Mechanisms of Disease

Parvovirus B19

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Yvonne Cossart, an Australian virologist working in London in the mid-1970s, noted an anomalous reaction of a normal blood donor’s serum (occupying position 19 in plate B) in an assay for hepatitis B. When Cossart excised the line of antigen–antibody precipitation, she saw the particles shown in Figure 1A, and in this way discovered a parvovirus in human blood. With the same technique, antibodies to parvovirus B19 were found in a large proportion of normal adults, indicating that infection is common and probably occurs in childhood. A disease was linked to parvovirus B19 infection by John Pattison and colleagues, who found either virus-specific antibodies or the virus itself in samples from children who had a severe complication of sickle cell disease called transient aplastic crisis. The most common illness caused by parvovirus B19 was identified a few years later, during outbreaks in the United Kingdom of fifth disease, a highly contagious childhood exanthem long suspected of having a viral cause. The virus has also been implicated in several other diseases (Table 1), and much has been discovered about how the virus causes these disorders. Diagnostic tests for the virus are commercially available, effective treatments are feasible, and a protective vaccine is under development.

The Parvovirus B19 and the Paroviridae

The Parvovirus family includes many pathogenic animal viruses that have long been of interest to veterinarians and virologists. These viruses include feline panleukopenia virus, canine parvovirus, Aleutian mink disease virus, and porcine parovirus. Adenoassociated viruses, also members of the Parvoviridae family, appear to infect humans without causing clinical manifestations and have been used as vectors for gene transduction and gene therapy.

The autonomous paroviruses propagate in actively dividing cells, whereas the adenoassociated viruses grow in tissue cultures infected with adenoviruses and herpesviruses. Parovirus B19 is the type member of the erythrovirus genus, which includes similar simian viruses, all of which propagate best in erythroid progenitor cells.

Genome, Transcription, and Proteins of Parvovirus B19

The paroviruses are broadly defined by their size (the name comes from parvum, the Latin word for small): they form small capsids, about 25 nm in diameter (Fig. 1A), and contain a genome consisting of single-stranded DNA. The approximately 5600 nucleotides in the genome of parvovirus B19 show remarkably few differences among isolates, with the exception of the sequences of two variants, V9 and A6, which are of uncertain clinical significance. Replication of a parovirus entails double-stranded intermediate forms, which can be detected in tissue culture and clinical specimens by simple methods of DNA hybridization.

The transcription map of B19 and the other erythroviruses differs markedly from...
that of other Parvoviridae, particularly in the use of a single promoter. The viral genome encodes only three proteins of known function (Fig. 2A). The nonstructural protein, from NS1, subserves multiple replicative functions and is cytotoxic to host cells.\(^{22,23}\) The two structural proteins, viral protein 1 (VP1) and viral protein 2 (VP2), arise from alternative splicing, so that VP1 is the same as VP2 except for an additional 226 amino acids at its amino terminal. The viral capsid of 60 capsomeres contains mainly VP2; VP1 accounts for only about 5 percent of the capsid protein. Folding of the proteins creates \(\alpha\)-helical loops that appear on the surface of the assembled capsids, where the host’s immune system can recognize them as antigenic determinants (Fig. 2B and 2C). The region unique to VP1 is external to the capsid itself and contains many linear epitopes recognized by neutralizing antibodies. (Epitopes are the parts of a molecule that are antigenic determinants.)

**Figure 1. Transmission Electron Micrographs Showing Native Parvovirus B19 in Serum and Cells and Recombinant Capsids.**

Panel A shows symmetric, icosahedral particles, about 25 nm in diameter, and empty capsids (arrowheads) in serum from an infected person (courtesy of Dr. Y. Cossart, \(\times 189,000\)). Panel B shows human erythroid progenitor cells infected in vitro with the virus; vacuoles and cytoplasmic pseudopods are present (\(\times 10,000\)). In Panel C, which shows part of Panel B at higher magnification, marginated chromatin contains assembled capsids (\(\times 100,000\)). Panel D shows empty recombinant parvovirus capsids produced in a baculovirus system (\(\times 154,000\)).

**Fifth Disease**

Most cases of parvovirus B19 infection are asymptomatic.\(^{32}\) The most common clinical presentation of infection is erythema infectiosum, or fifth disease, a childhood exanthem characterized by a “slapped cheek” rash (Fig. 3A). Fifth disease takes its name from a list of common childhood exanthems, named in the order of the dates when they were first
Table 1. Major Diseases Caused by Parvovirus B19.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Acute or Chronic</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fifth disease</td>
<td>Acute</td>
<td>Normal children</td>
</tr>
<tr>
<td>Arthropathy</td>
<td>Acute or chronic</td>
<td>Normal adults</td>
</tr>
<tr>
<td>Transient aplastic crisis</td>
<td>Acute</td>
<td>Patients with increased erythropoiesis</td>
</tr>
<tr>
<td>Persistent anemia</td>
<td>Chronic</td>
<td>Immunodeficient and immuno-compromised patients</td>
</tr>
<tr>
<td>Hydrops fetalis and congenital anemia</td>
<td>Acute or chronic</td>
<td>Fetus</td>
</tr>
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reported: measles, scarlet fever, rubella, Duke’s (or fourth) disease, fifth disease, and roseola, or sixth disease. (Most pediatricians question the validity of fourth disease.) Intranasal inoculation of normal volunteers has produced fifth disease, and the experimental setting allowed detailed correlation of clinical manifestations with virologic and immunologic events (Fig. 4A). Fever and nonspecific influenza-like symptoms occurred early, during the phase of parvoviremia. The later onset of a cutaneous eruption and rheumatic symptoms, about two weeks after the initial infection, corresponded to the appearance of antiviral antibodies. It is likely that these symptoms of parvovirus B19 infection are due to the formation and deposition of immune complexes in the skin and elsewhere. Serologic testing at this stage of infection generally shows seroconversion, IgM antibodies, or the new presence of IgG antibodies to parvovirus. The rash associated with fifth disease (Fig. 3A) may be evanescent, and recurrences can be provoked by exposure to sunlight, heat, emotion, or exercise. Fifth disease can be confused with rubella. In adults, the rash is less characteristic and may be difficult to see in persons with dark skin.

**Arthropathy**

In contrast to the mild course of the rash illness in children with parvovirus B19 infection, in adults, particularly middle-aged women, the infection may cause clinically significant arthropathy. Not only arthralgia but also inflammatory arthritis occur in about 50 percent of older patients; approximately 15 percent of new cases of arthritis may represent the sequelae of parvovirus B19 infection. Symmetric joint involvement, usually of the hands and occasionally of the ankles, knees, and wrists, can mimic rheumatoid arthritis, and the results of a test for rheumatoid factor may be positive. However, arthropathy associated with parvovirus B19 usually resolves within a few weeks, and even when symptoms persist for months or years, joint destruction does not occur. As in the case of the skin lesions of fifth disease, the pathogenesis of parvovirus B19 arthropathy is assumed to involve deposition of immune complexes.

A role of parvovirus B19 in rheumatoid arthritis has not been proved. Patients with rheumatoid arthritis are not more likely to be seropositive for parvovirus B19 than are controls; conversely, B19 arthropathy does not progress to rheumatoid arthritis. Parvovirus B19 DNA may be present in inflamed joints but is also found in an equivalent proportion of control samples of synovial tissue.

**Transient Aplastic Crisis**

In patients with increased destruction of red cells and a high demand for the production of erythrocytes, acute parvovirus B19 infection can cause an abrupt cessation of red-cell production, which exacerbates or, in compensated states, provokes severe anemia. Erythropoiesis is probably temporarily suppressed in all parvovirus B19 infections, since reticulocyte counts in normal volunteers fall to zero, but hemoglobin levels ordinarily remain stable because the erythrocyte has a long life span. Anemic crises in hereditary spherocytosis and in sickle cell disease have long been recognized, and their simultaneous or sequential occurrence in families led to the suspicion of an infectious cause. In retrospect, regenerative acute anemias ascribed to kwashiorkor, vitamin deficiency, bacterial infections, some medical drugs, and even glue sniffing were probably due to parvovirus infection. Examination of serially collected serum samples from a cohort of Jamaican patients with sickle cell anemia showed that virtually all cases of transient aplastic crises were related to recent parvovirus infection. Although patients with fifth disease typically have only antibodies against parvovirus and do not have viremia on
clinical presentation, viremia is present in transient aplastic crisis, and red-cell production resumes after antiviral antibodies that clear the infection have been produced (Fig. 4B).

Transient aplastic crisis is usually a unique event in the life of a patient, suggesting the induction of long-lasting, protective immunity. Although self-limited, an aplastic crisis can cause severe, occasionally fatal, anemia that precipitates congestive heart failure, cerebrovascular accidents, and acute splenic sequestration. The bone marrow in patients with transient aplastic crisis is characterized by an absence of maturing erythroid precursors and the presence of giant pronormoblasts (Fig. 3B); these pathognomonic cells result from the cytopathic effect of parvovirus.

White-cell and platelet counts may fall somewhat during transient aplastic crisis, especially in patients with functioning spleens. Occasional cases of agranulocytosis may be due to parvovirus B19, but evidence of the virus is infrequent in children with chronic neutropenia. Thrombocytopenia and pancytopenia have been reported in patients with well-documented acute parvovirus infection. Parvovirus B19 can precipitate the hemophagocytic syndrome, usually with a favorable outcome.

**Figure 2. Molecular and Structural Features of Parvovirus B19.**

Panel A shows a transcription map of the major genes and resulting transcripts. The nonstructural protein NS1 arises from the single unspliced transcript on the left-hand side of the genome (hatched areas). The capsid proteins VP1 and VP2 are encoded by genes with overlapping reading frames from the right-hand side of the genome (for simplicity, alternative splice messenger RNAs are not shown); aa denotes amino acids, nt nucleotides, (1), (2), and (3) reading frames, and P6, the single viral promoter. The capsid protein gene (Panel A), protein (Panel B), and placement of the protein in the capsid (Panel C) are shown in red. Panel B shows a ribbon diagram of a major capsid protein, adapted from Tsao et al. Like many other viruses, parvovirus B19 VP2 has a determined secondary structure, with eight antiparallel β-pleated sheets (labeled A through H) forming a compact “jelly-roll” core and α-helical loops extending to the surface of the virus. COOH denotes the carboxy terminal, and NH₂ the amino terminal. In Panel C, cryo-electron microscopy shows the surface topography of a parvovirus B19 particle, with superimposed positions of three identical capsid proteins and the two-, three-, and fivefold geometric axes of symmetry. The dashed and solid lines represent symmetric planes around a given point.

**PERSISTENT PARVOVIRUS INFECTION**

A lack of protective antibodies allows parvovirus B19 to persist (Fig. 4C). In the absence of antiviral immunity, fifth disease does not develop (because antigen–antibody complexes are not formed), but pure red-cell aplasia can be a manifestation of persistent B19 infection. The anemia is severe and requires transfusion; reticulocytes are absent from the blood, as are erythroid precursors from the marrow; giant pronormoblasts in a congruous clinical setting may
lead to the diagnosis. Antibodies to parvovirus are usually absent; however, the virus can be readily detected in the circulation, often at extremely high levels (>10^{12} genome copies per milliliter).

Failure to produce neutralizing antibodies to parvovirus B19 occurs in immunodeficiency states with congenital, iatrogenic, or infectious causes. The first patient in whom persistent parvovirus B19 infection was reported had the Nezelof syndrome, an inherited combined immunodeficiency disorder, in which susceptibility to parvovirus was the principal manifestation; the simultaneous onset of apparently acquired pure red-cell aplasia in the patient and his brother was highly unusual. Red-cell aplasia due to persistent parvovirus infection has been reported in patients receiving cytotoxic chemotherapy or immunosuppressive drugs, often after organ transplantation. Pure red-cell aplasia due to parvovirus B19 also occurs in the acquired immunodeficiency syndrome (AIDS), sometimes as the first manifestation of infection with the human immunodeficiency virus (HIV). Among HIV-positive men with anemia, persistent parvovirus B19 infection was a leading diagnostic possibility (at least in the era before highly active antiretroviral therapy).

**HYDROPS FETALIS**

Parvovirus B19 infection in a pregnant woman, followed by transplacental transmission to the fetus, can lead to miscarriage or hydrops fetalis (Fig. 3C). Parvovirus infects the fetal liver, the site of erythrocyte production during early development. The swollen appearance in hydrops is the result of severe anemia and perhaps also myocarditis, both of which contribute to congestive heart failure. Thrombocytopenia may accompany severe anemia. Seroprevalence data indicate that about half of pregnant women are susceptible to parvovirus infection. On the basis of prospective studies in the United Kingdom and the United States, the estimated risk of transplacental infection among women who are infected with parvovirus B19 during pregnancy is 30 percent, with a 5 to 9 percent risk of fetal loss. Infection during the second trimester poses the greatest risk of hydrops fetalis. Parvovirus B19 probably accounts for 10 to 20 percent of all cases of nonimmune hydrops fetalis. Approximately 8 percent of intrauterine fetal deaths were blamed on parvovirus B19 in a Swedish survey. The risk of infection is highest in epidemic years and is correlated with the ex-
tent of contact the pregnant woman had with children.\textsuperscript{75}

Most parvovirus B19 infections during pregnancy do not lead to loss of the fetus. The few reported cases of developmental anomalies in the eyes or nervous system of infants with in utero exposure may be coincidental. However, severe anemia at birth with bone marrow morphologic features that are consistent with constitutional pure red-cell aplasia (Diamond–Blackfan anemia) or congenital dyserythropoietic anemia may be due to transplacental transmission of parvovirus B19 infection.\textsuperscript{76}

**Other Suspected Parvovirus B19 Syndromes**

Some skepticism about case reports of illnesses associated with parvovirus B19 is warranted, because systematic studies that include controls have often failed to validate these reports.\textsuperscript{77} Frequently, the diagnosis of infection rests on detection of the B19 genome with the use of a polymerase-chain-reaction assay, but the notorious difficulty of ridding a laboratory of a highly stable contaminant that results from DNA amplification increases the rate of false positive results. Furthermore, parvovirus B19 can persist at low levels in the marrow,\textsuperscript{78} joints,\textsuperscript{43} and liver\textsuperscript{79} of normal persons for many months after infection, confounding the meaning of a single positive test.

Elevated levels of hepatic aminotransferases can accompany fifth disease, and parvovirus infection has been associated with severe but self-limited hepatitis in a few children.\textsuperscript{80} However, parvovirus B19 could not be implicated in larger numbers of patients with acute seronegative (non-A–E) hepatitis\textsuperscript{81,82} or chronic hepatitis.\textsuperscript{83} Parvovirus B19 has been postulated to cause fulminant hepatitis,\textsuperscript{84} but
we found no specific association. The presence of genetic sequences of B19 in cardiac tissue has led to the diagnosis of parvovirus myocarditis, and heart failure have followed fifth disease, and in one patient, chronic myocarditis was accompanied by persistent parvovirus B19 in the circulation. In some series, an association between parvovirus infection and myocarditis was rare, but in others, a high proportion of biopsy specimens from patients with inflamed hearts contained B19 DNA.

Sericologic and DNA evidence of parvovirus B19 infection has been reported in some patients with necrotizing vasculitis, Kawasaki’s disease, Henoch–Schönlein purpura, or giant-cell arteritis. The gloves-and-socks syndrome, an exanthem localized to the hands and feet, with edema, erythema, paresthesia, and pruritus, has also been linked to parvovirus B19. The chronic fatigue syndrome may follow infection with parvovirus B19, and their marrow erythroid progenitors proliferate normally in the presence of high concentrations of virus. The nonstructural protein of parvovirus is responsible for the death of erythroid progenitors, and some cells, such as megakaryocytes, may be lysed by restricted expression of viral proteins in the absence of viral propagation.

The simian parvoviruses, which cause outbreaks of severe anemia in caged animals, similarly target erythroid cells; experimental infection of monkeys results in conditions that mimic human pure red-cell aplasia and hydrops fetalis. Other parvoviruses that target hematopoietic cells more broadly are the feline panleukopenia virus and the minute virus of mice.

**Immunology to Parvovirus B19**

The antibody response is dominant in parvovirus B19 infection (indeed, it has been difficult to demonstrate a T-cell response to the parvovirus). Antibody production is correlated with the disappearance of virus from the blood, and IgG antibodies appear to confer lasting protection against reinfection. The basis of persistent parvovirus infection is a defect in immunoglobulin production. Serum from patients with persistent infection lacks antibodies to parvovirus B19 or contains low levels of nonneutralizing IgM or IgG antibodies. Antibodies to the unique amino-terminal region of VP1 are important for clearance of the virus. Serum samples from patients in the early phase of convalescence react to VP2, but serum samples from patients in the late phase (and commercial immunoglobulins derived from the plasma of normal subjects) have strong anti-VP1 activity. Experiments with recombinant capsids, in which VP2 or VP2 plus VP1 assembled in the empty capsids, demonstrated the crucial role VP1 has in the immune response. Capsids containing VP2 only and those containing VP2 plus VP1 differ markedly in their ability to elicit a neutralizing immune response in both animals and humans. VP1 is required for an effective immune response, whereas VP2 is ineffective. The function of VP1 is not known, but its phospholipase activity suggests that it has a role in viral entry into cells. Peptides derived from VP2 may be...
pathogenic in parvovirus B19 arthropathy, since they can induce cross-reactive autoantibodies against human keratin, collagen, and cardiolipin.\textsuperscript{118}

**Clinical Diagnosis**

Laboratory diagnosis of parvovirus B19 infection relies on serologic and DNA tests, because propagating the virus in standard tissue culture is difficult\textsuperscript{119,120} (Table 2). Virus-specific antibodies are measured in standardized commercial solid-phase, enzyme-labeled immunoassays, usually with the use of recombinant capsid proteins. IgM antibodies are detected in almost all cases of fifth disease at the time of presentation, and they appear within a few days after the onset of transient aplastic crisis; these antibodies persist for two to three months after acute infection. Substantial interindividual variation and the presence of IgG antibodies in a large proportion of the population make measurement of IgG less helpful than other tests for the diagnosis of parvovirus B19 infection. DNA assays are required to diagnose persistent infection, since antibody production is absent or minimal. Parvovirus DNA can also be found in serum early in the course of a transient aplastic crisis. Direct hybridization methods are reliable and can detect clinically relevant viral titers of more than 10\textsuperscript{6} genome copies. Gene-amplification methods are more sensitive, but the results may be false positive because of contamination or uninterpretable because low levels of virus may persist for months or years after an acute infection in a normal person. Virus can be detected in amniotic fluid, and both virus and IgM antibodies to parvovirus B19 are detectable in umbilical-cord blood\textsuperscript{122}; maternal serum will show seroconversion during pregnancy, but tests for maternal IgM antibodies may be negative at the onset of hydrops fetalis.

**Treatment and Prevention**

**Specific Therapies**

Most cases of parvovirus infection in children and adults do not require specific therapy. Isolation of infected persons is impractical, with the exception of hospitalized patients. Pure red-cell aplasia and the underlying persistent parvovirus B19 infection may be terminated rapidly by discontinuing immunosuppressive therapy, or by instituting antiretroviral drug therapy in patients with AIDS. Commercial immune globulins are a good source of antibodies against parvovirus; a persistent B19 infection responds to a 5- or 10-day course of immune globulin at a dose of 0.4 g per kilogram of body weight, with a prompt decline in serum viral DNA, as measured by hybridization methods, accompanied by reticulocytosis and increased hemoglobin levels.\textsuperscript{5,65} This regimen has been curative in patients with congenital immunodeficiency, but in patients with AIDS, parvovirus often persists at lower levels, detectable by gene-amplification methods; relapses of anemia may require repeated administration of immune globulin. Immune globulin therapy can precipitate the rash and joint symptoms of fifth disease. Hydrops fetalis may resolve spontaneously, but intrauterine blood transfusions have been used with apparent success.\textsuperscript{70,123-125} Chronic arthropathy has been treated symptomatically with antiinflammatory drugs. The benefit of immune globulin therapy is less clear in syndromes in which the virus does not circulate.

Avoiding both the misinterpretation of laboratory results — such as positive tests for IgG antibodies or borderline results of IgM and DNA tests — and the use of misguided therapies is as important as recognizing parvovirus infection. The administration of immune globulin in a patient with fulminant hepatitis or aplastic anemia not only is costly but can also delay an appropriate treatment, such as hepatic or hematopoietic stem-cell transplantation.

**Vaccine Development**

Effective vaccines are available for animal parvoviruses, and it is likely that parvovirus B19 infection

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**Table 2. Results of Diagnostic Assays for Diseases Caused by Parvovirus B19.\textsuperscript{a}**

<table>
<thead>
<tr>
<th>Disease</th>
<th>IgM</th>
<th>IgG</th>
<th>B19 DNA Hybridization</th>
<th>B19 DNA Amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fifth disease</td>
<td>++</td>
<td>++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Arthropathy</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Transient aplastic crisis</td>
<td>+/-</td>
<td>+/-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Persistent anemia</td>
<td>+/-</td>
<td>+/-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Hydrops fetalis and congenital infection</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>++</td>
</tr>
<tr>
<td>Previous infection</td>
<td>–</td>
<td>++</td>
<td>–</td>
<td>+/-</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The sensitivity of direct DNA hybridization methods is approximately 10\textsuperscript{6} genome copies per milliliter, and the sensitivity of DNA amplification techniques (polymerase chain reaction) is approximately 10\textsuperscript{5} genome copies per milliliter.\textsuperscript{118,121} Plus signs and minus signs denote positive and negative results, respectively, and greater numbers of plus signs indicate stronger positive results.
can also be prevented. The recombinant immunogen that is being developed as a vaccine for the human virus lacks DNA and is therefore noninfectious; empty capsids have been engineered to overexpress the highly immunogenic VP1, and a single dose of 2.5 µg of empty capsids elicited neutralizing antibody responses in normal volunteers. As with many other vaccines, commercial interest rather than lack of efficacy or safety has limited the development of a parvovirus B19 vaccine. Such a vaccine could prevent transient aplastic crisis in patients with sickle cell disease or other hemolytic anemias and pure red-cell aplasia in some immunodeficient persons, as well as hydrops fetalis, if seronegative women were inoculated early in pregnancy.

Chimeric viral capsids have been proposed as more general vehicles for the delivery of antigens, and parvovirus B19 is especially attractive for this purpose, because the VP1 unique region can be entirely replaced with other protein sequences, allowing, for example, the presentation of a conformationally and functionally intact enzyme on the surface of the empty viral capsid. This method is now being adapted for protection against an agent of bioterrorism: a domain of protective antigen of anthrax is being incorporated on a parvovirus B19 particle.

**CONCLUSIONS**

Parvovirus B19 is an excellent example of the dependence of the clinical manifestations of disease on the intrinsic properties of a pathogen and the peculiarities of the infected host. Distinct B19 syndromes are prominent in pediatrics and obstetrics, dermatology, rheumatology, and hematology, but the full spectrum of virus-induced disease has not yet been defined. Symptoms and signs follow from the infected person’s hematopoietic and immune status, and parvovirus infection can range from an asymptomatic condition to life-threatening disease. In the quarter century since Cossart’s discovery, our knowledge of parvovirus B19 has led to the recognition of new human diseases, as well as the development of diagnostic assays, effective treatments, and a candidate vaccine.

We are indebted to M.G. Rossmann for his assistance with Figure 2C.

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