Parvovirus B19 infection of bone marrow in systemic sclerosis patients

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ABSTRACT

Objective
To investigate the prevalence of human parvovirus B19 (B19) infection in the bone marrow of systemic sclerosis (SSc) patients.

Methods
Twenty-one consecutive SSc patients and 15 sex- and age-matched subjects without immunological rheumatic diseases were studied for: (i) the presence of circulating anti-B19 antibodies (anti-B19 IgG and IgM type and anti-B19 NS1 IgG) detected by means of standard methodologies, and (ii) B19 genomic sequences in sera and bone marrow biopsy specimens using a nested-PCR technique.

Results
The presence of B19 DNA was demonstrated in a significant percentage of bone marrow biopsies from SSc patients (12/21; 57%) and was never detected in the control group ($p < 0.01$). In no case was the B19 viremia observed, while serum anti-B19 NS1 antibodies, possible markers of B19 persistent infection, were more frequently detected in SSc patients than in controls (33% vs 13%). SSc patients with bone marrow B19 infection showed a shorter mean disease duration than B19-negative patients ($5.6 \pm 4.2$ vs $12.7 \pm 7.8$ yrs; $p < 0.01$).

Conclusions
This is the first demonstration of bone marrow B19 infection in a significant percentage of SSc patients. The possible etiopathogenetic role of B19 should be verified in a larger patients series and further investigated by means of molecular biology studies.

Introduction
Systemic sclerosis (SSc) is a connective tissue disease characterised by skin and visceral organ involvement (1). The immunological abnormalities observed in SSc patients can cause both microvascular alterations and collagen overproduction by altered fibroblasts which represent the pathobiological hallmarks of the disease (2).

Numerous environmental and infectious agents have been suggested as possible triggering factors of SSc (1, 2). Since the human parvovirus B19 (B19) has been proposed as a causative agent for some rheumatic disorders, such as rheumatoid arthritis and the systemic vasculitides (3), we began to study the prevalence of serum B19-related markers in SSc patients (4). Viremia was detected in 4% of SSc patients, a very high rate in comparison with that of healthy blood donors, which does not exceed 0.6% (5). Moreover, the presence of anti-B19 IgG, but not anti-B19 IgM, in the serum of B19 DNA-positive SSc patients suggested a persistent infection (4).

This preliminary observation prompted us to further investigate the possible pathogenetic involvement of this virus in SSc. Given the B19 tropism for various organs, due to the broad distribution of its cellular receptor (6), particularly in bone marrow tissue, we investigated the prevalence of B19 infection in bone marrow biopsies from patients with SSc compared with a control group of subjects without immunological rheumatic disorders.

Patients and methods
Twenty-one unselected SSc patients (5 M, 16 F, mean age ± SD: $49 \pm 12$ yrs., mean disease duration: $9 \pm 7$ yrs.) and a control group of 15 sex- and age-matched subjects without immune-mediated rheumatic disorders (6 healthy bone marrow donors, 1 monoclonal gammopathy, 4 non-Hodgkin’s lymphoma, and 4 multiple myeloma patients) were included in the study. All of the SSc patients met the American College of Rheumatology (formerly, American Rheumatism Association) 1980 preliminary criteria for the classification of the disease (7). Patients were consecutively recruited during the course of routine check-up visits at our clinic. Both the SSc patients and the controls gave their informed consent before entering the study.

A thorough clinical assessment of the SSc patients was carried out. In particular, the extent of cutaneous involvement, and the presence of telangiectasias, cutaneous calcinosis, Raynaud’s phenomenon, nailfold capillary abnormalities, arthritis, and esophageal, cardio-pulmonary, and renal involvement were evaluated as previously described (8). In addition, the following serological markers were detected by means of standard
techniques: anti-nuclear (ANA) antibodies, anti-centromere antibody (ACA), and anti-extractable nuclear antigen (ENA) antibodies, including anti-Scl70, -Sm, -SSB, -SSA, -PCNA, -SL and Jo1 (8).

Virological studies were carried out in both the SSc patients and controls. Serum anti-B19 IgG and IgM type antibodies were detected by commercially available kits (EIA, Biotrin, EIRE). In addition, IgG-type antibodies against the non-structural viral protein NS1 (anti-B19 NS1; Western blot, Mikrogen, Germany) were evaluated because of their possible link to persistent infection (9). The presence of B19 DNA was evaluated in both serum and bone marrow biopsy specimens by means of a nested-PCR technique. In order to remove PCR inhibitors serum specimens were heated at 70°C for 45 min. before being analysed. DNA was extracted from the biopsy specimens by treatment with proteinase K followed by adsorption to glass fibers in the presence of a chaotropic salt (High pure PCR template preparation kit, Boehringer, Mannheim).

Two B19 DNA sequences were amplified by two nested PCRs to exclude the possibility of carry-over contamination: one sequence from the non-structural region (NSR) and one from the region of the viral promoter P6. For NSR amplification the primers P1 (1399 nt -1422 nt) and P6 (1682 nt -1659 nt) were used in the first reaction, whereas the primers P2 (1498 nt -1525 nt) and P5 (1600 nt -1576 nt) were employed as inner primers in the second reaction (10). The first as well as the second reactions were performed for 35 cycles, each at 94°C for 45 sec, at 55°C for 60 sec, and at 72°C for 90 sec. For the P6 promoter region, primers and reaction conditions according to Gareus et al. were used (11).

Statistical analysis was performed by means of Student’s T-test and the chi-square test with continuity correction.

**Results**

Table I summarises the principal clinico-serological and virological features of the SSc patients compared to the control subjects.

Anti-B19 IgG type antibodies were detected in 19 out 21 (90%) of SSc patients (Table I).
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and in a comparable percentage of controls (80%), while serum anti-B19 IgM and B19 DNA were not found in either series. On the contrary, B19 genomic sequences were demonstrated in a significant percentage of bone marrow biopsy specimens from SSC patients (12/21 = 57%) and were never detected in the control group (p < 0.01).

Antibodies to the anti-B19 NS1 protein, possible markers of persistent B19 infection, were more frequently found in the SSC patients than in the controls (33% vs 13%). There was no correlation between B19 infection and other clinicorheumatological features, with the exception of a significantly shorter disease duration in SSC patients with bone marrow B19 infection compared to B19 DNA-negatives (5.6 ± 4.2 vs 12.7 ± 7.8 yrs; p < 0.01).

Discussion

The present study demonstrates for the first time the significantly high prevalence of B19 infection of the bone marrow in an unselected series of SSC patients compared with control subjects without immunological rheumatic disorders. B19 is the etiologic agent of fifth disease affecting children in three-quarters of the cases (3). Generally it is a mild, benign infection which in a minority of predisposed subjects may be complicated by hematological manifestations such as aplastic anemia or pancitopenia (3).

In adults, the most common manifestations of symptomatic B19 infection are sudden onset symmetrical polyarthalgias with frequent joint swelling, fever, myalgia, and evanescent rash (3). In some instances a chronic rheumatoid-like arthropathy, with non-erosive synovitis, vasculitis or systemic lupus-like manifestations have been reported (12-15). A still open and controversial question in the literature concerns how often B19 causes chronic arthritis and whether this can evolve to frank rheumatoid arthritis or other connective tissue diseases (3, 12-15). High titer anti-B19 IgG antibodies against whole virus or capsid as an antigen have been detected in adult fifth disease complicated by chronic arthritis; however, sera from the same patients failed to recognize certain peptide epitopes, thus suggesting that these subjects might have a selective defect in their antibody response to viral antigens that allows the B19 persistence (3). Similarly, an impaired immune surveillance could be the underlying defect responsible for the development of B19-associated SSC. This is a complex disorder that has been correlated to vascular, fibroblast, and immune system alterations.

An early pathogenetic mechanism of SSC seems to be the activation of both T and B lymphocytes (2). In predisposed individuals exogenous toxic or infectious factors may trigger both cellular and humoral autoimmune alterations including molecular mimicry phenomena, as suggested by the presence in SSC patients of different cross-reactive autoantibodies against target antigens (DNA Topoisomerase I, U1 snRNP, U3 snRNP) and some viruses, i.e. human cytomegalovirus, feline sarcoma virus, and herpes simplex type I (2). SSC comprises a spectrum of clinicorheumatological variants with a highly variable picture in terms of organ damage and prognosis (1). This clinical heterogeneity could be due to a multi-factorial and multi-step pathogenetic process.

The present study suggests that B19 may be included among the different infectious agents so far proposed as causative factors of the disease. The bone marrow infection serves as a B19 reservoir (12-16) from which the virus could spread to other peripheral tissues, depending on the presence of cellular receptors. On the other hand, the persistence of B19 infection could also represent a chronic stimulus for the immune system, leading to the immunological abnormalities observed in SSC. The significantly shorter mean disease duration of patients with B19 infection is suggestive of a triggering role of the virus in the early steps of the disease.

In conclusion, the present data further support our preliminary observation suggesting a possible role of B19 infection in SSC (4). Obviously, this hypothesis remains to be verified by epidemiological studies on larger patient series and analysed by means of immunological and molecular biology studies.

References