

## SHORT COMMUNICATION

## Studies on the Production and Regulation of Interleukin, IL-13, IL-4 and Interferon- $\gamma$ in Human Schistosomiasis *Mansoni*

Clarice N Lins de Morais, Joelma Rodrigues de Souza, Wladimir Gomes de Melo, Marcílio L Aroucha\*, Ana Lúcia C Domingues\*, Thomas Wynn\*\*, Frederico Guilherme Coutinho Abath, Sílvia ML Montenegro/+

Centro de Pesquisas Aggeu Magalhães-Fiocruz, Av. Moraes Rego s/n°, Cidade Universitária, 50670-420 Recife, PE, Brasil

\*Universidade Federal de Pernambuco, Recife, PE, Brasil \*\*National Institutes of Health, Bethesda, USA

*The production and regulation of interleukin (IL) IL-13, IL-4 and interferon- $\gamma$  was evaluated in different clinical forms of human schistosomiasis. The mechanisms of immune regulation are apparently different in the various clinical stages of the disease, some of them being antigen specific.*

Key words: *Schistosoma mansoni* - cytokines - human schistosomiasis

In the murine model of schistosomiasis type 1 cytokine interferon- $\gamma$  (IFN- $\gamma$ ) and activated macrophages have been correlated with immunity, and type 2 associated cytokines such as interleukin (IL), IL-4, IL-13, and IL-10 inhibit classical macrophage activation and have been implicated in granuloma formation and fibrogenesis around tissue deposited eggs (Wynn & Cheever 1995). A distinct and most important function of IL-13, was the observation that IL-13 and not IL-4 was the major type 2 cytokine driving type I and III collagen mRNA production and hepatic fibrosis in infected mice (Wynn et al. 1993, Chiaramonte et al. 1999). In the context of the type 1/type 2 cytokine paradigm, data from mice and humans have categorized schistosomiasis as a predominantly type 2 disease (Wynn & Cheever 1995, Abbas et al. 1996, Allen & Maizels 1997, Fallon 2000), implicating type 2 responses as the cause of morbidity and being detrimental to the host. In fact, infection of human with schistosomes and other helminths is characteristically associated with elevated IgE and eosinophilia, hallmarks of a type 2 cytokine response (Abbas et al. 1996). This concept has resulted in a body of research that has attempted to prevent putative type 2 responses, to reduce morbidity. However, recent data in the mouse suggest that the original distinct categorization of schistosome pathology being caused by type 2 cytokines is misleading and, more convincingly evidence from schistosome infected humans suggest that proinflammatory type 1 responses are the cause of morbidity (Fallon 2000). Previous studies have shown that the pattern of cytokine production changes in different stages of human schistosomiasis and more specifically demonstrated that early

infection is associated with a significant IFN- $\gamma$  response and IL-10 plays an important downregulatory role in that response during late infection (Montenegro et al. 1999). The disease is more severe when the immunopathological mechanisms are dominant or the effective control of the parasite is not attained (Chensue et al. 1997). However, the mechanisms underlying the transition to the most severe forms of the disease are not clear. The immunopathology of severe chronic schistosomiasis has been widely studied in experimental models, however studies in humans are relatively scanty.

In the present work some aspects of the immune response in different clinical forms of human schistosomiasis were evaluated, focusing the production and regulation of IL-13, IL-4 and IFN- $\gamma$ .

The patients selected for the study, displayed acute (n = 12) and chronic (n = 31) clinical forms of schistosomiasis *mansoni*. The control group was from non-endemic areas of Brazil. All human studies described were reviewed by ethical and scientific boards.

The whole peripheral blood was drawn with heparin (10 U/ml), and diluted in RPMI 1640 medium (1:3) plus penicilin (100 U/ml) and streptomycin (100  $\mu$ g/ml) and whole blood cultures (Montenegro et al. 1999) were stimulated with SEA (soluble egg antigen) and SWAP (soluble adult worm preparation) (Boros & Warren 1970, Pearce et al. 1991), mitogens (PMA/Iono), recombinant IL-13 and neutralizing antibodies for 96 h in a humidified atmosphere with 5% CO<sub>2</sub>, and then the supernatants were collected and stored at -70°C for subsequent determination of cytokine production.

The measurements of IFN- $\gamma$  and IL-4 were performed by ELISA using specific capture and detection mAbs as previously described (Montenegro et al. 1999). The IL-13 production was quantitated using Quantikine Human IL-13 Immunoassay (R & D systems), following instructions of the supplier. All statistical comparisons were performed by paired or unpaired student t test. P < 0.05 was considered significant.

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\*Corresponding author. Fax: + 55-81-3453.1911. E-mail: silvia@cpqam.fiocruz.br.

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The production of IL-13 in schistosomiasis patients stimulated by SEA and SWAP was evaluated and no statistical difference was found in comparison to normal individuals. However, after IFN- $\gamma$ /IFN- $\gamma$ Receptor neutralization plus stimulation with SWAP, chronic patients, particularly hepatointestinal, expressed higher levels of IL-13 than normal individuals ( $p = 0.04$ ) and patients with the acute form of schistosomiasis ( $p = 0.045$ ). The significant increase of IL-13 production in SWAP stimulated patients, after IFN- $\gamma$  neutralization indicates Th1/Th2 cross-regulation. After mitogen stimulation, normal individuals produced more IL-13 than chronic patients ( $p = 0.018$ ). Experimental studies demonstrated that type 2 cytokine IL-13 has profibrotic activity, and IL-13 could regulate hepatic fibrosis during murine schistosome infection (Fallon et al. 2000). Because of the paucity of information available it is difficult to indicate what mechanisms delineated in murine models are applicable to human disease.

Respecting IL-4, production of this cytokine in schistosomiasis patients after *S. mansoni* antigen stimulation, did not differ significantly from normal individuals. In addition, this production did not seem to change after IFN- $\gamma$  neutralization. On the other hand, acute patients produced more IL-4 after mitogen stimulation than chronic patients ( $p < 0.001$ ) and normal individuals ( $p = 0.007$ ). Recombinant IL-13 addition to the cultures of schistosomiasis patients stimulated with SWAP resulted in the production of high levels of IL-4 by acute patients in comparison to antigen stimulation only ( $p = 0.03$ ).

Regarding IFN- $\gamma$  production, egg and parasite antigen specific stimulation demonstrated production of higher amounts of this cytokine in chronic hepatosplenic patients in comparison to acute patients and this observation was statistically significant. Despite the presence of a predominantly type 2 response in infected humans, there is currently no evidence to suggest that immunopathology in humans is driven by type 2 cytokines. In fact, the available evidence suggests that hepatosplenic disease in humans is associated with type 1 cytokine responses (Fallon 2000). Mwatha et al. (1998) associated high levels of TNF- $\alpha$  and IFN- $\gamma$ , and low levels of IL-5 with the presence of hepatosplenomegaly. Similarly, Montenegro et al. (1999) demonstrated that there was marked production of IFN- $\gamma$ , but not of IL-4 and IL-5 by parasite antigen stimulated splenocytes from *S. mansoni* patients with hepatosplenic disease.

Addition of recombinant IL-13 to cultures of schistosomiasis patients stimulated with SEA resulted in the expression of higher levels of this cytokine in acute patients in comparison to the same patients under SEA stimulation only ( $p = 0.027$ ). However, after combined stimulation with SWAP and recombinant IL-13, chronic patients produced lower levels of IFN- $\gamma$  when compared with the

same patients stimulated with SWAP only ( $p < 0.001$ ). Thus, the effects of exogenous IL-13 on IFN- $\gamma$  production is apparently antigen specific, since the IFN- $\gamma$  expression is differentially regulated by IL-13.

Finally, the production and regulation of Th1 and Th2 cytokines seem to be different in clinical stages of human schistosomiasis, indicating a very complex orchestration of the immune response during the transition to the severe forms of the disease.

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