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# Human schistosomiasis mansoni: Immune responses during acute and chronic phases of the infection

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ABSTRACT

*Schistosoma mansoni* infection may occur either as an acute infection in individuals who have recently visited an endemic area, with no previous contact with the parasite, or as a lasting chronic disease, if not interrupted by specific chemotherapy. The acute phase is characterized by symptoms such as fever, cough, diarrhea, anorexia, and arthralgias in combination with leukocytosis and eosinophilia, and a high cellular immune response to schistosome antigens especially those from the parasite's eggs. In the chronic phase, most patients living in endemic areas are asymptomatic, and their immune responses to egg antigens are modulated. A few develop periportal fibrosis of the liver, which may result in the hepatosplenic form of the disease. The humoral response (IgG, IgM and IgE) in acute patients to egg and worm antigens does not differ from the chronic phase. However, a high level of IgG and IgM antibodies to KLH were detected in acute patients. Acute patients express a considerably higher in vitro cellular responsiveness than do chronic patients, especially to egg antigens. They present a mixed profile of Th1 and Th2 cytokines. Ultrasound examinations of endemic population reveal a high heterogeneity between the patients as regards the presence and intensity of periportal fibrosis. Most patients are asymptomatic and their immune responses to schistosoma egg antigens (SEA) are modulated. In contrast, a high percentage of patients with incipient fibrosis (early stage of hepatosplenic) responded strongly to SEA. Patients with advanced hepatosplenic disease were likely to be non-responders to SEA. Most of the chronic patients presented a Th2 profile with low production of interferon-gamma (IFN-γ). The intensity of infection favors the production of interleukin (IL)-10. After adjusting for age, sex, and intensity of infection, a strong correlation was observed between the production of IL-13 and the degree of fibrosis. Chronic asymptomatic patients and those with incipient fibrosis expressed very high levels of heterogeneity of their antibody responses. IgG response to soluble worm antigen preparation (SWAP) was distinct and significantly higher in hepatosplenic patients than in those asymptomatic or with incipient fibrosis. Levels of IgG4 to SEA were significantly higher in sera from patients with incipient fibrosis as compared to uninfected and hepatosplenic groups. Polyclonal idiotypic antibodies and their fragments F(ab′)2, directly stimulate in culture T cells of schistosomiasis patients in presence of IL-1. Polyclonal idiotypic antibodies are able to modulate in vitro granuloma formation around SEA-polyacrylamide. The importance of idiotypes for protection or pathology in schistosomiasis is still not clear.



## 1. Introduction

*Schistosoma mansoni* has a wide geographical distribution in Africa, South America and the Caribbean. The disease is mainly due to eggs deposited in the host tissue by the adult female worm. The egg antigens induce granuloma formation and fibrosis mainly in intestine and liver portal system. There are two immunologically distinct phases during schistosomiasis infection: acute and chronic.

The acute phase usually occurs in individuals who have recently visited the endemic area without having had any previous contact with the parasite. They present acute symptoms such as fever, cough, diarrhea, anorexia, and arthralgias in combination with leukocytosis and eosinophilia, and the immune responsiveness (Gazzinelli et al., 1985).

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In the chronic phase, most patients living in endemic areas are asymptomatic, but a few develop Symmers' periportal fibrosis of the liver, which results in the severe form of the disease with subsequent portal hypertension, splenomegaly, oesophageal varices and recurrent hematemesis. The sequential development of hepatosplenic (HS) disease is not completely known. It has been suggested that the hepatointestinal (HI) and compensated hepatosplenic clinical forms represent early forms of the severe clinical disease found in the hospital (decompensated hepatosplenic) (Colley et al., 1986).

In order to identify the reliable parameters indicative of the phase, stage and prognosis of the disease, we have been studying the immune response of chronically infected individuals from endemic areas with a different form and/or stage of the disease, and also, in groups of individuals in the acute phase. A review of these studies is presented herein.

## 2. The acute phase of schistosomiasis

Acute schistosomiasis is a toxemic disease following the primary infection with the parasite. The acute phase of schistosomiasis mansoni is seldom observed in endemic populations. This could be due to the early age (2–4 years) at which most children in endemic areas receive their initial environmental exposure. In contrast, visitors to endemic areas appear to be more likely to develop symptomatic acute infections. The endemic and non-endemic individuals are likely to differ in various ways. Upon primary contact with the schistosome disease environment, a person from a non-endemic area is immunologically naive, responding to schistosome antigens for the first time. On the other hand, many children in endemic areas were probably born from mothers who had schistosomiasis during their pregnancy, and thus they first encounter schistosome antigens and anti-schistosome idiotypes *in utero* and/or the breast milk (Montesano et al., 1999; Novato-Silva et al., 1992). These children are probably already primed for many anti-schistosome responses at the time of their first environmental exposure to cercariae and may respond quite differently from truly naive individuals. This hypothesis is reinforced by studies in an experimental model that demonstrated that mice born from infected mothers were apparently able to rapidly modulate the egg-lesions, forming early fibrotic granulomas (Andrade and Warren, 1964).

The anatomic features of the acute disease in humans include a massive military dissemination of granulomas around the eggs, especially in the liver, lung, pancreas and lymph nodes. Acute, diffuse, severe hepatitis and enterocolitis have also been described (Bogliolo, 1958; Neves and Raso, 1963). In a case-study of 25 individuals with acute infection of *S. mansoni*, it was shown by Rabello et al. (1995) that morbidity, evaluated by clinical and sonographic index was more severe in patients with high-levels of egg output.

The humoral response (IgG, IgM and IgE) to egg and worm antigens has been shown to be equivalent in patients with acute and chronic schistosomiasis (Rabello et al., 1995; Kanamura et al., 1979). However, as high level of IgG and IgM antibodies to KLH was detected in acute patients, this becomes a simple diagnostic tool, with high sensitivity and specificity, for differentiation between acute and chronic schistosomiasis (Alves-Brito et al., 1992; Rabello et al., 1995). The cellular responsiveness of acute patients clearly differs from those observed in most chronically infected patients. Gazzinelli et al. (1985) have shown that acute patients express considerably higher *in vitro* responsiveness than do intestinal chronic patients, especially in regard to responses of their peripheral blood mononuclear cells (PBMC) to soluble schistosoma egg antigens (SEA). Total leukocyte levels and peripheral blood eosinophilia were higher in these individuals than in similar individuals with chronic schistosomiasis mansoni. In contrast to the patients with chronic infections, the eosinophilia of these acute cases decreased (rather than elevated) upon treatment. Total lymphocyte population (T and B cell) percentages were not altered during acute infection. Lymphoid subset ($CD3^+$, $CD4^+$ and $CD8^+$) analysis yielded elevated levels of both $CD4^+$ and $CD8^+$ in acute patients.

Studies in human and experimental models have demonstrated that cytokines are important factors in the formation as well as the modulation of the granulomatous immune response to the egg. Malaquias et al. (1997) demonstrated that the addition of blocking anti-IL-10 monoclonal antibodies to PBMC cultures from acute patients did not have any significant effect on the cellular proliferation to either SEA or soluble worm antigen preparation (SWAP). The opposite was observed when the PBMC individuals with chronic intestinal schistosomiasis were tested. Anti-IL-4 antibodies decreased the PBMC response of the intestinal individuals to SEA and SWAP, and the PBMC response of acute patients to SEA but not to SWAP. Addition of anti-IL-5 mAb did not decrease the PBMC response of acute patients to SEA or SWAP.

Montenegro et al. (1999) found that patients with an acute infection responded to SEA and SWAP by producing significantly higher amounts of IFN-γ than patients with chronic intestinal infection and that the production of IL-10 was modestly elevated in cells from both acute and chronic patients. IL-5 was detected in SEA-stimulated cultures from a majority of acutely infected patients, whereas only a subset of patients with chronic infection showed a response above background.

The immune responses of 31 patients with acute schistosomiasis exposed to the same water source of infection and for a similar time period were evaluated by de Jesus et al. (2002), 33–60 days after infection. They showed a high production of the proinflammatory cytokines IL-1, IL-6, and TNF-α in cultures of unstimulated PBMC and detected TNF-α in the serum of 87% of these patients. A higher level of IFN-γ production was found in PBMC from patients with acute than from those with chronic schistosomiasis. In contrast with Montenegro results, fewer patients with acute disease produced IL-10 and IL-5 in response to specific antigens, when compared with patients with the chronic disease. This discrepancy may be attributed to period of time after infection in which the assays were done as suggested by Abath et al. (2006).

PBMC from acute and chronic patients were cultured *in vitro* in the presence of polyacrylamide soluble egg antigen-coated beads (PB-SEA), and reactivity of these cells to the PB-SEA was scored, by measuring the artificial granuloma size (Falcão et al., 1998). The addition of anti-IL-10 monoclonal antibodies to the PB-SEA cultures containing PBMC from acute patients resulted in a small increase in the *in vitro* granuloma size, but was not statistically significant. In contrast, the addition of anti-IL-10 antibodies to PBMC from asymptomatic infected patients significantly increased the *in vitro* granuloma formation. These results indicate that the production of IL-10 by PBMC from acute cases is insufficient to regulate the granuloma formation (Falcão et al., 1998).

Recently, a study of the cytokine profile of leukocytes in the PBMC from acute patients showed an increase in the expression of CCR5 and CCR2 chemokine receptors in $CD4^+$ T cells (personal communication Silveira-Lemos and Alves-Oliveira).

Acute patients, as well as chronic, have a lower percentage of overall circulating $CD3^+$ T lymphocytes in comparison with those of uninfected persons and the frequency of $CD4^+$ is lower in comparison to chronic asymptomatic patients. However, in acute phase, these lymphocytes expressed a higher percentage of major histocompatibility complex (MHC) class II molecules suggesting that this subpopulation cells was activated. In contrast acute patients pre-

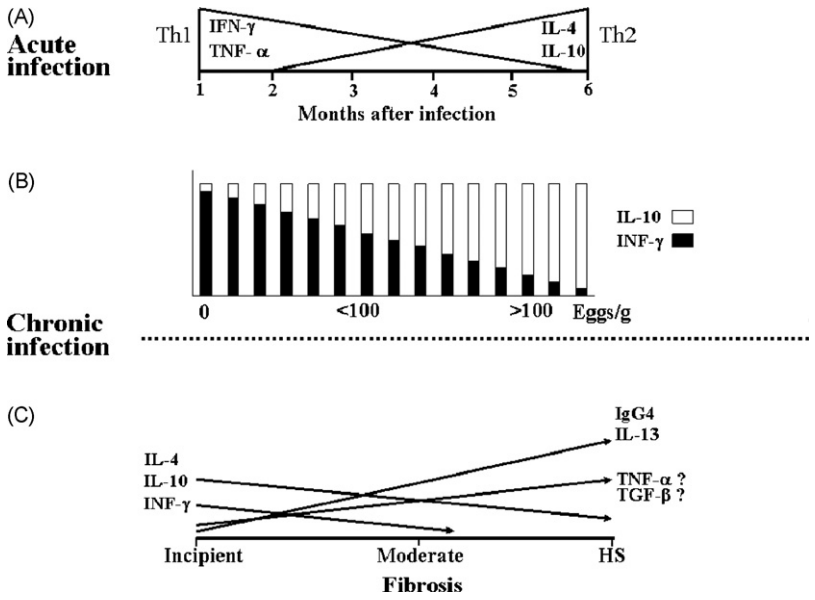


**Fig. 1.** Cytokines cascades in human *S. mansoni* infection. (A) Change from Th1 to Th2 profile according to post-infection month. (B) Schematic diagram of the inverse relationship between IFN-g and IL-10 according to intensity of infection. (C) Change of cytokine profile during development of severe fibrosis.

sented a decreased number of activated $CD8^+$ in relation to chronic patients (Martins-Filho et al., 1999).

From these data, we may conclude that in the acute phase there is a mixed expression of Th1 and Th2 cytokines with predominance of Th1 in the early infection (Fig. 1A). The higher and lower productions of IFN-γ and IL-10, respectively, in acute as compared to chronic schistosomiasis, partially explain the lack of modulation of the immune response in acute patients. In addition there is an increase of eosinophils and a decrease of T lymphocytes in the percentage of blood cells. The contribution of each cell population to the cytokine production in humans is not yet established.

## 3. Definition of the chronic phase of schistosomiasis

In endemic areas most individuals infected with *S. mansoni* exhibit a chronic, relatively asymptomatic infection referred to as the intestinal form of the disease. Based on clinical criteria (liver enlargement, left lobe consistency as well as spleen size) they were considered either as intestinal (INT; liver <2 cm under the costal edge), hepatointestinal (HI; liver >2 cm under the costal edge) or hepatosplenic (HS) (Bogliolo, 1958; Warren, 1979; Prata and Bina, 1968; Andrade, 2004).

The introduction of ultrasound in field surveys expanded the criteria for evaluation of schistosomiasis patients and it became clear that the physical examination of the patients by itself no longer reflected the stage of the disease. This conclusion is based on the observation of thickness of periportal fibrosis as determined by ultrasound echogenicity which correlates with liver biopsies in pathological studies (Cerri et al., 1984; Abdel-Wahab et al., 1992; Homeida et al., 1988; Doehring-Schwerdtfeger et al., 1992a; Magalhães et al., 2005). Therefore, in addition to the clinical examination, ultrasound measurements, such as liver size, portal-vein diameter, thickness of the walls of central and peripheral portal branches, spleen size and splenic veins diameters improve the determination of the stage of the disease. However, there is a variation in the assessment of images provided by ultrasound (Doehring-Schwerdtfeger et al., 1992b), especially in incipient pathology, frequently seen in field surveys and, in these cases, liver biopsies are not indicated and therefore the interpretation of ultrasound images could not be checked.

Ultrasound examinations in field surveys demonstrated a marked heterogeneity between individuals of the INT and HI groups when the degree of central and peripheral periportal fibrosis of the liver were considered. This pathogenic heterogeneity may be the result of susceptibility/resistance to the infection, degree of water contact and/or intensity of contamination of the infection focus. In addition, the susceptibility/resistance itself is related to sex, age and genetic background (Butterworth et al., 1985; Kloos et al., 1998; Fulford et al., 1998; Karanja et al., 2002).

Additional complications to the selection of homogeneous study groups are the presence in endemic areas of reinfected individuals after specific chemotherapy, coinfected individuals with hookworms, and a group of naturally resistant individuals named "endemic normals" (Viana et al., 1995). Thus the interpretation of immune responses of individuals from endemic areas becomes complex. Nevertheless, immunological studies of these individuals have contributed to the understanding of the processes involved in the susceptibility to the infection as well as to the development of pathology. This subject is treated here, and includes the comparison of cellular and humoral immune response of infected groups in different stages of morbidity with non-infected controls. The non-infected groups are composed of individuals with three negative fecal examinations by the Kato-Katz technique (Katz et al., 1972). Some of these had been treated at least 3 years previously.

## 4. Proliferation of PBMC from chronically infected individuals

Initially, we compared proliferation of PBMC from several different groups of patients, representing progressively the more severe clinical form of endemic schistosomiasis mansoni, to three schistosome-derived preparations: SEA, SWAP and soluble cercarial antigens (CERC). When compared with earlier data from patients with acute infections, considerable differences were found in the distribution of non-responders, moderate responders and high responders for the three antigenic preparations. Essentially all patients were highly responsive to SEA after acute infection (Gazzinelli et al., 1985). Most patients in the chronic INT group expressed less responsiveness to SEA but continued to express substantial response to SWAP. In contrast, many of those patients who

had entered the HI form of the disease responded strongly to SEA. Patients apparently in the early stage of the hepatosplenic disease had a high percentage of high responders to SEA. On the other hand, most of the hospitalized patients with advanced hepatosplenic disease were likely to be non-responders to SEA. Chemotherapeutic cure of chronic patients led to long-term expression of high responsiveness to SEA (Gazzinelli et al., 1987).

The possibility of differentiation of T lymphocytes involved in the pathogenesis of schistosomiasis, determinant of the clinical forms, emerged with the development of monoclonal antibodies which reacted with different human T lymphocyte subsets (Reinhertz and Schlossman, 1980). The quantification of T lymphocyte subsets (T4 and T8) in PBMC suspensions by the use of monoclonal antibodies was done by immunofluorescent microscopy (Colley et al., 1983). Our data indicated that the three mean values of $CD8^+$ for uninfected, INT and HS did not differ. However, HS patients had a significantly lower percentage of $CD4^+$ cells than either INT patients or uninfected subjects, reflecting a dramatic decrease in $CD4^+$:$CD8^+$ percentage ratio. The mean ratio values ± S.D. were $2.22 \pm 0.82$, $1.86 \pm 0.62$ and $0.94 \pm 0.34$ for the uninfected, INT and HS groups, respectively. This $CD4^+$:$CD8^+$ subset imbalance observed in HS groups was correlated with an *in vitro* functional inability to respond to SWAP. Other immunological studies of HS patients have also described a degree of unresponsiveness in this population (Reiner et al., 1979; Ellner et al., 1980; Kamal and Higashi, 1982). Confirming earlier studies (Ellner et al., 1980; Reiner et al., 1979) we found also that splenic cells obtained from a significant percentage of HS patients do not respond to external stimuli in a blastogenic assay. In addition, we observed a low $CD4^+$:$CD8^+$ lymphocyte ratio in splenic populations which is similar to that observed in the PBMC populations from HS patients (Colley et al., 1983).

## 5. Idiotype/anti-idiotipic interactions in different clinical forms of schistosomiasis

It is generally accepted that immune-regulation of the granuloma size protects the host against the development of periportal fibrosis. Therefore, most of the studies of schistosomiasis immunology have been centered on the relationship between the mechanism of immune modulation and the development of pathology in the host. Recent review articles on this subject have been published (Wynn et al., 2004; Harnett and Harnett, 2006). We have studied the potential role of idiotypic/anti-idiotype interactions on the process of regulation and morbidity in human population from endemic area. Previously, we developed an experimental method to evaluate patient cellular reactivity against potential idiotypes expressed by antibodies against soluble schistosomal egg antigens. These SEA-idiotypes antibodies were prepared from sera of patients with schistosomiasis by immune-affinity chromatography. We reasoned that these anti-SEA antibodies, of polyclonal origin, would express a wide variety of private and potentially cross-reactive idiotypes (Lima et al., 1986). We have demonstrated that these preparations, and their fragments F(ab)2, specifically stimulate T cells in the PBMC of schistosomiasis patients and former patients (patients infected and treated many years ago) (Fig. 2). Cell populations moderately enriched for B cells did not respond to anti-SEA eluates, while highly B cell-depleted populations of T cells responded well. Additional studies demonstrate that anti-idiotypic T cells can recognize and respond to anti-SEA idiotypes directly (Parra et al., 1988). By using chloroquine in the culture media, an inhibitor of antigen processing by macrophages, it was demonstrated that chloroquine inhibited the SEA stimulation, but failed to interrupt proliferation stimulated by anti-SEA idiotypes (id). Furthermore, the stimulatory combination of covalently bound F(ab)-sepharose plus IL-1, in the absence of adherent cells, indicates that the recognition of these id by T cells does not require MHC-associated recognition. Some murine systems have been used to demonstrate the existence of T cells that recognize id directly, in a non-MHC-restricted manner (Bottomly and Maurer, 1980; Cerny and Cronkhite, 1983). To demonstrate the idiotypic/anti-idiotipic disease-specific interactions, we compared the stimulatory effect of antibody preparations (anti-epimastigote and anti-SEA) from sera of Chagas disease, schistosomiasis or both infections. Schistosomiasis or Chagas disease (American trypanosomiasis) are examples of long-tem infections that may result in severe morbidity. Only cells from patients with schistosomiasis or both infections proliferated upon exposure to anti-SEA antibodies. Conversely, only cells from patients with Chagas' disease or both infections responded to anti-epimastigote antibodies. These studies demonstrated the

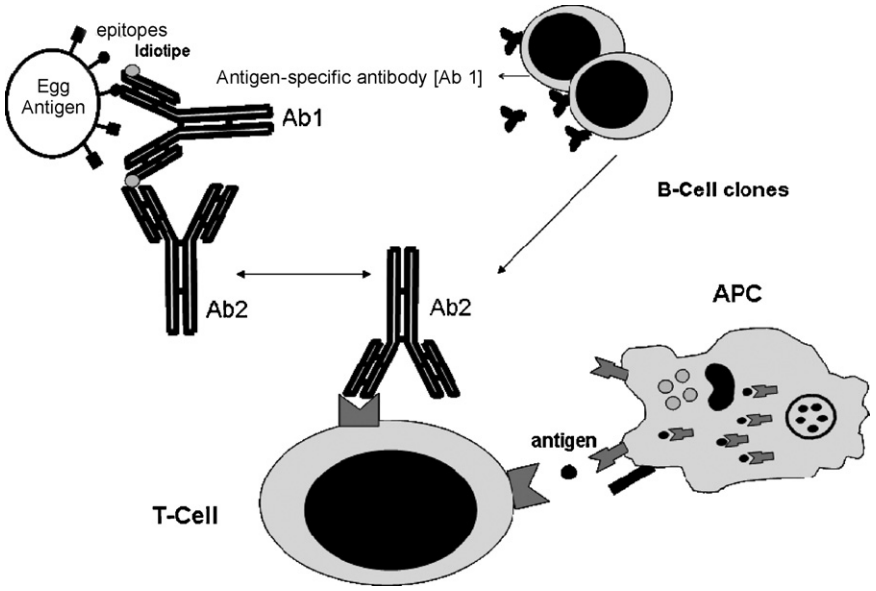


**Fig. 2.** Epitope structure (idiotype) of antigen-binding site of Ab1 stimulates the immune system to generate Ab2 which mimics epitope of the antigen. To stimulate T-cell the Ag is previously processed, but the idiotype binds directly to T-cell. The balance between id/anti-id may exacerbate or regulate the granuloma.

presence of anti-idiotypic T lymphocytes in PBMC of patients with Chagas' disease or schistosomiasis which are specific for idiotypes generated during these infections (Gazzinelli et al., 1988). Furthermore we demonstrated that cord blood mononuclear cells (CBMC) of neonates born of mothers with Chagas' disease or schistosomiasis exhibited strong proliferative responses against idiotypes expressed on antibodies with specificity for *Trypanosoma cruzi* or *S. mansoni* antigens. These immunoaffinity purified preparations were stimulatory if they were prepared from pools of patients or from the mother's own serum, taken early during her pregnancy. We proposed that *in utero* exposure of a fetus to some idiotypes expressed on placentally transferred antibodies induces the anti-id T lymphocyte sensitization (Eloi-Santos et al., 1989). The ability of PBMC from patients with different clinical forms of schistosomiasis mansoni to respond to immune affinity purified anti-SEA antibodies prepared from the pooled sera of patients with different clinical forms of the infection was also investigated. The anti-SEA antibodies from each clinical group were also evaluated by ELISA and immunoblotting. The data indicates that anti-SEA antibodies from patients with chronic INT and HI clinical forms share id and are highly stimulatory for PBMC from most chronic patients, regardless of the clinical form. In contrast anti-SEA antibodies in two different pools from HS patients minimally share these "intestinal form-related" id and do not stimulate proliferation of PBMC from patients in their own or other clinical categories. Anti-SEA antibodies from patients with acute schistosomiasis do not share id with those from patients with INT, HI, or HS forms of the infection and are not stimulatory for PBMC from these patients (Montesano et al., 1989, 1990a,b). In addition, we demonstrated that anti-SEA antibodies from serum pools of chronic asymptomatic patients stimulate proliferation of spleen cells from mice infected with *S. mansoni*. However, antibodies from HS patients did not stimulate these spleen cells (Montesano et al., 1990a,b). Interestingly, polyclonal idiotypic antibodies are able to modulate *in vitro* granuloma formation around SEA-polyacrylamide beads (Parra et al., 1991).

It remains unclear how the idiotypic/anti-idiotypic events may influence the progress of the disease in schistosome infections. It is possible, however, that in the protracted chronic stage of the infection, the induction of idiotypic network triggered by schistosome antigens, eventually generate epitopes on the antibody molecules, which react with specific T cells changing the course of the disease. However, we know that due to the complexity of the idiotypic network it is hard to prove that it may influence the progression of the disease from INT to HS.

## 6. Cytokines production during *Schistosoma mansoni* infection

### 6.1. Interferon gamma (IFN)-γ

The level of IFN-γ production by PBMC stimulated with SWAP was low in the majority of infected patients, independent of clinical form, but was elevated in the normal endemic group (Bahia-Oliveira et al., 1992). This agrees with earlier observations of a general suppression of IFN-γ production during *S. mansoni* infection and its return to normal following chemotherapic cure (Zwingenberger et al., 1989). Since it is thought that normal endemic individuals are resistant to infection (Correa-Oliveira et al., 1989), the data are consistent with the idea that IFN-γ and, by influence, delayed-type cellular hypersensitivity reactions, contribute to protective immunity. In addition an even lower IFN-γ response was detected in HS patients than in those with INT disease. Since it has been suggested that IFN-γ plays a role in the regulation of fibroblast activity and collagen synthesis (Kovacs, 1991), the lower levels of this cytokine in those patients with the more severe form of the disease explain the increased scar tissue and fibrosis in these individuals. In this context, it has been demonstrated that IFN-γ inhibits collagen deposition in murine schistosomiasis (Czaja et al., 1989). In addition, the level of IFN-γ is also affected by the intensity of infection which has been reported to be related to morbidity in schistosomiasis (Silveira et al., 2004). The intensity of infection (evaluated by the number of eggs excreted per gram of feces) depends on a number of epidemiological factors, such as the frequency and intensity of water contact (Gazzinelli et al., 2001), innate host factor such as age (Gryseels, 1994), and genetic disposition (Bethony et al., 1999; Dessein et al., 1992). The prevalence of hepatosplenic disease and intensity of infection has also been previously reported (Arap-Siongok et al., 1976).

### 6.2. Interferon-γ vs interleukin-10 (IL-10)

In contrast to the report of Dunne et al. (2006) from communities on Lake Albert (Uganda) we found a relationship between the intensity of infection and production of both cytokines IFN-γ and IL-10 by PBMC from Melquiades residents (Brazil). *In vitro* stimulation of PBMC with SEA (but not SWAP) resulted in significantly higher secretion levels of IFN-γ *in* egg-negative individuals compared with those with an intensity of infection of more than 100 epg which produced significantly higher amounts of IL-10 (Fig. 1B) (Silveira et al., 2004). Since in endemic areas the intensity of infection is related to the morbidity (Arap-Siongok et al., 1976; Gryseels, 1991) increasing IL-10 and consequently decreasing IFN-γ in association with the intensity of infection may be important factors to trigger the process of fibrosis. However, they are not necessary for the progress to severe fibrosis as presented by the HS form, since this process clearly depends on a number of other important factors, including the genetic background of the individuals. Dessein et al. (1999) investigating genetic control of severe schistosomasis infection in Sudan identified a major human locus closely linked to IFN-γ R1 gene, which encodes the receptor for IFN-γ. The authors suggested that polymorphisms within the IFN-γ R1 gene might influence the development of hepatic fibrosis.

### 6.3. Interleukin-10 (IL-10), interleukin 5 (IL-5) and interleukin 4 (IL-4)

As schistosomiasis infections progress, the majority of the infected individuals evolve to the asymptomatic INT form of the infection and modulate many of their specific Th2 immune responses (Ottesen et al., 1978; Gazzinelli et al., 1985, 1987; Dunne and Riley, 2004).

The chronic HS schistosomiases do not seem to develop the same modulatory mechanisms as the INT (Colley et al., 1986; Tweardy et al., 1987). Malaquias et al. (1997) evaluate the role of three Th2 cytokines on the *in vitro* proliferative responses of PBMC from infected patients after stimulation with schistosomal antigens. The results obtained demonstrated that the proliferative response from chronic INT patients to SEA and SWAP is increased by the blockage of IL-10 with specific monoclonal antibodies. The effects of these antibodies were readily reversed by the addition of recombinant IL-10. In contrast, no effect was observed on the PBMC response of acute and HS patients in the presence of anti-IL-10. Anti-IL-4 antibodies decreased the PBMC response of the INT and HS individuals to both antigens (SEA and SWAP), and the PBMC response of acute patients to SEA but not to SWAP. These results suggested that IL-10 has an important role in the modulation of the Th2 immune response in chronic asymptomatic patients and that this cytokine

may be an important factor in controlling morbidity. The involvement of IL-10 in modulating the proliferative response in PBMC of chronic patients during schistosomiasis mansoni or hematobium has been reported previously (Araujo et al., 1996; King et al., 1996). These results also suggested that in the severe form of the disease the deficiency of IL-10 might be correlated with the pathogenesis of schistosomiasis. The decrease in cell proliferation observed when IL-10 is present may be caused by inhibiting the expression of MHC class II molecules or accessory molecules such as B7 (Mahanty and Nutman, 1995). In either case, IL-10 may be acting through inhibition of IL-4 secretion, which has a T-cell growth promoting activity for Th2 lymphocytes. In addition, it was demonstrated that anti-IL-4 antibodies induce a significant decrease of the proliferative responses to SEA. Addition of anti-IL-5 mAb did not decrease the PBMC response of acute patients to SEA or SWAP (Malaquias et al., 1997). In experimental schistosomiasis it has been demonstrated that this cytokine is required for blood and tissue eosinophilia but not for granuloma formation (Sher et al., 1990; Reiman et al., 2006).

### 6.4. Interleukin 13 (IL-13)

An important role of IL-13 in the development of liver fibrosis in mice and humans has been pointed out by a number of investigators (Brunet et al., 1998; Fallon et al., 2000; Chiaramonte et al., 2001; de Jesus et al., 2004,). Therefore, we examined the level of IL-13 in groups of schistosomiasis patients evaluated by ultrasound and classified as without fibrosis (echogenicity <0.3 cm), with incipient fibrosis (echogenicity >0.3 and <0.5 cm) and with moderate to severe fibrosis (echogenicity >0.5 cm). After adjusting for age, sex and intensity of infection, a strong correlation was observed between IL-13 and the group with moderate to severe fibrosis (Fig. 1C). It appears that IL-13 is the main cytokine involved in the development of hepatic fibrosis. Interestingly, polymorphisms within the IL-13 promoter and IL-13Rα1 has already been reported, reinforcing the role of the genetic background in association with IL-13 for the development of the HS form in schistosomiasis (Ahmed et al., 2000). Nevertheless, as IL-13 was not found to be related with the intensity of infection, we believe that this cytokine is involved only in the development of severe fibrosis, but not to trigger the incipient process. This idea is in accordance with a recent study in knockout mice by Reiman et al. (2006). These authors conclude that IL-5 augments the progression of liver fibrosis by regulating IL-13 activity. Further studies in humans are necessary to elucidate this point.

### 6.5. Transforming growth factor (TGF)-β

TGF-β is a pleiotropic cytokine that is known to be involved in fibrosis due to its ability to induce collagen deposition (Zhu et al., 2000). In a recent study Alves-Oliveira et al. (2006), studying groups of patients with different degrees of fibrosis, found that high levels of TGF-β appeared to be associated with protection against fibrosis, however the strength of this association was low (Fig. 1C). de Jesus et al. (2004) did not find differences in TGF-β levels in SEA-stimulated PBMC supernatants between groups of patients with different degrees of hepatic fibrosis. Indeed, these authors suggested the occurrence of an intermittent induction of fibrosis by TGF-β, as has been shown in baboons infected with *S. mansoni* (Farah et al., 2000). Several reports indicate that TGF-β is a regulatory cytokine that is mainly produced by regulatory T cells which provides an effective mechanism of control of the progression of fibrosis in association with IL-10 (Kitani et al., 2003; Hesse et al., 2004).

### 6.6. Interferon (IFN)-γ vs tumor necrosis factor (TNF)-β

Alves-Oliveira et al. (2006) did not find an association between TNF-β and fibrosis in a study in patients from Virgem das Graças locality, Minas Gerais (Brazil). Booth et al. (2004) in a study in Africa, in whole-blood samples in the absence of *in vitro* antigen stimulation or after stimulation, found that the production of a lower IFN-γ and a high TNF-β were consistent with a high risk of fibrosis. However, this relationship between risk of fibrosis and levels of both cytokines varied with sex and age. Similar observations were made in a study of both sexes and all ages in Brazil (Zwingenberger et al., 1989, 1990), and by Henri et al. (2002) in Sudan. They report a higher TNF-β in sera and a lower IFN-γ production in PBMC assays in cases of periportal fibrosis when compared with uninfected controls.

### 6.7. Humoral response and morbidity

In the chronic phase, a few studies have reported some differences of antibody level and specificity between the INT, HI and HS clinical groups. Cercaricidal antibody levels are higher in patients with hepatosplenism (Capron et al., 1977). Western blot analysis of sera from INT or HI patients indicate that patients of both groups expressed very high levels of heterogeneity of their antibody responses, but there is a major antigen from adult worm of approximately 31 kDa that is recognized by 82% of INT patients and by only 13% HS individuals. In contrast sera from all HS patients recognized 14 kDa and 66 kDa antigens. The 14-kDa molecule was also seen by sera of 25% of INT patients but the latter was unrecognized by sera of any of the INT patients assayed (Correa-Oliveira et al., 1987). It has also been reported that IgG response to SWAP was distinct and significantly higher in HS patients than in those with INT and HI clinical groups (Simpson et al., 1990). Earlier reports associated protection with IgE and susceptibility with IgG4 in schistosomiasis haematobium (Hagan et al., 1991). And more recently, Grogan et al. (1997) reported a positive correlation between IgG4 to SEA and the intensity of infection. Intensity of infection has also been associated with a high risk for the development of periportal fibrosis (Smith et al., 1979; Chever, 1958). However studies linking humoral immune response with morbidity are few (Camus et al., 1977; Goodgame et al., 1978; Guimarães et al., 1979). We compared levels of IgG4 groups with fibrosis with/without organomegaly, as determined by clinical and ultrasound examinations, with a control group without periportal fibrosis, liver and spleen enlargements. Levels of IgG4 to SEA were significantly higher in sera from patients with incipient fibrosis as compared with the patients from the control and organomegaly (severe hepatosplenic disease) groups, indicating a relationship between a specific humoral response and initial fibrosis, a form of schistosomiasis disease transient between intestinal and severe hepatosplenic (Fig. 1C) (Silveira et al., 2002). Other immunological parameters have been correlated with disease severity, such as the occurrence of circulating immune complexes (IC) (Galvão-Castro et al., 1981) and higher levels of anti-worm glycoprotein, but not anti-egg, antigens in HS patients (Simpson et al., 1990).

### 6.8. Immunogenetics and hepatosplenomegaly

The immunogenetics of schistosomiasis has been investigated by several groups. Various different HLA haplotypes have been associated with hepatosplenism, and it appears that some patients have an immunogenetic predisposition to severe disease (Abdel-Salam et al., 1979; Ohta et al., 1982; Hafez et al., 1991). There is also evidence that host genetics could play a hole in human resistance/susceptibility and pathology (Abel et al., 1991; Dessein et al., 1999).

In summary, this report provides an analysis of immune response of peripheral blood mononuclear cells antigen-stimulated *in vitro*, and in sera of acute and chronic patients from endemic areas in Minas Gerais (Brazil). It shows differences in reactivity of T and B cells between patients in different phases of the disease (acute and chronic) as well as in different clinical forms of chronic schistosomiasis (asymtomatic, incipient fibrosis and hepatosplenic). Although the molecular mechanisms underlying the development of morbidity in schistosomiasis are not completely known, it is dependent of the immunological phenotype of the host, as a result of binding of ligands from the parasite to appropriate cell receptor. Much of this is determined by gene expression.

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