Parvovirus B19 Infection, Systemic Lupus Erythematosus and Behçet's Disease: Coincidence or Triggering Agent?

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Abstract

Autoimmune diseases arise from an overactive immune response of the body against substances and tissues normally present in the body.

What causes the immune system to no longer tell the difference between healthy body tissues and antigens is unknown. One theory is that some microorganisms (such as bacteria or viruses) or drugs may trigger some of these changes, especially in genetically predisposed individuals.

Studies have suggested that parvovirus B19 infections have been frequently implicated as a cause or a trigger of various forms of autoimmune diseases.

In this study, we propose to seek the role of B19 in two clinical situations of autoimmune origin: Systemic Lupus Erythematosus (SLE) and Behçet's disease (BD).

Key Words: Autoimmune diseases, Parvovirus B19, Systemic Lupus Erythematosus, Behçet's disease.

1. Introduction

Parvovirus B19 was discovered fortuitously in 1975 then systematic research of virus hepatitis B particles in the blood of healthy donors. This virus was called successively SPLV (Serum Parvovirus Like Virus), HPV (Human Parvovirus), and B19, number of blood bag where it was discovered for the first time (Cossart et al, 1975).
The pathogenic role of parvovirus B19 was discovered in 1981, where it was recognized as the causative agent of erythroblastopenic crisis of chronic hemolytic anemia (Pattison et al, 1981) (example sickle cell disease) and in 1985 as the causative agent of erythema infectiosum or fifth disease (Anderson et al, 1985). Rheumatic manifestations associated with parvovirus B19 were reported in 1985 and occur in young adults while they are rare in children with erythema infectiosum (Naides, 1998; Moore, 2000). Other proposed disease associations include several autoimmune disorders such as Systemic lupus erythematosus (Cope et al, 1992) and Behçet's disease (Kiraz et al, 2001).

The question arises of the relationship between parvovirus B19 infection and the onset of systemic lupus erythematosus or vasculitis: coincidence or triggering agent?

2. Patients and Methods

2.1. Patients: We included twenty six patients with SLE and nine patients diagnosed with BD. Patients selected are in the active phase of disease.

2.2. Controls: The control population is composed of forty three volunteer blood donors free of any infectious diseases. All tests are performed on serum samples.

2.3. Laboratory Studies

2.3.1. Detection of Antibodies to B19

IgM and IgG antibodies to B19 were determined in sera by Enzyme Linked Immunosorbent Assay (ELISA); (Biopharm, Germany; Ridascreen, Tunisia).

Recent B19 infection was defined as the presence of positive IgM antibodies (with or without IgG). However, past B19 infection was defined as the presence of IgG antibodies in the absence of IgM antibodies.
The Rheuma test was used to investigate the presence or absence of rheumatoid factors in sera to be tested in order to ovoid cross-reactions in ELISA and to eliminate false positive for either IgM or IgG antibodies. Its corresponding box is marketed by Pasteur Institute of Tunis (Tunisia).

2.3.2. B19 DNA Extraction and Polymerase Chain Reaction (PCR) Amplification

Sera positive to IgM antibodies are subjected to molecular analysis to search parvovirus B19 DNA, using as a tool of detection the Nested PCR as it was described by Yamakawa et al (1995). The step of amplification is preceded by a step involving DNA purification by affinity chromatography on columns (QIAamp blood kit, QIAGEN, Germany). The extraction protocol includes four steps: a lysis of cells in the sample, the binding of the genomic DNA in the cell lysate to the column membrane, washing the membrane and elution of the genomic DNA from the membrane.

Viral DNA extract was sought by Nested PCR. It is an amplification technique performed on two rounds. In the first one, the extracted DNA is amplified in the presence of the first pair of outer primers: sense (S1) **+5'-CAAAAGCATGTGGAGTGAGG-3';** antisense (S2) **-5'-CTACTAACATGCATAGGCGC-3'.** Concerning the second round, the amplicon obtained after the first period is again amplified in the presence of a second pair of inner primers : sense (S3) **+5'-CCCAGAGCACCATTATAAGG-3'** and antisense (S4) **-5'-GTGCTGTCAGTAACCTGTAC-3'.** The first round of amplification was programmed as follows: a DNA denaturation for one minute at 94°C, annealing of outer primers for two minutes at 55°C and a phase of primers extension for one minute at 72°C.

The primers extension is followed by a step of further extension for five minutes. Thirty cycles were programmed in the first period of amplification. In the second round, the amplification was performed in the presence of the pair of inner primers (S3and S4). The cycling parameters of the second amplification round were same as the first one expect that the primers annealing step is carried out at a temperature of 57°C instead of 55°C.

Amplified DNA fragments were analyzed by 2% agarose gel electrophoresis. The size of each fragment was 288 base pairs (**see figure 1 below**).

3. Results

IgM anti-B19 antibodies and parvovirus DNA (figure 1) were detected in four patients with systemic lupus erythematosus (15.3 %) and in three patients with Behçet's disease (33.3 %). While, no IgM antibodies were detected in the control group. Additionally, IgG anti-B19 antibodies were detected significantly in 15 patients with SLE (57.6 %), three patients with BD (33.3 %) and seventeen blood donors (39.5 %).

4. Discussion

Autoimmune diseases are common diseases of multifactorial origin involving intrinsic mechanisms (genetic predisposition) and extrinsic (role of viruses). Several studies sought a possible correlation between the pathogenesis of these diseases and infection with certain viruses. Among the viruses mentioned in the literature, Parvovirus B19 may play a role in triggering certain diseases of autoimmune origin such as SLE (Chassagne et al, 1993; Diaz et al, 2002) and BD (Kiraz et al, 1996; Baskan et al, 2007).

In the light of these studies, given the role of parvovirus B19 in the installation of these disorders and given the significant frequency of these pathologies in Tunisian hospitals, a prospective epidemiological study was conducted by our team in order to elucidate the possible role of this infectious agent in the pathogenesis of these disorders. This study uses common methods for diagnosis. In fact, ELISA was applied for the research of serological markers IgM and IgG antibodies against Parvovirus B19. This technique showed perfect reproductibility and good sensitivity without interposants such as rheumatoid factors were observed. Molecular diagnosis was based in the search for viral DNA by Nested PCR. It was undertaken on a group of seven subjects seropositive for IgM antibodies. This technique had the advantage of being reproductible and very sensitive. Indeed, after enzymatic amplification and revelation of amplicons obtained on agarose gel, it was possible to highlight seven identical DNA bands of 288 base pairs each, compared with a positive control and a molecular weight marker.

If we repeat the PCR technique applied by Yamakawa team (1995), this single band probably corresponds to a DNA sequence of parvovirus B19 positioned in a region of the genome coding for the VP1 protein (**Figure 1**). In the case of Behçet's disease, the results observed in our study would support a direct relationship between Parvovirus B19 infection and the pathogenesis of this disease. However, the study conducted by kiraz et al (2001) which focused on forty one patients affected by the disease indicated above and forty subjects in control group, was able to detect IgM anti-B19 antibodies in six patients. Whereas, this serological marker was absent in the control population. However, IgG antibodies were detected in both populations with similar prevalence. These results showed that the direct involvement of Parvovirus B19 in Behçet's disease was not quite obvious and IgM antibodies detected and which are considered as witnesses of acute infection, are found by chance and fortuitously. Therefore, we felt it was necessary to complement the serological study by molecular analysis which can demonstrate the involvement or not of B19 in Behçet's disease.

Other study undertaken by Baskan et al (2007) had led to the search of viral DNA in lesions through skin biopsies in patients with Behçet's disease. This team used the Real time PCR to quantify viral DNA separately in a group of patients with Behçet's disease and a group of control subjects. This study showed that the presence of viremia argues for a direct correlation between Parvovirus B19 infection and installation of Behçet's disease. Nevertheless, this study still faces to the problem of the very limited number of patients with Behçet's disease.

In the case of Systemic lupus erythematosus, and taking into account the results of virological diagnosis obtained in our study, we found that the Parvovirus B19 likely play a role more or less directly in the pathogenesis of lupus. Thus, infection with this virus is able to induce a gradual exacerbation and a slow evolution of lupus (Cope et al, 1992; Chassagne et al, 1993; Roblot et al, 1997). In addition, it appears that the Parvovirus B19 is the cause of the outbreak of the production of auto antibodies by mimicking self-antigens (Kerr and Boyd, 1995) to contribute to the development of the characteristic symptoms of lupus erythematosus. However, in patients with SLE, the clinical symptoms associated with this disease have emerged in parallel with Parvovirus B19 infection. Therefore, we can conclude that autoimmune phenomena are probably triggered by infection. Thus, we can assume that B19 infection is not the main cause of lupus but rather a trigger for symptoms (Nesher et al, 1995). However, we cannot exclude the possibility that, in some cases, Parvovirus B19 infection is the cause of lupus as it has been described by other authors (Nigro et al, 1997; Tanaka et al, 1998).

5. Conclusion

To date, it has not been possible to demonstrate the exact mechanism by which Parvovirus B19 induces or triggers lupus or Behçet's disease. The exact relationship between Parvovirus B19 and these disorders requires further investigations based primarily on further studies in the presence of a large-scale sampling.

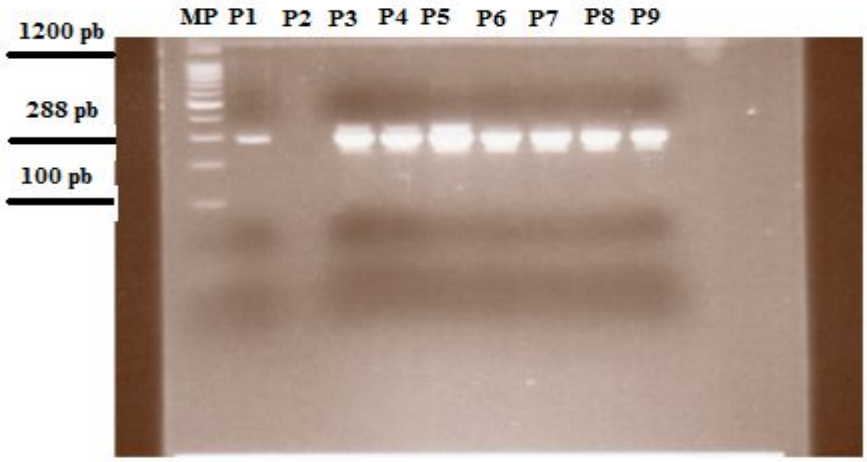

Figure 1: Electrophoretic profile of the nested PCR products in the presence of inner primers S3 and S4

MP: Molecular weight marker (100 bp DNA ladder, Biogen)

P1: Positive Control (purified DNA Regaya et al, 2007)

P2: Negative Control (DNA extracted from leukocytes of a volunteer donor blood)

P3, P4 and P9: amplicons of DNA extracts purified from sera of patients with Behçet's disease.

P5, P6, P7 and P8: amplicons of DNA extracts purified from sera patients with systemic lupus erythematosus.

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