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DEPRESSION OF BLOOD MONOCYTES CHEMOTAXIS IN HUMAN CHRONIC CHAGAS DISEASE

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SUMMARY

Blood monocytes from patients with chronic Chagas Disease showed a chemotactic index 40% below that of normal individuals. The presence of myocardiopathy in some of these patients did not influence the results obtained. Chagasic sera showed no modulatory effect on locomotion of monocytes from normal persons.

INTRODUCTION

Mononuclear phagocytes are known to be an essential component in host defence in infections caused by intracellular parasites, where they participate in both afferent and efferent limbs of the immune response. Defects in chemotaxis of human monocytes have been described in several pathologic conditions [1,2,4,5,6,7,8,9,11,12,13], but nothing is known about this subject during infections caused by intracellular parasites.

In this paper we present the results of studies carried out on two topics: 1) the chemotactic activity of blood monocytes from patients with chronic Chagas Disease; 2) the effect of chagasic sera on chemotactic migration of normal blood monocytes.

MATERIAL AND METHODS

Patients — Seventeen adult patients admitted to the Cardiology Division of the University Hospital (UFRJ), with the diagnosis of chronic Chagas Disease, were selected for studies. They were classified in two groups: 1) patients without heart involvement; 2) patients with mild or severe myocardiopathy. Patients with concurrent diseases, or receiving medications, were not included. Eleven healthy adults served as controls.

Chemotactic factor — Bacterial chemotactic factor was prepared from 18h cultured filtrates of **Escherichia coli** in nutrient brot and used diluted 1:4 in Hanks' solution.

Sera — Sera were obtained from patients with chronic Chagas Disease and from healthy volunteers and stored at -20°C until used.

Chemotactic assay — Peripheral blood monocytes were isolated by Ficoll — Hypaque centrifugation, washed three times in Hanks' solution and standardized to contain 3 x 10^6 cells/ml. Four-tenths of this suspension were placed in the upper compartment of a modified Boyden chamber [10]. The cells were separated from the chemotactic factor, or Hanks' solution by 5.0 μm polycarbonate (Nuclepore) filter. All assays were done in triplicate. The chambers with cells were incubated for 120 min at 37°C in humidified air. Cell migration was quantified by counting and avaraging the number of cells which completely migrate through the filter, in 20 oil immersion fields. The percentage of migration was calculated by the following formula:

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$$\% \text{ Migration} = \frac{\text{Mean migration of cells from patients}}{\text{Mean migration of cells from patients}} \times 100$$

Serum inhibition experiments — In order to investigate the role of serum factors on mononuclear locomotion, the following studies were performed. Five-tenths of normal human mononuclear leukocytes (6 x 10^6 cells) in Hanks' solution were mixed with equal volume of control and test serum and incubated at 37°C for 30 min. Five-tenths of the mixture were then transferred to the chamber and challenged with chemotactic factor, as described above. The results were expressed as percentage of migration of monocyte chemotaxis in the presence of serum, according to the formula:

$$\% \text{ Migration} = \frac{\text{Mean cell migration in the presence of patient serum}}{\text{Mean cell migration in the presence of control serum}} \times 100$$

RESULTS

As we can see (Table I and Fig. 1) blood monocytes from patients with chronic Chagas Disease showed a chemotactic index of 60%, that is, 40% below the chemotactic activity of blood monocytes of normal individuals. The disperson of the locomotion response of monocytes of our patients with Chagas Disease can be seen in Fig. 2. The migration of monocytes from patients with chronic Chagas myocarditis (migration index 58%) was not found significantly different ($p > 0.05$) from those of patients without heart lesions (migration index 63%, Fig. 3). The sera obtained from patients with chronic Chagas Disease and assayed at 50% dilution, showed no modulating effect on locomotion of monocytes from normal persons (Table II and Fig. 4).

TABLE I

Chemotactic migration of mononuclear leukocytes from patients with chronic Chagas Disease

Group	No.	Cells/Field Mean ± 1 S.D.	Range	Migration Index	P-Value
Patient	17	59.5±13.0	26-78	60%	
Normal	11	99.6±20.0	72-133	100%	0.01

TABLE II

Chemotactic migration of normal mononuclear leukocytes incubated with patients' serum

Group	No.	Cells/Field Mean ± 1 S.D.	Range	Migration Index	P-Value
Patient	17	64.0±13.0	48-92	99%	
Normal	11	64.9±10.0	51-85	100%	NS

NS = not significant

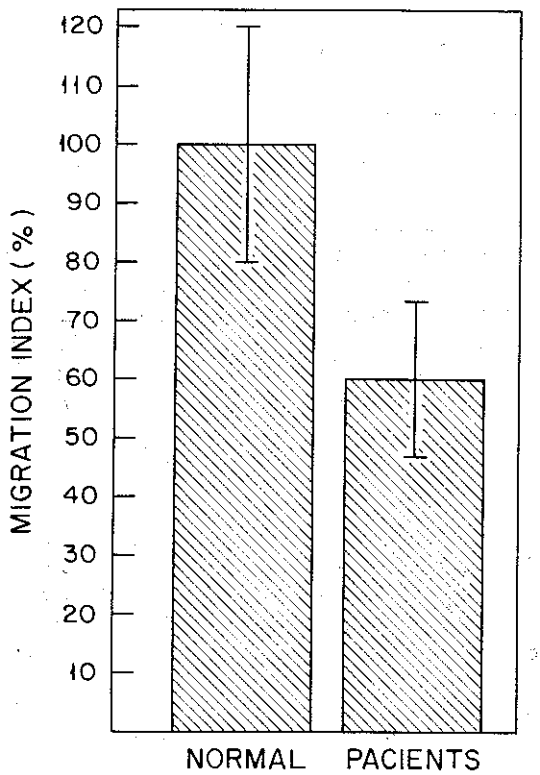

Fig. 1 — Migration index of mononuclear leukocytes from patients with chronic Chagas Disease. Cell migration was quantified by counting the number of cells which completly migrate through the filter in 20 oil immersion fields, under the influence of a chemotactic factor.

DISCUSSION

According to our results, blood monocytes from patients with chronic Chagas Disease showed a decreased migration when compared with that of normal monocytes. The occurrence of cardiopathy in some of these patients, exert.

ed no influence on the results obtained. No inhibitory effect on chemotactic migration of normal blood monocytes could be found in the sera from these patients.

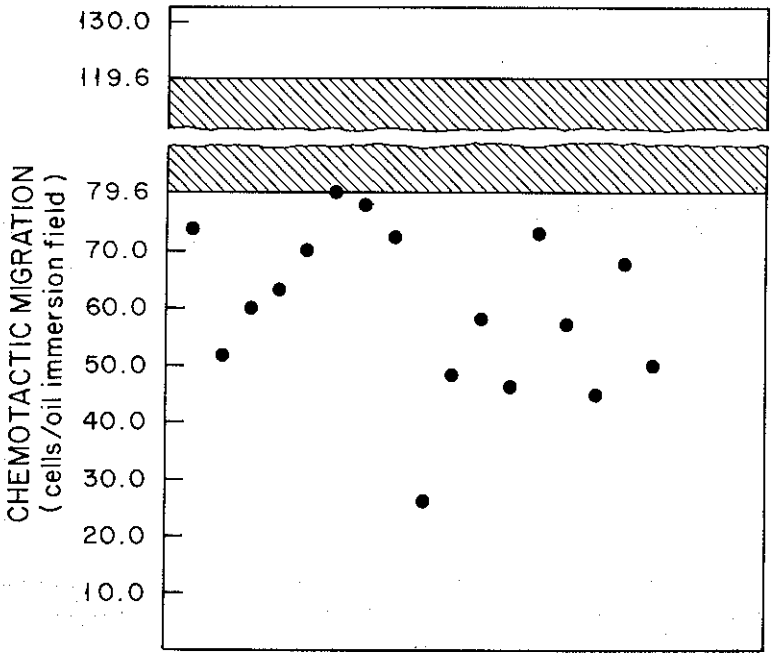

Fig. 2 — Chemotactic migration of mononuclear leukocytes from patients with chronic Chagas Disease. Points are single determination of different patients. Shaded area: mean chemotactic migration of normal mononuclear leukocytes (± 1 S.D).

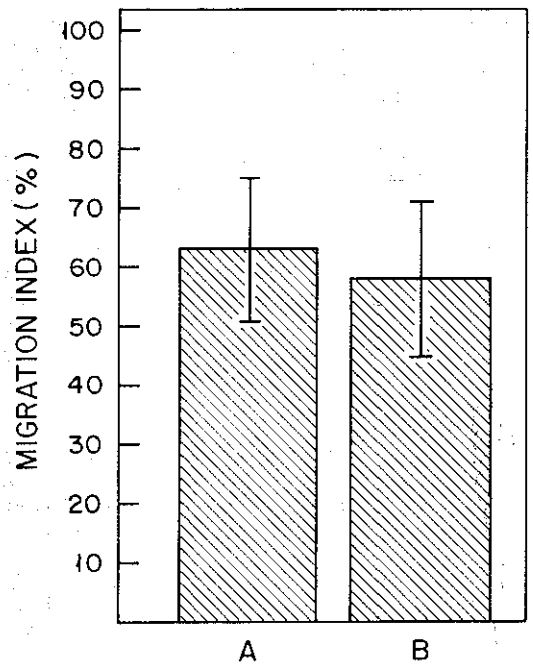

Fig. 3 — Migration index of mononuclear leukocytes from patients with chronic Chagas Disease (A) and from chagasic patients without heart disease. (B) The difference between mean of two groups was not found significant ($p > 0.05$).

The cause of the defect on monocyte migration in chronic Chagas Disease could not yet be determined, but our results suggest that the factor is not found in patients' sera. There are several mechanisms by which the different factors could modulate the locomotion activity of monocytes and neutrophils. Inhibitors which act directly on these cells include several drugs [13], chemotactic serum inhibitors [13], deficiency of substrates needed for generation of chemotactic factor, as seen in deficiences involving C3 and C5 [13]. The possibility that in chronic Chagas Disease the defect on monocyte migration might be caused directly by factors elaborated by **T. cruzi**, is supported by previous results from our laboratory [3]. We could show that both filtrates and lysates from **T. cruzi** exerted a inhibitory effect on immunologic phagocytosis by mouse peritoneal macrophages. It has also been reported that depressed macrophage mobilization in tumor-bearing mice can be attributed to a low molecular substance present in neoplasma which directly inhibits macrophage chemotactic responsiveness [12].

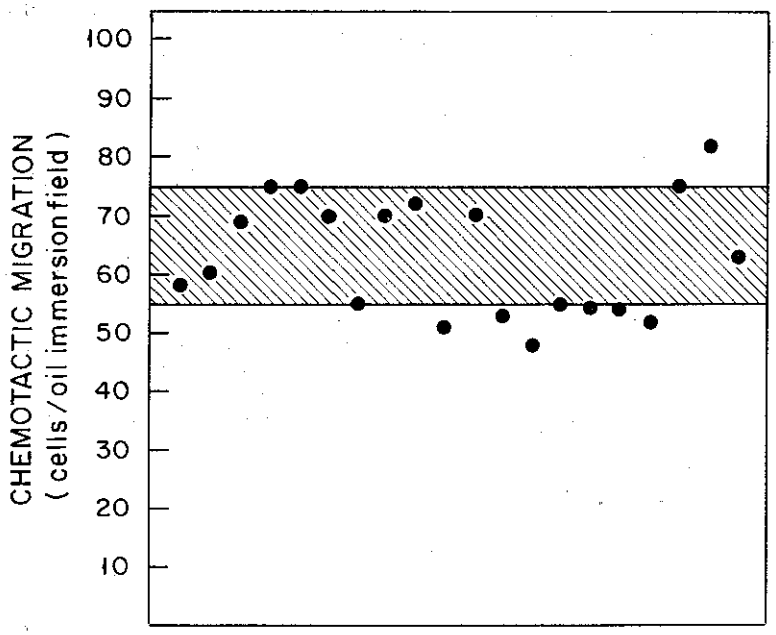

Fig. 4 — Chemotactic response of mononuclear leukocytes from normal individuals incubated with patients' sera. Shaded area: mean locomotion response of normal mononuclear leukocytes (± 1 S.D.) incubated with normal serum.

The biologic consequence of depressed monocyte function which occurs in chronic Chagas Disease has yet to be determined. Studies are now under way to determine whether this defect can be abrogated by treatment of patients with drugs. BCG vaccination is also a new approach to the correction of certain types of leukotactic defect [13].

If we consider that an adequated expression of delayed type hipersensitivity reaction requires the production by lymphocytes of factors capable of atracting and retaining phagocytic cells in certain inflammatory sites, and that in **T. cruzi** infection mononuclear phagocytes are an essencial component in host defence, then any defect in monocyte locomotion may seriously interfere with the evolution of the infection.

RESUMO

Depressão da quimiotaxia dos monócitos do sangue na doença de Chagas crônica do homem

Os monócitos do sangue de pacientes com doença de Chagas crônica acusaram índice quimiotático 40% abaixo do índice das pessoas normais. A presença de miocardiopatia em alguns dos doentes não influenciou nos resultados. O soro dos pacientes chagásicos não exerceu nenhum efeito modulador sobre a locomoção dos monócitos obtidos de pessoas normais.

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