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Dermatomyositis associated with the presence of parvovirus B19 DNA in muscle

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Abstract

We report a case of dermatomyositis associated with molecular evidence of parvovirus B19 DNA in two muscle biopsies collected 5 months apart. IgG- but not IgM-specific antibodies were detected in serum. None of four serum samples was positive for parvovirus B19 DNA. The two biopsies contained B19 VP1 sequences and the second one was also positive for NS1. This is the first report of viral parvovirus B19 DNA in muscle of a patient with dermatomyositis. Latent muscle infection may contribute to the clinical picture.

KEY WORDS: Dermatomyositis, Autoimmune disease, Parvovirus B19, Muscle biopsy, Polymerase chain reaction.

Dermatomyositis (DM) is a rare muscle disorder classified as an autoimmune disease [1]. However, the acute onset of myositis as a viral-like syndrome in some patients is one of the reasons for suspecting the involvement of infectious agents in various systemic connective tissue diseases. In particular, parvovirus B19 has been demonstrated in the synovium of patients with rheumatoid arthritis [2]. We report here a case of DM associated with the presence of parvovirus B19 DNA in muscle biopsy.

Case report

A 48-yr-old woman, without previous medical history, suffered from polyarthralgia in 1995 for 4 months. Recurrence of these complaints, associated with fatigue, an urticarian rash, muscle pain and weakness, started in December 1996, without pulmonary or gastrointestinal manifestations or hepatitis. Because of the persistence of these symptoms, the patient was hospitalized in February 1997. Joint examination showed painful second and third metacarpophalangeal joints and feet, without evidence of swelling. There was significant muscle weakness associated with a purple rash of the upper eyelids. An electromyographic study showed a myogenic pattern. The erythrocyte sedimentation rate was 100 mm but C-reactive protein was normal. Serum creatinine phosphokinase was 724 IU/l (normal value 30–125) and aldolase 22 IU/l (normal value <7.6). Tests for rheumatoid factor and antinuclear antibodies were positive [1/7016 (latex assay) and 1/4096, respectively]. Antibodies SSA, SSB, Sm, RNP, Scl70 and Jo1 were negative. HLA genotype was A2-A4-B45-B50-DR4B1*0406-DR7-DR53-DQ2-DQ4. An EMG-guided deltoid muscle biopsy was performed 10 weeks after the onset of symptoms. It showed perivascular lymphocyte and plasmocyte infiltration with areas of muscular necrosis. A diagnosis of DM was considered. No family history of neuromuscular and autoimmune diseases was found. The patient was not exposed to chemicals or particular drugs before or during the onset of disease. No recent history of infection, in particular with parvovirus B19, was found in the family.

In February 1997, treatment with prednisone (20 mg/day) and i.m. methotrexate (MTX) (12.5 mg/week) was started. The clinical outcome was good and 3 months later the patient did not complain of any pain but had some proximal lower limb muscle weakness. A biopsy of the right quadriceps femoris muscle was performed in June 1997. In this biopsy, the initial lesions were less severe. In June 1997, serum creatinine phosphokinase was normal but aldolase was 9 IU/l. However, doses of prednisone and MTX were decreased gradually from June to September 1997 without relapse. The patient was maintained on MTX alone for a total of 2 yr and has been disease-free since MTX was stopped.

Because of the association of arthralgia and a rash, serum and muscle biopsies were analysed for the presence of parvovirus B19 antibodies and DNA.

Serological analysis of parvovirus B19 antibodies was performed on four sequential serum samples (4 January, 24 February, 17 June and 9 September 1997), using different commercial kits for enzyme immunoassay (EIA). The presence of specific IgG was tested with a synthetic peptide derived from two capsid proteins, viral proteins 1 (VP1) and 2 (VP2) (Parvoscreen-B19™ IgG; Euro-Diagnostica, Gentilly, France). A μ-antibody-capture assay based on a recombinant VP2 antigen

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(Parvovirus B19 IgM EIA; Biotrin, Dublin, Ireland) was used for detection of specific IgM. Serological analysis for other viruses [coxsackie virus, echo virus, influenza virus, adenovirus, human immunodeficiency virus (HIV), human T-cell leukaemia–lymphoma virus type I (HTLV-1)] were performed on the same samples.

Detection of parvovirus B19 DNA in the four serum samples and two muscle biopsies from the patient was performed after amplification by the polymerase chain reaction (PCR). Vastus lateralis muscle biopsies of three patients undergoing hip replacement for osteoarthritis were used as controls. Briefly, muscle biopsies were mechanically disrupted in RBS buffer (10 mM Tris–HCl, pH 7.4, with 10 mM NaCl and 25 mM EDTA) then digested by proteinase K (1 mg/ml in sodium dodecyl sulphate 10%; Sigma, St Louis, MO, USA) at 37°C overnight. After phenol extraction and ethanol precipitation, air-dried DNA pellets were resuspended in 0.1 M Tris–EDTA. The same procedure was applied to two parvovirus B19-positive sera used as a positive PCR control and a negative control (200 μl H_2O instead of serum). Serum samples were treated with proteinase K. Serum samples (200 μl) were mixed with 100 μl 10 × lysis buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$ and 0.45% Tween 20) and 100 μg/ml proteinase K, then incubated at 56°C for 1 h, then at 95°C for 10 min. The total volume of PCR mixture was 100 μl, and included either 1 or 10 μl of muscle and either 0.1 or 1 μl of serum DNA extracts.

Two sets of primers were selected to amplify two genomic sequences, the first coding for VP1 and the second for non-structural protein 1 (NS1). Primers A (5′ GTG CTT ACG TGT CTG GAT TGC 3′, sense nucleotides 2408–2422) and B (5′ GCT AAC TTG CCC AGG CTT GT 3′, antisense nucleotides 2809–2790) amplified (34 cycles at an annealing temperature of 48°C) a 402-base pair (bp) fragment located within the VP1 coding sequence [3]. Amplified products were detected by ethidium bromide staining after agarose gel electrophoresis. Specific identification was performed with a biotin-labelled probe (5′ AAT ATT AAA AGA TCA TTA TAA TAT TTC TTT AGA TAA TCC CC 3′, nucleotides 2560–2600) according to the hybridization procedures of GEN-ETI-K®DEIA; DiaSorin, Saluggia, Italy.

A second set of primers, C (5′ AAT ACA CTG TGG TTT TAT GGG CCG 3′, sense nucleotides 1399–1422) and D (5′ CCA TTG CTG GTT ATA ACC ACA GGT 3′, antisense nucleotides 1682–1659) [4], was used for the amplification (35 cycles at an annealing temperature of 55°C) of a 284 bp fragment corresponding to the NS1 coding sequence. PCR products were visualized by gel staining.

Specific parvovirus B19 IgG antibodies were present at a stable level in the different samples. No specific IgM antibodies were detected. The samples were also negative for antibodies against other viruses (coxsackie virus, echo virus, influenza virus, adenovirus, HIV and HTLV-1).

None of the four serum samples was positive for parvovirus B19 DNA. Conversely, the two muscle biopsies (27 February and 17 June) contained parvovirus B19 VP1 sequences (Fig. 1). The second one was also positive for NS1. Parvovirus B19 DNA was not detected in muscle biopsies of three control individuals.

Parvovirus B19 is a single-stranded DNA virus that causes erythema infectiosum, arthralgia, aplastic crisis (particularly in patients with red cell defects) and chronic anaemia in immunocompromised patients [6]. More recently, parvovirus B19 has been associated with autoimmune connective tissue diseases [7]. In particular, a large proportion of rheumatoid arthritis synovial biopsies was found to contain parvovirus B19 DNA [2] but the significance of this remains controversial [8].

Regarding muscle diseases, several cases of myositis

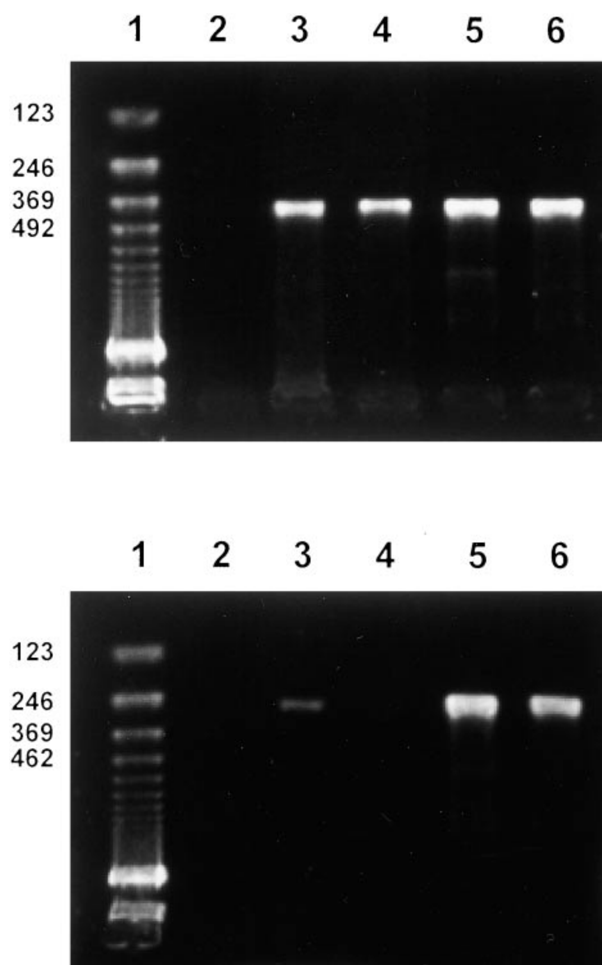

Fig. 1. Agarose gel electrophoresis of parvovirus B19 DNA sequences encoding viral structural protein VP1 and non-structural protein NS1, amplified from the second muscle biopsy (June 1997) by the PCR. DNA bands were visualized by ethidium bromide staining. Lane 1, bp ladder; lane 2, negative buffer control; lane 3, biopsy (5 μl); lane 4, biopsy (1 μl); lane 5, positive serum control 1; lane 6, positive serum control 2. Top panel shows the detection of VP1 (see text for details); bottom panel shows the detection of NS1. Results with the first biopsy were identical except for the NS1 products, which were not seen.

with the possible involvement of parvovirus B19 have been described. In two patients with a clinical picture highly suggestive of systemic lupus erythematosus, increased creatinine phosphokinase and aldolase levels were associated with acute parvovirus B19 infection, as indicated by elevated IgM and IgG antibodies, but no viral examination was done in the muscle biopsy [9]. One child with recent onset of juvenile dermatomyositis showed serological evidence of acute parvovirus B19 infection. Amplification of viral DNA by PCR was positive in an early serum sample but was negative in muscle biopsy and peripheral blood leukocytes drawn at the time of biopsy [10]. A 7-yr-old girl exposed to parvovirus B19 developed acute hepatitis and myositis followed by a life-threatening interstitial lung disease [11]. Parvovirus B19 DNA in bone marrow, lung and serum and elevated IgM and IgG antibodies were detected. PCR-amplified viral DNA remained positive in serum until the end of the survey. Muscle biopsy showed type II fibre atrophy without significant inflammation, but parvovirus B19 DNA detection was not performed.

The absence of IgM in our patient could be explained by the recurrence of parvovirus B19 infection in December 1996. However, the presence of specific IgM is not sufficient to link a parvovirus B19 infection to the diagnosis of DM. Viral detection directly at the site of the disease by PCR or Southern hybridization was therefore necessary. The presence of parvovirus B19 DNA in muscle biopsy was confirmed twice in our patient, using two different sets of primers. However, the extent of the contribution of a latent parvovirus B19 infection to myositis remains to be determined. Parvovirus B19 DNA has been shown to persist in synovial membrane, bone marrow or skin, not only in patients with autoimmune diseases or chronic urticaria but also in healthy immunocompetent individuals [12–14]. Our control muscle samples were negative.

Since the cause and pathogenesis of DM remain unclear, the contribution of an infectious agent is also difficult to establish. Many factors are probably involved, including genetic and hormonal components. Conversely, many viruses have been studied for their possible contribution to DM [1, 5]. Regarding this case, it is likely that the virus itself was not the cause of muscle damage, but that an undefined aberrant host immune response was triggered by the parvovirus, leading to muscle destruction. The presence of parvovirus B19 DNA in our two muscle biopsies was established using primers for VP1 and NS1. At a 5-month interval, the two samples were positive for VP1, which may be active in the formation of infectious viral progeny. Only the second sample was positive for NS1. In this context, it is of interest to note that NS1 has been associated with persistent infection and may therefore be involved in the pathogenesis [15]. Similarly, recent data suggest a link between the apoptotic pathways activated by the combination of tumour necrosis factor α and NS1 in human erythroid cells [16].

This is the first report of viral parvovirus B19 DNA in muscle of a patient with all the classical features of DM. The demonstration of a direct link will need a prospective study with large numbers of patients with such a rare disease.

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