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BRIEF COMMUNICATION

Autoimmune manifestations in patients with visceral leishmaniasis

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Visceral leishmaniasis (VL) is a vector-borne protozoal infection caused by replication of *Leishmania* species in macrophages. VL is characterized by fever, hepatosplenomegaly and cytopenia. Apart from those classic clinical characteristics, VL has been associated with autoimmune clinical and laboratory features. Reported herein are 16 consecutive patients with VL who were checked for laboratory autoimmune manifestations. A variety of autoimmune antibodies including elevated titers of antinuclear antibodies and rheumatoid factor were detected in all patients. Of note, no laboratory autoimmune manifestations were detected in the seven patients who were re-evaluated 3 months after therapy. It is concluded that autoimmune laboratory manifestations during VL infection are common. These may mistakenly lead to diagnosis of an autoimmune disorder.

Introduction

Visceral leishmaniasis (VL) is a vector-borne protozoal infection caused by replication of *Leishmania* species in macrophages.[1] There is a broad range of manifestations and VL can even be asymptomatic or subclinical in some cases. Classic clinical presentation of VL includes fever, abdominal discomfort, weight loss, cough, pallor, splenomegaly and hepatomegaly.[2] Of note, splenomegaly may be absent in immunocompromised patients, or in the early stages of the disease.[3] Common laboratory findings include pancytopenia and hypergammaglobulinemia.[2] However, VL may present with both clinical and laboratory autoimmune manifestations including arthralgia, cutaneous vasculitis, increased titers of rheumatoid factor (RF), antinuclear antibodies (ANAs), presence of cryoglobulins, and low serum complement levels.[4—6] Of note, patients with VL who were initially treated with corticosteroids have been described.[7,8]

The incidence of laboratory autoimmune manifestations during VL infection remains unknown. In this study, we checked 16 consecutive patients with VL for the presence of laboratory autoimmune manifestations.

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Patients and methods

Consecutive hospitalized patients with VL (from August 2003 to April 2011) were studied. The study was approved by the Ethics Committee of the University of Ioannina. VL diagnosis was made by the presence of high titers of anti-leishmania antibodies via indirect immunofluorescence assay and indirect hemagglutination antibodies, as well as demonstration of intracellular parasites on bone marrow aspiration. All patients were negative for hepatitis B, hepatitis C and human immunodeficiency viruses. All patients were administered liposomal amphotericin B (5 mg/kg/day on days 1–5, 14 and 21). On admission, ANA (MB Laboratories, Sidney, BC, Canada), anti-smooth muscle cell antibodies (Siemens Healthcare Diagnostics, Erlangen, Germany), anti-neutrophil cytoplasmic antibodies (ANCAs), including protoplasmic-staining and classical (MB Laboratories) were assessed by immunofluorescence. A commercial enzyme-linked immunosorbent assay was used to evaluate the levels of various autoantibodies including anti-cardiolipin, anti-thyroid peroxidase, anti-thyroxine binding globulin, anti-myeloperoxidase, anti-proteinase 3 (DRG Instruments GmbH, Marburg, Germany), anti-extractable nuclear antigen (ENA) (AESKU Diagnostics, Wendelsheim, Germany), anti-Ro, anti-La and anti-double-stranded-DNA. Anti-Smith (Sm) antibodies were evaluated via immunoprinting (Inogenetics, Ghent, Belgium), while levels of serum globulins, C3 and C4 fractions of complement and RF were evaluated using nephelometry (Siemens Healthcare Diagnostics, Erlangen, Germany). The presence of cryoglobulins and direct and indirect Coombs' test were also checked on admission. Seven patients were followed up and re-evaluated 3 months after therapy.

All statistical analyses were carried out with SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Sixteen consecutive patients (9 men and 7 women, aged 21–83 years) were studied (Table 1). No clinical autoimmune manifestations such as arthralgia or cutaneous vasculitis were reported in any patient. Laboratory investigation revealed the presence of a broad spectrum of autoantibodies in all patients (Table 2). Specifically, elevated titers of serum ANA were reported in 88% of patients. Two patients also had elevated titers of anti-ENA antibodies and two others had elevated titers of anti-ENA and anti-Ro antibodies. It was noteworthy that neither the titer of anti-leishmania antibodies (detected by immunofluorescence assay and indirect hemagglutination antibodies) nor the duration of fever was significantly correlated with the increase in ANA titer (data not shown). Elevated titers of serum RF were reported in 63% of patients studied, whereas C3 and C4 factors of complement were decreased in 13% and 50% of patients, respectively. Hypergammaglobulinemia was revealed in 63% of patients; in 60% of them, it was monoclonal, in 30%, polyclonal, and in 10%, biclonal. In addition, in 50% of patients, serum cryoglobulins were detected. Coombs' direct test was positive in one patient, whereas both direct and indirect tests were positive in another patient. Other autoimmune manifestations included the detection of anti-smooth muscle cell antibodies (25%), protoplasmic ANCAs (25%), and anti-myeloperoxidase (6%), anti-Sm (6%), anti-thyroid peroxidase and anti-thyroxine binding globulin antibodies (6%), whereas anti-double-stranded DNA and anti-proteinase 3 antibodies were not detected in any patient. Of note, no laboratory autoimmune manifestations were detected in the seven patients who were re-evaluated 3 months after therapy.

Table 1 Patient characteristics on admission ($n = 16$)[a]

Sex (M/F)	9/7
Age (y)	47.4 ± 21.0
Weight (kg)	74.7 ± 9
Smoking (yes/no)	8/8
Anti-leishmania antibody titer (median, range)	IFA: 1/960 (1/160–1/10,240) IHA: 1/384 (1/8–1/26,568)
Duration of fever before hospitalization (d)	28 ± 19

[a] Values are expressed as mean ± standard deviation, except for IFA and IHA which are expressed as median (range). F = female; IFA = indirect fluorescent antibody; IHA = indirect hemagglutination antibody; M = male.

Discussion

All 16 consecutive patients with VL who were tested on admission were found to have a variety of autoantibodies present without any clinical autoimmune manifestation. Elevated autoantibody titers subsided to normal levels 3 months after therapy in the seven patients who were followed-up. The most common features of VL-associated autoimmunity were elevated titers of ANA and RF.

VL infection has been occasionally associated with autoimmune clinical and laboratory features.[4–6] Small case series have reported the presence of immunocomplexes, complement consumption, and elevated RF levels in approximately 80% of patients with VL.[9,10] However, this is believed to be the first time that autoimmune features have been reported for all patients who were studied, suggesting that autoimmune laboratory features may universally characterize VL infection.

It has been suggested that VL infection may, especially in patients with high parasitic load, stimulate T helper 2 lymphocytes to produce interleukins 4 and 10, which in turn activate B cells to produce a wide spectrum of antibodies.[11,12] In contrast, *Leishmania* parasites themselves may cause tissue destruction, releasing self antigens, which in turn may stimulate autoreactivity.[13] Especially for the formation of cryoglobulins, a common mechanism between hepatitis C and VL has been proposed, based on the phenomenon of molecular mimicry.[5] Patients with VL have high titers of autoantibodies against a common epitope of the lymphocyte activation gene 3 (LAG-3.1), whereas patients with hepatitis C but no cryoglobulinemia do not have any autoantibodies against this epitope.[5] Of note, molecular mimicry has been associated with the formation

Table 2 Immune laboratory findings on admission[a]

Parameter (reference range)	
ANA (<1/160)	Elevated in 14 patients (88%): 1/480 (1/160—1/2560)
RF (<20 IU/mL)	Elevated in 10 patients (63%): 42.7 ± 35.9 IU/mL
C3 factor of complement (88—201 mg/dL)	Decreased in 2 patients (13%) 1st patient: 38 mg/dL 2nd patient: 44 mg/dL
C4 factor of complement (16—47 mg/dL)	Decreased in 8 patients (50%): 11.2 ± 4.5 mg/dL
Anti-cardiolipin antibodies (IgG)	Elevated in 2 patients (13%): 1st patient: 35 U/mL 2nd patient: 40 U/mL
Cryoglobulins	Detected in 8 patients (50%)
Coombs' test	• Direct: positive in 2 patients (13%) • Indirect: positive in 1 patient (6%)
β2-microglobulin (0—1900 μg/L)	Elevated in 4 patients (25%): 37,900 ± 23,800 μg/L
Hypergammaglobulinemia	• Polyclonal: 3 patients (19%) • Biclonal: 1 patient (6%) • Monoclonal: 6 patients (38%)
ASMA	Detected in 4 patients (25%) 1/160 (1/80-1/240)
p-ANCA	Detected in 4 patients (25%) 1/40 (1/20-1/80)
Anti-TPO/anti-TBG	Detected in 1 patient (6%)
Anti-ENA	Detected in 4 patients (25%)
Anti-MPO	Detected in 1 patient (6%)
Anti-Ro	Detected in 2 patients (13%)
Anti-Sm	Detected in 1 patient (6%)
Anti-ds-DNA	Not detected (0%)
Anti-PR3	Not detected (0%)

[a] Values are expressed as mean ± standard deviation, except for ANA, ASMA and p-ANCA, which are expressed as median (range). ANA = anti-nuclear antibody; anti-ds-DNA = anti-double-stranded-DNA antibody; anti-ENA = anti-extractable nuclear antigen antibody; anti-MPO = anti-myeloperoxidase antibody; anti-PR3 = anti-proteinase 3 antibody; anti-Sm = anti-Smith antibody; anti-TBG = anti-thyroxine binding globulin antibody; anti-TPO = anti-thyroid peroxidase antibody; ASMA = anti-smooth muscle cell antibody; p-ANCA: protoplasmic-staining anti-neutrophil cytoplasmic antibody; RF = rheumatoid factor.

of a wide variety of lupus-related autoantibodies including anti-nucleoprotein, anti-Ro, anti-La and anti-Sm.[14] Thus, the formation of autoantibodies during VL infection is probably not only due to polyclonal B cell activation but also to molecular mimicry between *Leishmania* antigens and ribonucleoproteins.[14]

On practical grounds, it is important that patients with VL can be initially misdiagnosed as having an autoimmune disease (especially systemic lupus erythematosus),[15] thus potentially being treated with immunosuppressive drugs[8] with even fatal consequences. Therefore, we should always keep in mind that, especially in endemic areas, VL has to be ruled out before starting immunosuppressive drugs in patients with autoimmune laboratory manifestations. Moreover, VL should be considered in patients with autoimmune disorders who do not respond to immunosuppressive treatment, because VL can mimic a flare of the pre-existing autoimmune disease. This is especially true when immunosuppressive treatment in these patients provides the background on which VL may occur as an opportunistic infection.[6,16]

Conflict of interest

This paper was written independently; no company or institution supported it financially. Some of the authors have given talks, attended conferences and participated in trials and advisory boards sponsored by various pharmaceutical companies.

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