

IMMUNOGLOBULINS IN VISCERAL LEISHMANIASIS

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SUMMARY

We have done assays on the sera from patients with Kala-azar using as antigen for double diffusion test extract of *Leishmania donovani*.

The antibodies formed precipitin lines characterized as IgM (19 S) and IgG (7 S), by immunoelectrophoresis in diffusion against the antigen.

The Authors feel that immunodiffusion could be used to study the pathophysiology of the leishmaniasis. They also recommend these methods for controlling the action of the therapeutic agents and as screening test.

INTRODUCTION

The plasma proteins during the course of the Kala-azar in humans, are characterized by a great increase of the gamma-globulin and a decrease of the albumin when electrophoresis was used.

SILVER et al.⁴, in 1961, have shown an increase of IgM by immunoelectrophoresis. These data were confirmed by us, although our observations were also concerned to the morphology of the precipitin line of this immunoglobulin during the period that the symptoms of the disease are clear.

Some of our patients were followed for a period of two years after Glucantime therapy with clinical cure, returning to normal³ the IgM morphology and level.

At the present, there is no data concerning either the nature or the immunochemical properties of these globulins. This short communication deals with double diffusion and immunoelectrophoresis of the serum antibodies against *L. donovani* antigen.

We shall not discuss extensively the data presented here because it will be the subject of the doctoral thesis of one of us (J. CHAVES).

MATERIAL AND METHODS

1) Serum from twenty patients with Kala-azar were used all of them with confirmed parasitologic diagnosis and positive complement fixation test⁵. We received sixteen lyophilized sera from the "Universidad de Carabobo, Venezuela" and four were kept under freezing conditions and were obtained from "Faculdade de Medicina da Universidade de Minas Gerais". Eleven of the patients were adults, ten of which were male and one female; the other nine were children less than ten years, five male and four female.

2) Anti-human serum from "Centro de Pesquisas Imunoquímicas" was prepared in horses against human sera, for the identification of the precipitin lines.

3) *L. donovani* antigen was obtained from cultural forms of the parasites grown in Lit's broth¹ with 6-7 days of incubation. Then the culture was desintegrated with the aid of glass beads and after centrifugation the supernatant was used as antigen.

4) Ouchterlony's double diffusion and Grabar's immunoelectrophoresis according to FERRI & COSSERMELLI², were also used.

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RESULTS AND CONCLUSIONS

Gel double diffusion against the antigen were used as screening test to select the Kala-azar sera with high content of antibodies. We feel that the gel diffusion can be used as screening test for the diagnosis of Kala-azar because ten out of twenty samples were positive by this test, and only with few changes in the techniques it would be possible to obtain better results.

After electrophoresis of the sera followed by simultaneous diffusion against anti-human serum and *L. donovani* antigen, precipitin lines were obtained in the region of the IgG and IgM respectively.

Figure 1 shows one of the serum containing precipitin lines of the IgM-type and IgG-type as antibodies.

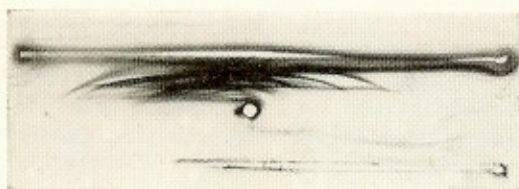


Fig. 1 — Immunoelectrophoresis. Center well — Kala-azar serum. Upper trough — Horse anti-human serum. Lower trough — *L. donovani* antigen

The change of the morphology of the IgM line after treatment of the patients is well demonstrated using specific anti IgM serum in the immunoelectrophoresis (Fig. 2).

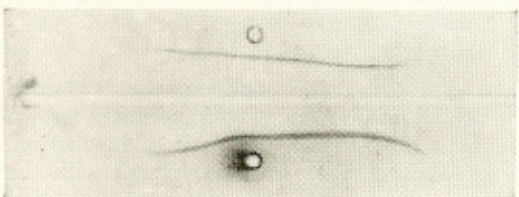


Fig. 2 — Immunoelectrophoresis. Upper well — Kala-azar serum after therapy. Lower well — Serum of the same patient before treatment. Center trough — Specific anti-IgM serum (Behringwerke)

The IgG and IgM increase in Kala-azar demonstrated by other Authors was confirmed by us. Return of the IgM to the normality after treatment with Glucantime suggests that the antibodies against *L. donovani*,

are not only IgG but also IgM-type. In this communication we demonstrated, by immunoelectrophoresis that IgM and IgG antibodies are simultaneously present.

RESUMO

Imunoglobulinas na leishmaniose visceral

Os Autores estudaram soros de pacientes com calazar, usando como antígeno extratos de *Leishmania donovani* por dupla difusão em gel de ágar.

Os anticorpos formaram arcos de precipitação caracterizados como IgM (19 S) e IgG (7 S), por imunoelectroforese em difusão contra o antígeno.

Os Autores sugerem que a imunodifusão pode ser usada para o estudo da fisiopatologia da leishmaniose visceral, e recomendam tais métodos para o controle da ação de agentes terapêuticos, bem como reação diagnóstica preliminar.

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