideoxyinosine due to failure of or intolerance to zidovudine therapy over a two-year period, four of whom suffered severe anaemia.

The majority of patients with lymphoproliferative disorders are children with lymphoblastic leukaemia (70, 172–177). As with other immunodeficiencies, patients presented with persistent anaemia, persistent presence of B19 DNA in the serum, and absent or low levels of B19-specific antibody. In general, bone marrow examination revealed giant pronormoblasts and immunoglobulin was curative (172). Another effective therapy was the temporary cessation of maintenance chemotherapy (173, 177), suggesting that a decrease in the level of immunosuppression may allow antibody production and virus clearance.

Persistent B19 infection has occurred following allogeneic bone marrow transplantation for myeloid leukaemia (145, 178). The source of the virus was unknown in both cases, but may have been transmitted by platelet transfusions in the second case. Chronic infection has also been described following procedures requiring potent immunosuppression, such as cardiac and liver transplantation (179). A prolonged anaemia following B19 infection has also been noted in patients with systemic lupus erythematosus on steroid therapy (180), and atypical manifestations of B19, such as prolonged rash and IgM response, in cancer patients on chemotherapy (181).

Persistent, as well as acute, B19 infection may affect the myeloid lineage (182). A previously healthy female developed recurrent granulocytic aplasia as the primary presentation of chronic B19 infection. The erythroid precursors appeared unaffected. B19 DNA was detected in serum by dot-blot hybridisation and PCR. The patient responded to one dose of immunoglobulin, but B19 viraemia recurred ten months later and a five-day course of immunoglobulin subsequently led to virus clearance. This patient, like that reported by Kurtzman et al. (169), had no prior history of increased susceptibility to infection. Immunoglobulins and T-cell responses to mitogens were normal, but a reduced cellular immune response was demonstrated by cutaneous testing, again suggesting the existence of a subtle immunodeficiency.

Kerr et al. (22) demonstrated persistent infection in 7 of 53 persons (13.2%) an average of 57 months after acute B19 infection. None of these were known to be immunosuppressed. All seven patients with persistent infection were women. Four patients were asymptomatic and three were symptomatic; one had arthralgia, one chronic anaemia, and one arthralgia and chronic fatigue syndrome. In the serum of the patient with chronic fatigue syndrome, two virus types were demonstrated by single-stranded conformational polymorphism (SSCP) assay. This phenomenon has not, until now, been demonstrated for B19 but is analogous to persistent Aleutian mink disease parvovirus infection in which multiple virus types occur in infected minks (183). In two of the four cases for which both the acute and follow-up PCR product was available, the SSCP type of the follow-up product was different from that of the acute product, showing genetic variation of the virus during persistent infection.

Myocarditis. There have been two fatal cases of B19-associated myocarditis. A one-year-old child with erythema infectiosum developed cardiac failure and died two weeks later (1). At postmortem examination, there was active myocardial inflammation with necrosis and B19 capsid proteins were detected in myocardial tissue sections. The myocardial findings were similar to those found in fetuses infected in utero with B19 (56, 155). In the second case, a three-year-old child, B19 DNA was detected in liver and spleen but not in the myocardium (184). Tsuda et al. (185) describe a patient with both haemophagocytosis and acute myocarditis associated with B19 infection. In view of these cases, the role of B19 in the pathogenesis of myocarditis requires further investigation, especially as B19 is known to cause myocarditis in the fetus.

Neurological Disease. Prior to the discovery of B19 as the causative agent of erythema infectiosum, two cases of encephalitis associated with fifth disease were reported (186, 187). In both cases, encephalitis followed appearance of the rash and, despite extensive investigation, no other cause for the encephalitis was found. In the first case (186), the eight-year-old child made a full recovery, but in the second, there were permanent neurological sequelae (187). In an immunocompetent patient with acute meningitis, B19 DNA was detected repeatedly in the blood and cerebrospinal fluid (CSF) in the nine months following presentation (188). In a second case of meningitis, B19 DNA was detected in CSF during the acute phase (189).

Pruritis is not uncommon in erythema infectiosum, and one study showed that 50% of patients with serologically confirmed B19 infection experienced neurological symptoms of tingling and numbness in fingers and toes; mild slowing of nerve conduction velocities and decreased amplitudes of motor and sensory potentials were also demonstrated (190). In most patients, neurological examination was normal apart from decreased sensation to light touch. However, one patient developed progressive weakness of one arm. Brachial plexus neuropathy with weakness and sensory loss has also been described in two other patients with B19 infection (191, 192). The mechanism for the neurological symptoms is thought to be immune mediated due to the prior appearance of the rash.

Kawasaki Disease. Nigro et al. (193) investigated B19 involvement in 15 children with Kawasaki disease. Active or recent infection, as shown by serum B19 DNA, anti-B19 IgM, or both, was detected in ten patients (67%). A high frequency of all major criteria for diagnosis of Kawasaki disease was found in children with the B19-associated disease, anaemia (60%), coronary aneurysms (30%), and arthropathy (30%) being documented. However, others have found no evidence of a role for B19 in Kawasaki disease (194, 195).

Vasculitis. B19 infection has been associated with vasculitis on the basis of serological evidence of acute infection in a few patients with various vasculitic syndromes (196–199). Recently, Finkel et al. (53) described three patients who had B19 infection with onset of systemic necrotising vasculitis syndrome, two with features of polyarteritis nodosa and one with features of Wegener's granulomatosis. These patients had persistent B19 infection, as shown by antibody testing and PCR for serum B19 DNA. The patients had atypical serological responses to B19, although none had a recognisable immunodeficiency. Corticosteroids and cyclophosphamide did not control the vasculitis, but human immunoglobulin led to long-term remission and virus clearance. Interestingly, human immunoglobulin is known to induce remission in some patients with polyarteritis nodosa and Wegener's granulomatosis (200, 201). P antigen, the erythrocyte receptor for B19 (46), is also present on vascular endothelial cells, suggesting a role for localised B19 infection. Histological evidence of vasculitis and endothelial damage in human fetuses after intrauterine B19 infection supports the concept that B19 can cause vascular endothelial damage (155).

Other Clinical Syndromes. B19 infection may mimic the presentation of systemic lupus erythematosus (SLE) (202) and has been associated with onset and exacerbation of SLE (203, 204) and antinuclear antibody production (205). One study of autoantibodies following B19 infection found that 14 of 53 test patients and 2 of 53 control patients had one or more serum autoantibodies (antinuclear antibody, anti – smooth muscle antibody, gastric parietal cell antibody, antireticulin antibody, antimitochondrial antibody, rheumatoid factor); the difference was significant ($p = 0.004$) (206). However, in only one patient (with rheumatoid factor) was this clinically relevant. B19 infection has been linked with three cases of systemic lupus erythematosus associated with necrotising histiocytic lymphadenitis (Kikuchi's disease) (207), a case of severe pulmonary disease after paediatric heart transplantation (208), two cases of hepatic dysfunction (209, 210), and one case of erythema infectiosum with

conjunctivitis, in which B19 DNA was detected in a specimen of conjunctival fluid (211). Kerr et al. (22) described chronic fatigue syndrome in 2 of 53 patients a mean of 57 months following acute B19 infection; in one of these patients, B19 DNA was detected in serum at follow-up. Weirenga et al. (212) described glomerulonephritis and nephrotic syndrome following B19-related aplastic crisis in seven patients with homozygous sickle cell disease.

Treatment

Symptomatic therapy for erythema infectiosum is rarely necessary, especially in children. Saline baths or calamine lotion may reduce pruritis and paracetamol may reduce the temperature. The prognosis in virtually all cases of erythema infectiosum is excellent. In TAC, once a satisfactory haemoglobin concentration has been obtained by erythrocyte transfusion, the prognosis is excellent.

If B19 infection complicates pregnancy, the pregnancy should be carefully monitored. At delivery, examination of cord blood for virus and specific IgM will reveal whether or not the virus has crossed the placenta and infected the fetus. Pregnant women may be monitored by ultrasonography for evidence of hydrops fetalis. Fetal blood sampling is possible after 18 weeks' gestation, and this blood may be tested for B19 DNA and anaemia. Intrauterine blood transfusion has been used to treat a few cases of B19-related fetal hydrops, but this high-risk procedure has not been properly evaluated (97). In the absence of convincing evidence for B19-induced congenital abnormalities, as is the case at present, there is no indication for therapeutic abortion (213).

Persistent B19 infection occurring in the immunocompromised patient may be treated with administration of human immunoglobulin which is a good source of neutralising antibodies as most of the adult population have been exposed to the virus. One patient with congenital immunodeficiency was cured by a ten-day course of immunoglobulin followed by intermittent injections until the virus had disappeared from his serum (8). Patients with AIDS initially respond to a five- to ten-day course. In the event of relapse, which may occur months later, a second course has been shown to be curative (214). Measurement of serum virus is helpful in predicting relapse and may assist in determining optimal treatment regimens. Recently, persistent B19 infection in an immunosuppressed patient after liver transplant was successfully treated with empirical plasmapheresis followed by human immunoglobulin (215). However, the role of plasmapheresis in persistent B19 infection remains to be evaluated.

Prevention of Infection

Passive and Active Immunisation. Due to the content of neutralising B19 antibodies (216), commercial immunoglobulin from normal donors can cure or ameliorate persistent B19 infection in immunocompromised patients (170, 214).

Recombinant B19 capsids produced in baculovirus-infected insect cells induce neutralising antibodies in inoculated animals (43). Capsids consisting of $\geq 25\%$ VP1 protein efficiently and consistently produced vigorous B19 virus – neutralising responses (217). These recombinant capsids are viable candidates for a human B19 vaccine and should soon be evaluated in clinical trials (1).

Animal Model for B19 Infection. O'Sullivan et al. (218) have recently identified a novel simian parvovirus (SPV) in cynomolgous monkeys with severe anaemia. Based on the sequence of a 723 bp fragment of cloned viral DNA extracted from serum, there was 65% homology between SPV and B19 but little homology between SPV and other parvoviruses. Light microscopy of bone marrow from infected animals showed intranuclear inclusion bodies and marked dyserythropoiesis, suggesting that B19 infection may underlie human dyserythropoietic syndromes. Affected animals had concurrent infection with the immunosuppressive type D simian retrovirus; the clinical situation was therefore analogous to that in which HIV-infected patients develop severe anaemia due to persistent B19 infection. Evidence of bone marrow regeneration was noted and the dyserythropoiesis was similar to that found in human congenital dyserythropoietic anaemias.

This inevitably leads to a recognition of the potential importance of SPV infection in monkeys as a model for studying one of the major remaining questions in the study of B19: i.e. what is the full range of consequences of intrauterine infection? In particular the association with human congenital dyserythropoietic anaemias or conditions such as Diamond-Blackfan anaemia warrants study. It would take a very large series of human cases to prove or disprove these associations, and

it is in this context that the new potential for experimental infection with SPV in monkeys is important (219). There is already a recombinant B19 vaccine under development, and assuming rapid progress towards expression of SPV structural proteins, SPV vaccination of monkeys will inform the process of prevention of B19 by vaccination (219).

B19 Inactivation in Blood Products. Since it is thermostable and without a lipid envelope, B19 is not destroyed by chemical and physical treatments used to inactivate lipid-enveloped viruses, such as HIV and hepatitis B and C viruses. Because of the frequency of B19 in blood donations and the large number of blood donations (5,000) used in a plasma pool to produce a batch of clotting factor concentrate, a high proportion of batches could be infected. As an alternative to testing of individual donors, the strategy of screening of small pools (500 donations) for B19 by PCR and the exclusion of positive batches from manufacture into clotting factor concentrates has been recommended by McOmish et al. (101) and Lefrère et al. (220). However, the lack of in vitro recovery of B19 from clotting factor concentrates may not necessarily indicate a lack of infectivity.

After donor selection and laboratory testing, the third component of the "safety net" needed to protect persons who receive clotting factor concentrates is virus inactivation. Solvent/detergent treatment is effective for enveloped viruses, but not for nonenveloped viruses such as B19 (106). Various methods of heating appear to reduce the incidence of B19 transmission (103, 106), relatively intensive heating (80°C for 72 h) seeming to be the most promising (105).

As Mosley (221) states, more important than laboratory results is the fact that solvent/detergent and dry-heat– and wet-heat–treated concentrates, whether PCR positive or negative, have continued to transmit B19 to previously untreated, anti-B19 negative haemophiliacs (103, 106). Considerable work remains to define more clearly the magnitude of transfusion-associated B19 infection, and to improve laboratory methods of virus inactivation, especially in clotting factor concentrates. Until an efficacious method of viral inactivation is instituted, the surest measure would be to screen batches of such products for B19 DNA by PCR, especially those to be used in immunocompromised HIV-infected haemophiliacs, following the principle used for prevention of transfusional cytomegalovirus infection.

Future Prospects

Further research is required in a number of areas. The question of whether congenital abnormalities, either functional or anatomical, occur at a rate less than 1% remains unanswered. A 13.2% incidence of B19 persistence among normal persons following acute infection challenges the accepted natural history of B19 infection; the finding of two co-existent B19 virus types in one of these patients with chronic fatigue syndrome also requires investigation (22). There are still several unanswered questions regarding the pathogenesis of B19 arthropathy. The question of whether disease represents an altered immune response or B19 persistence or both needs to be further explored.

In transient transfection experiments B19 NS1 protein has been shown to transactivate the long terminal repeat of HIV-1 in the presence of HIV-1 Tat protein. The relation of these in vitro experiments to the clinical situation is unknown at present and requires further study as B19 may be more than an opportunist in HIV-infected persons (38).

Although the coding regions of B19 and adeno-associated viruses are clearly different from one another, their left and right termini are identical inverted repeat sequences. Adeno-associated viruses are known to integrate into the human chromosome (222), an ability conferred by the termini. In view of this, and the fact that the mechanism of B19 persistence is unknown, B19 integration is one of a number of possible explanations which must be investigated.

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