IgG and IgG subclass antibodies in patients with active cutaneous leishmaniasis

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Received 7 April 2006

Accepted 9 May 2006

This research was planned to detect IgG and IgG subclasses in sera of patients with active cutaneous leishmaniasis (CL). Sera from 30 patients with active CL aged between 10 and 50 years and from 30 healthy controls aged between 8 and 50 years were included in the study. Levels of IgG and its subclasses were measured by a nephelometer. Levels of IgG, IgG1 and IgG3 in the CL patients were higher than in the controls. In addition, IgG and IgG1, and IgG and IgG3 levels showed a significant positive correlation. These results showed that IgG subclasses could possibly be used as a helpful diagnostic marker in CL.

INTRODUCTION

Leishmania spp. are obligately intracellular protozoa that cause a spectrum of diseases, including cutaneous (CL), mucocutaneous and visceral leishmaniasis (VL) (Trujillo *et al.*, 1999). Sanliurfa is an area highly endemic for CL caused by the protozoan *Leishmania tropica* and for many years it has been an important focus in southern Anatolia, Turkey (Gurel *et al.*, 2002). A lot of research to understand the varied aspects of regulation in the immune system has been focused on the immunopathology of leishmanial infections. With respect to the humoral immune response, a successively higher specific antibody titre can be observed in localized CL, mucocutaneous leishmaniasis and diffuse CL. The intensity of the antibody response appears to reflect both the parasite load and the chronicity of the infection (Gutierrez *et al.*, 1991). An exceptionally high antibody titre against *Leishmania* antigens can be detected in the most severe form of disease, VL, as a consequence of polyclonal activation of B cells as a result of the presence of large numbers of parasites in the bone marrow and spleen (Galvao-Castro *et al.*, 1984).

It has been shown that IgG not only fails to provide protection against this intracellular pathogen, but that it actually contributes to disease progression (Miles *et al.*, 2005). In addition, studies have shown that large CL lesions were correlated with a higher frequency of lymphocytes producing *Leishmania* soluble antigen specific inflammatory cytokines (IFN- γ or TNF- α) (Antonelli *et al.*, 2005).

Analysis of *Leishmania* antigen-specific immunoglobulin isotypes and IgG subclasses in VL patient sera revealed

elevated levels of IgG, IgM, IgE and IgG subclasses during disease (Atta *et al.*, 1998; Anam *et al.*, 1999a; da Matta *et al.*, 2000; Ryan *et al.*, 2002; Ravindran *et al.*, 2004). The differential patterns of immunoglobulin isotypes observed during disease progression, drug resistance and cure were specific for antigens of *Leishmania donovani*. IgG subclass analysis revealed expression of all of the subclasses, with a predominance of IgG1 during disease (Anam *et al.*, 1999b). The role of antibody titres in resolution of CL and protective immunity is largely unknown. Although some studies have shown the advantage of using specific subclass antibodies for the diagnosis of VL, only a few reports are available for CL. The goal of this study is to evaluate serum IgG levels and IgG subclass distribution and the correlation between them in CL patients, and to find out whether this may be used as a helpful diagnostic tool for this disease.

METHODS

Patients. This study was conducted at the Harran Kapi Leishmania Center in Sanliurfa, southern Anatolia, Turkey. Sixty people were included (30 patients with CL and 30 healthy controls selected from the same region) in this study. The control group was selected from healthy volunteers (19 females and 11 males aged 8–50) who had not been exposed to CL. The patient group (21 females and 9 males aged 10–50) was selected from people who had come to the Harran Kapi Leishmaniasis Center for CL therapy. The patients were not under treatment at the time of study. Patients were selected with active CL, i.e. had lesions that were wet, large in size and mostly ulcerated for less than 3 months. Before blood collections all patients were informed about the content of the study and a written agreement was obtained from all of them. They were diagnosed by established clinical and epidemiological criteria. Diagnosis had been confirmed by direct microscopy after staining with Giemsa and by parasite culture in NNN (Novy, Nicolle, Neal) medium (15 %

Abbreviations: CL, cutaneous leishmaniasis; VL, visceral leishmaniasis.

defibrinated rabbit blood and 200 U penicillin ml⁻¹). Cultures were incubated at 25 °C and checked for growth of *Leishmania* promastigotes twice a week.

Immunoglobulin analysis. All venous blood samples were collected in polystyrene tubes. The tubes were centrifuged at 500 g for 15 min. Serum was then removed and stored at -20 °C until use. IgG and IgG subclass levels were measured with a nephelometer (Behring 100) according to the protocol provided by the manufacturer with a commercially available kit (Behring Diagnostics).

Statistical analysis. Data were analysed using the program SPSS 10.0 for Windows. Parametric statistical methods were used to analyse the data. The Student's *t*-test was used for pairwise comparisons. Bivariate comparisons were examined using Pearson rank correlation coefficients (*r*) and values were corrected for duplicates. Two-tailed significance values were used. A *P* value of 0.05 or less was considered to be significant.

RESULTS

IgG and IgG subclass levels were measured both in patients with active CL and in healthy controls, and the results were then compared with each other (Table 1). In our study, IgG, IgG1 and IgG3 levels were significantly higher in sera of the patients with active CL than in sera of the control group (*P* < 0.001 for all). There was no statistically significant difference between the sera IgG2 and IgG4 levels of the study groups (*P* > 0.05). A high correlation between IgG and IgG1 levels (*r* = 0.805, *P* < 0.001) and a weaker correlation between IgG and IgG3 levels (*r* = 0.404, *P* < 0.05) was found. There was no significant correlation between IgG and its other subclasses, or among the IgG subclasses.

DISCUSSION

Cytokines elaborated by activated T cells induce the switching of B lymphocytes to several IgG isotypes and are thus obligatory for some humoral responses. IFN- γ , the Th1 cytokine, probably upregulates IgG1 and IgG3 in humans. IL-4 and IL-5 and Th2 cytokines stimulate the production of high levels of IgG subclasses such as IgG1 (Reiner & Locksley, 1995; Abbas *et al.*, 1996; Rothman & Coffman, 1996). Little information is currently available about IgG subclass distribution in human CL. In this study, we aimed to explore the distribution of IgG and its subclasses in active CL cases and to ascertain whether this

can be used as a diagnostic marker for this disease. In agreement with a recent report in the literature (Skeiky *et al.*, 1997), levels of all IgG subclasses were increased in the sera of patients with active CL, but IgG1 and IgG3 appeared to be predominant.

The possible role of antibody-mediated protection against *Leishmania* signifies the importance of identification of antigens that may elicit protective antibodies. Post-treatment VL sera tested for most of the IgG subclasses indicate a potential role of gp63 in protection against this disease (Ravindran *et al.*, 2004). We previously characterized parasite cell surface antigens of CL and identified at least four antigenic structures different in size and biochemical composition (Aksoy *et al.*, 2004).

Results obtained by Deplazes *et al.* (1995) appear to support the data obtained from this present study. They showed that there were increased levels of IgG subclasses with a predominance of IgG1 and IgG3 in patients with active CL. Our results strongly suggest that levels of IgG and its subclasses, particularly IgG1 and IgG3, in sera of patients with active CL are significantly upregulated and may have an important role in the antibody-dependent defence mechanisms of the host against CL.

In the light of these findings, it should be possible to conclude that in human CL, levels of IgG and all its subclasses (IgG1 and IgG3 predominantly) are upregulated, and that there is a significant correlation between IgG and IgG1, and between IgG and IgG3 isotypes. We may also conclude that measurement of the sera concentrations of these immunoglobulins could be used as a diagnostic or prognostic marker to evaluate patients with active CL. To ascertain this, complex studies with a higher number of patients obviously need to be planned.

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Table 1. Comparison of serum IgG and IgG subclass levels of patients with active CL and controls (mean $\pm$ SD)

Parameter	Controls (<i>n</i> = 30)	Patients with CL (<i>n</i> = 30)	<i>P</i> value
IgG (g l ⁻¹)	12.196 $\pm$ 0.52	16.894 $\pm$ 0.81	<i>P</i> < 0.001
IgG1 (g l ⁻¹)	8.069 $\pm$ 0.35	13.657 $\pm$ 0.72	<i>P</i> < 0.001
IgG2 (g l ⁻¹)	2.087 $\pm$ 0.16	2.440 $\pm$ 0.19	<i>P</i> > 0.05
IgG3 (g l ⁻¹)	0.711 $\pm$ 0.05	1.403 $\pm$ 0.15	<i>P</i> < 0.001
IgG4 (g l ⁻¹)	0.299 $\pm$ 0.03	0.322 $\pm$ 0.02	<i>P</i> > 0.05

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