Slow Clearance of Human Parvovirus B19 Viremia following Acute Infection

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Parvovirus B19 is a common, clinically significant pathogen. Reassessment of the viral kinetics after acute infection showed that the virus is not rapidly cleared from healthy hosts, despite early resolution of symptoms. These findings challenge our current conception of the virus’ pathogenesis and have implications for the management of the infection.

Human parvovirus B19 (B19) is ubiquitous throughout the world and causes a variety of symptoms, ranging from mild febrile illness to life-threatening anemia and fetal death. The infection is primarily thought to be controlled by humoral immune responses, because peripheral viremia decreases concurrently with the development of virus-specific antibodies, and the virus has been shown to be cleared in healthy hosts weeks to months after infection. Establishment of persistent infection is well characterized in immunocompromised individuals, primarily in association with congenital, iatrogenic, or infectious causes. However, cases of immunocompetent, symptomatic individuals with detectable B19 DNA in bone marrow and peripheral blood specimens for long periods of time have also been described [1, 2]. Recently, investigations of the cellular immune responses to B19 have shown a surprisingly large pool of circulating B19-specific CD8+ T lymphocytes remaining for >2 years after infection, with maintained effector function in healthy subjects [3]. Because this would indicate that viral antigen is present for a much longer time than has previously been shown, we reassessed the viral kinetics after primary B19 infection with a newly developed real-time quantitative PCR.

Materials and methods. Five individuals were identified prospectively after their serum samples had been referred to the clinical virology laboratory at the Karolinska University Hospital and were found to be positive for B19 IgM. The patients had presented their general practitioners with symptoms of fever, arthralgia, fatigue, and rash. None of the patients had received immunosuppressive treatment or had showed clinical symptoms of any other underlying chronic infection. Furthermore, they did not have any medical history of increased frequency of reactivation of latent herpes virus infection, recurrent respiratory infection, or mucocutaneous infection and did not recall having previous episodes of symptoms that resembled those of B19 infection. During the subsequent 128 weeks after inclusion of the first individual in the study, samples of serum and PBMCs were collected at intervals from all individuals, together with medical history and data regarding clinical symptoms. In addition, 15 B19 IgG-positive and IgM-negative healthy laboratory workers who did not recollect having parvovirus-related symptoms were included as control subjects.

Serum samples were analyzed for B19 IgG and IgM using a commercial EIA (Biotrin International). For assessment of B19 DNA levels, a novel, parvovirus genotype 1–3–specific TaqMan real-time PCR assay was developed. In brief, 200 μL of serum was extracted with use of an automated MagnaPure extractor (Roche Diagnostics) using the LC Total Nucleic Acid Isolation Kit (Roche). The assay was performed in a ABI 7700 sequence detection system (Applied Biosystems) in a 50-μL reaction mixture containing 25 μL of TaqMan Universal PCR Master Mix (Applied Biosystems), 5 μL of template DNA, 3 μmol/L of each primer, and 1.5 μmol/L probe for 40 cycles consisting of 15 s at 95°C and 20 s at 60°C. The following primers were used in the amplification: sense, 5′-ACAAAGGCTGGCGAAGTTAGC-3′, and antisense, 5′-GGCCCAAGCTGTAGCTCATT-3′, positioned at B19 genomic nucleotide positions 854–873 and 910–928, respectively (numbers refer to GenBank AY083239). Detection was provided by an FAM-TAMRA–labeled probe (Applied Biosystems) with the sequence 5′-CAACTACCCGGTGACTAACT-ATGTTGCGGT-3′ at B19 genomic nucleotide positions 877–908. A B19 viremic plasma, determined to contain 1.4 × 10^11 genome equivalents (geq)/mL, lot BPL9 (kindly provided by Dr. Kerr, Biotrin International), was used as standard. The sensitivity of the assay was 2 geq/reaction, as determined by repeated testing of serial dilutions of the BPL9 standard. Negative controls were extracted and analyzed between every 5 patient samples throughout the procedure. Extraction, preperation of the master mix, and template and standard addition were performed in separate laboratories. Samples that had pos-
itive results of quantitative PCR were partially sequenced to assess viral genotype using a separate assay. Outer primers in this assay were as follows: sense, 5'-GTGGTGAAAGCTCTGAAGAAGCTCA-3', and antisense 5'-GCCAGGCTTGTAAGTCCTTCA-3', at B19 genomic nucleotide positions 37–60 and 844–865, respectively. The inner primers were as follows: sense, 5'-CGGGACCAGTTCAGGAGAATCA-3', and antisense, 5'-GGGGTGGTCAGATAACTGTCCATG-3', at B19 genomic nucleotide positions 137–158 and 757–780, respectively (numbers refer to GenBank AY083237). Amplification was performed in a volume of 50 µL in 1× buffer II (Applied Biosystems) and 25 mmol/L MgCl₂ and 10 pmol/L primer at an annealing temperature of 55°C and for 40 cycles. The amplified product was sequenced using the Big Dye Termination Kit (Applied Biosystems) in an ABI 3100 sequencer (Applied Biosystems).

CD4+ and CD8+ T lymphocyte counts were determined by direct staining of PBMCs isolated by Ficoll-Paque (Amersham Biosciences) by fluorochrome-labelled monoclonal antibodies (BD), and subsequent analysis was performed by fluorescence-activated cell sorting (FACS). IFN-γ responses to phytohemagglutinin (Sigma-Aldrich) were assessed by enzyme-linked immunospot (ELISpot), which was performed as described elsewhere [4], using nitrocellulose plates (Milipore) and IFN-γ antibody (Mabtech AB). Approval for the study was obtained from the local ethics committee at the Karolinska University Hospital (Stockholm, Sweden).

Results. Serum and PBMC samples were obtained from patients for the first time 5 days (at the earliest) to 10 days (at the latest) after the onset of symptoms. FACS analysis revealed normal distribution of CD4+ and CD8+ T lymphocytes, as well as normal IFN-γ response to phytohemagglutinin in PBMCs obtained from all patients (data not shown). Symptoms present in all patients were arthralgia and erythematous eruptions. Additional symptoms, such as fever, malaise, pronounced myalgia, and peripheral edema, were present in some patients. All patients reported cessation of acute clinical symptoms (i.e., fever, exanthema, myalgia, and peripheral edema) 4–6 weeks after the onset of disease. The patient group was observed for a mean duration of 105 weeks (range, 77–128 weeks).

At the first point at which samples were obtained, serum samples contained a mean of 1.2 × 10⁷ B19 geq/mL serum (range, 1.7 × 10⁶–4.1 × 10⁷ geq/mL) (figure 1) and all isolates were shown to cluster in genotype 1 (B19) [5]. At that point, all patients tested positive for both B19 IgM and IgG. The viral load peaked at the time that the first sample was obtained or earlier, after which the virus levels stabilized in the range 10³–10⁵ geq/mL. Patient 3 exhibited an increase in viral load after week 80, but no epidemiological or clinical information correlated with this observation. During the study period, only 1 patient (patient 1) had clearance of the peripheral viremia (in the interval between weeks 85 and 106). All other patients had persistently detectable B19 DNA levels during the entire follow-up period, whereas all control subjects were found to be B19 DNA negative (data not shown). B19 IgM was detected for 5–17 weeks in all patients, except for patient 2, in whom B19 IgM was detectable for 91 weeks.

Discussion. We assessed the kinetics of acute B19 infection by quantitative PCR in 5 immunocompetent individuals who presented with classic symptoms of parvoviral infection. The average initial virus level was in line with what was earlier published [6]. A rapid decrease in the viral load was observed to be inverted to the development of B19 IgG and coincidental with resolution of acute clinical symptoms. By week 17, B19 IgM cleared in all patients, except for patient 2, who continued to have positive results for 90 weeks; this could have been the result of cross-reacting antibodies. Detectable DNA levels were maintained after development of B19 IgG and symptom resolution in all patients. Only 1 patient had clearance of peripheral viremia during the study period. If we assume that these 5 individuals are representative of the general population, we can conclude that B19 exhibits delayed clearance after acute infection. Similarly, B19 DNA has been detected in specimens of skin, synovia, and testis obtained from healthy, IgG-positive individuals [7]. In contrast, dot-blot and nested-PCR assays...
have shown that peripheral viremia clears weeks to months after acute infection [8, 9]. No comparable, quantitative data are available, because previous studies have described patients with long-term symptoms, documented persistent infections, and severe presentations when the immune status was not characterized [10–12]. Recent investigations of the cellular immune responses against B19 have revealed that these responses increase during the first year after infection, despite resolution of clinical symptoms [3].

B19-specific CD8+ T cells were shown to possess strong effector function and proliferative capacity and to maintain an activated CD38+ phenotype, with strong expression of perforin and CD57 and down-regulation of CD28 and CD27. The likely explanation for these observations, which supports the present findings, is low-level antigen persistence. The facts that none of the healthy control subjects included in this study had any detectable B19 DNA in serum samples and that the smaller populations of antigen-specific CD8+ T cells detected in individuals who had been infected in the past indicate that the virus is eventually cleared from peripheral blood [13].

The emerging evidence that B19 exhibits slower clearance of peripheral viremia after acute infection than previously thought challenges our current understanding of the virus pathogenesis and suggests a new entity of viral persistence. Furthermore, this evidence has practical implications on the means of diagnosing B19 infection, the means of preventing nosocomial transmission of infection, and vaccine development—areas of research that are all currently evolving. Additional studies that use novel and sensitive techniques are warranted to elucidate the relationship between B19 and the host, to readdress the same questions asked when the pathogen was discovered >25 years ago.

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