BRIEF COMMUNICATION

"IN VIVO" LEUKOCYTE CHEMOTAXIS IN EXPERIMENTAL MICE SCHISTOSOMA MANSONI INFECTION

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SUMMARY

The "in vivo" chemotaxis was studied in C57Bl/10 mice 10, 30, 50 and 60 days after a Schistosoma mansoni infection in comparison to a control group (uninfected mice). Staphylococcal protein A was injected into a connective tissue air pouch of control and experimental mice and the leukocyte chemotaxis was counted. A decrease in polymorphonuclear (PMN) leukocyte response was found in infected mice in comparison to the control group (p<0.05). The 10 day infected mice showed a decreased PMN leukocyte response respecting the control group (p<0.05) and this finding became more evident 30 and 50 days post-infection. Although the PMN leukocyte response of 60 day infected mice increased in comparison to 50 day infected animals, it was still significantly lower the control response. The mononuclear leukocyte response was not significantly different between infected or uninfected mice.

KEYWORDS: Infection Schistosoma mansoni; Leukocyte chemotaxis.

INTRODUCTION

Several assays have been described to evaluate leukocyte chemotaxis of isolated cell populations "in vitro" ^{3, 6, 13}. However, these assays are not affected by the local and systemic interactions between inflammatory mediators ^{1, 10}. Thus, the validity of extrapolating these results to responses "in vivo" is questionable.

Although the leukocyte chemotaxis can occur in the absence of immunological control, they play an important role in the anti-parasite effector response, in conjuction with antibodies and complement ¹².

LAWMAN et al. (1984) developed an air pouch technique to estimate "in vivo" the response of leukocytes to chemotatic agents in mice. This technique,

as modified by ABATH et al. ¹, was used in the present work to investigate the "in vivo" chemotaxis in *Schistosoma mansoni* infected mice.

The protein A was used in this work as a indirect chemoattractant. Its action is dependent on the cross linking of IgG molecules via their Fc region, resulting in complement activation and the generation of C3a and C5a 4, 5, 8, 10.

MATERIAL AND METHODS

Six to eight week old C57Bl/10 mice were infected percutaneously with 90 cercariae from *Biomphalaria glabrata*, BH (Belo Horizonte) strain.

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The "in vivo" leukocyte response was evaluated 10, 30, 50 and 60 days after S. mansoni infection. Ten mice were used per group and the control group comprised uninfected mice. The data obtained were analysed according to Student T-test.

One tenth milliliter of a solution of Staphylococcal protein A (100ng/ml) was injected into an air pouch produced subdermally on the back of mice. Two hours later, the connective tissue air pouch was excised and the thin membranous lining of the pouch was microscopically examined after Giemsa staining. The number of leukocytes in 10 randomly selected microscopic (x400) fields were scored ¹.

RESULTS

As shown in Table 1, the polymorphonuclear (PMN) leukocyte response of mice studied 10, 30, 50 and 60 days after S. mansoni infection was significantly lower in comparison with uninfected mice (p<0.05). The decrease in the PMN chemotaxis of S. mansoni infected mice was observed as early as 10 days after infection and continued to decrease at 30 and 50 days post-infection. Interestingly, at 60 days after infection the PMN leukocyte response increased, reaching levels significantly different from 30 and 50 day infected mice (p<0.05). However, the response was still lower than the control (p<0.05). The mononuclear leukocyte response was not significantly different between infected or uninfected mice (Table 1).

TABLE 1

Leukocyte response of uninfected and S. mansoni infected mice to staphylococcal protein A.

The results are reported as mean ± standard deviation.

Groups	Polymorphonuclear Mean ± S. D.	Mononuclear Mean ± S. D.
Uninfected	193.85 ± 80.11	2.78 ± 2.16
10 days after* infection	38.69 ± 14.18	3.24 ± 1.08
30 days after* infection	14.00 ± 11.09	3.32 ± 2.27
50 days after* infection	11.98 ± 7.83	2.36 ± 1.25
60 days after* infection	64.43 ± 20.60	2.26 ± 1.50

*p<0.05

DISCUSSION

The PMN chemotaxis decrease found in mice infected with *S. mansoni*, may be resulted from a disturbance of the complement system or an intrinsic defect of the PMN cells ¹⁰. As far as we know there is no reduction on complement in schistosomiasis, but it was observed that schistosomiasis significantly reduced the PMN production and mobilization ¹¹. Maybe this reduction of mature PMN, could be explained by a delay in the maturation of the granulocytopoietic cells, described in experimental and human schistosomiasis ^{7, 12, 13}.

Further research (observing infected mice for a longer time) is necessary to define if the PMN response will reach normal levels after 60 days of *S. mansoni* infection.

RESUMO

Quimiotaxia de leucócitos "in vivo" na infecção experimental por Schistosoma mansoni

A quimiotaxia de leucócitos "in vivo" foi avaliada em camundongos da linhagem C57B1/10 e estudada 10, 30, 50 e 60 dias após a infecção por Schistosoma mansoni. A proteína A foi utilizada como quimiotático e injetada no tecido conjuntivo no dorso dos camundongos dos grupos experimentais e controle. Nos grupos experimentais foi observado uma diminuição na resposta dos leucócitos polimorfonucleares (PMN) em comparação com o grupo controle (p<0.05). Os camundongos estudados 10 dias após a infecção, mostraram uma diminuição na resposta quimiotática de leucócitos PMN, comparando com o grupo controle (p<0.05) e este dado tornou-se mais evidente nos grupos experimentais estudados 30 e 50 dias após a infecção. Apesar da resposta quimiotática dos leucócitos PMN nos camundongos estudados 60 dias após a infecção aumentarem em comparação aos animais analisados 50 dias após a infecção, este aumento foi bem menor em relação ao grupo controle. A resposta quimiotática dos mononucleares não apresentou diferença significativa entre camundongos experimentais e controles.

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REFERENCES

- ABATH, F. G. C.; MONTENEGRO, S. M. L. & CARVALHO, A. B.
 Modificação da técnica da bolsa de ar para mensuração da quimiotaxia in vivo. Ciênc. e Cult., 38: 1430-1433, 1986.
- BOROJEVIC, R.; SANTOS-DA-SILVA, C. & CARVALHO, E. A. Chronic schistosomiasis mansoni: splenic myelopoiesis and
 inhibition of neutrophil granulocytopoiesis mediated by sera of
 patients. J. Infect. Dis., 148: 422-426, 1983.
- BOYDEN, S. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J. exp. Med., 115: 453-466, 1962.
- FARID, Z.; PRASAD, A. S.; SCHULERT, A. R.; SANSTEAD, H. H. & ROOBY, A. S. Bilharzial spenomegaly. Arch. intern. Méd., 113: 37-41, 1964.
- FORSGREN, A. & SJOQUIST, J. Protein A from S. aureus. I. Pseudo-immune reaction with human - globulin. J. Immunol., 97: 822-827, 1966.
- GALLIN, J. I.; CLARK, R. A. & KIMBALL, H. R. Granulocyte chemotaxis: an improved in vitro assay employing labelled granulocytes. J. Immunol., 110: 233-238, 1973.
- JAMRA, M.; MASPES, V. & MEIRA, D. A. Types and mechanisms of anemia in schistosomiasis mansoni. Rev. Inst. Med. trop. S. Paulo, 6: 126-136, 1964.

- KRONVALL, G. & GEWURZ, H. Activation and inhibition of IgG mediated complement fixation by staphylococcal protein A. Clin. exp. Immunol., 7: 211-220, 1970.
- LAWMAN, M. J. P.; BOYLE, M. D. R.; GEE, A. P. & YOUNG, M.

 A rapid technique for measuring leukocyte chemotaxis in vivo.
 J. immunol. Meth., 69: 197-206, 1984.
- MONTENEGRO, S. M. L.; TEIXEIRA, K. M.; COUTINHO, E. M.; ABATH, F. G. C. & CARVALHO, A. B. - Efeitos de imunopotenciadores não específicos na infecção experimental pelo Schistosoma mansoni. I. Levamisole. Rev. Inst. Med. trop. S. Paulo, 33: 69-73, 1991.
- SANTOS-DA-SILVA, C.; CARVALHO, E. A.; GONÇALVES, M. S. & BOROJEVIC, R. Experimental murine schistosomiasis mansoni: inhibition of neutrophil granulocyte inflammatory reaction. Braz. J. med. biol. Res., 21: 273-279, 1988.
- SMITHERS, S. R. & DOENHOFF, M. J, Schistosomiasis. In: COHEN, S. & WARREN, K. S., ed. - Immunology of parasitic infection. Oxford, Blackwell, 1982.
- ZIGMOND, S. H. & HIRSCH, J. G. Leukocyte locomotion and chemotaxis. J. exp. Med., 137: 387-410, 1973.

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