

Gender-Related Cytokine Patterns in Sera of Schistosomiasis Patients with Symmers' Fibrosis

David Nascimento Silva-Teixeira,¹ Cristiane Contigli,¹ José Roberto Lambertucci,²
José Carlos Serufo,² and Virmondes Rodrigues, Jr.^{1*}

Laboratory of Immunology, Faculdade de Medicina do Triângulo Mineiro, Uberaba,¹ and Departamento Clínica Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte,² Minas Gerais, Brazil

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Cytokine levels were compared between schistosomiasis patients affected by intense fibrosis defined by ultrasound examination and graded from F-0 to F-3. The concentrations of interleukin-1 β (IL-1 β), IL-4, IL-5, IL-10, IL-13, gamma interferon, and tumor necrosis factor alpha (TNF- α) were determined by enzyme-linked immunosorbent assay of serum samples. Levels of IL-4, IL-5, and TNF- α in the sera of F-3 patients were significantly higher than those found in F-0 individuals, while levels of IL-13 were lower. Levels of IL-4, IL-5, and TNF- α in serum were significantly higher in F-3 males than in F-0 males or F-3 females. Conversely, levels of IL-13 were significantly lower in F-3 females than in F-0 females and males.

The pathology resulting from parasitic infection caused by the helminth *Schistosoma mansoni* includes an acute inflammatory granulomatous reaction, followed by a tissue repair process that has evolved to block the toxic effects of parasite eggs trapped in host tissues (especially in the liver) and prevent extensive cell damage (25). If this healing process is excessive, it may lead to the development of more severe forms of disease, accompanied by severe clinical manifestations, such as hepatosplenomegaly, portal-vein hypertension, gastrointestinal bleeding, and heart or kidney failure, ultimately caused by an extensive fibrosis in hepatic periportal spaces designated Symmers' fibrosis. Direct evaluation of the degree of fibrosis development was enabled by abdominal ultrasonography, an accurate, noninvasive, and reliable technique and a more precise procedure than clinical examination (14, 20). The reasons why severe fibrosis occurs only in a fraction of infected individuals, even when they are submitted to the same environmental conditions, are still unknown.

Epidemiologic studies of populations living in areas where *S. mansoni* is endemic have suggested that fibrosis development is related to infection intensity and duration, gender-related factors, and inherited factors (21, 24). A field study of a Sudanese population demonstrated the prevalence of high fibrosis grades (F-2 and F-3) in young adult and old males but only of F-2 in young adult females, suggesting that males have a tendency to earlier development of higher fibrosis grades (24).

A codominant major gene located in the 5q31-q33 region (*Sm1*) controls human resistance to the parasite, influencing the intensity of infection, probably by acting on cytokine production (23, 28). Another codominant major gene, named *Sm2*, that accounts for familial distribution of the fibrosis-affected phenotype and gender-dependent penetrance was mapped on chromosome 6, in the 6q21-q22 region, closely linked to the gamma interferon (IFN- γ) receptor alpha gene (10).

Several Th2 cytokines, such as interleukin-4 (IL-4), IL-5, IL-10, and IL-13, were associated with the murine immune response against *S. mansoni* (2, 3, 5, 12, 16, 22, 29, 32). Recent studies have demonstrated that tumor necrosis factor alpha (TNF- α) and some Th2 cytokines, such as IL-4 and IL-13, increase fibrosis development in experimental models of *S. mansoni* infection (2, 4). IFN- γ seems to act in the opposite direction, since it elicits antiproliferative and antifibrogenic activities (33).

However, there are still large gaps in our understanding of the role of lymphocytes and effector cells, and their cytokines, in initiating and extending the fibrotic process. Differences in severity between individuals could be related to diverse levels of cytokine production, and therefore it is essential to examine the cytokine profile associated with the physiopathology of severe fibrosis development in human schistosomiasis mansoni.

This study was performed with Brazilians who were born and live in rural areas where the helminth *S. mansoni* is highly endemic: the Caraiá district, located in the central region of the Jequitinhonha Valley in the northeastern part of Minas Gerais State, and Caatinga do Moura, a village in the northeastern part of Bahia State (20, 28). Different degrees of hepatic fibrosis were diagnosed by ultrasound in accordance with World Health Organization guidelines (14, 18). All infections were treated with oxamniquine. Studies were conducted with the informed consent of all blood donors, following approval of the patient protocols used throughout this study by the Ethics Committee of Human Subjects. Grades of fibrosis were determined by echography with a Hitachi linear-array scanner with a 3.5-MHz transducer. Degrees of involvement were estimated on the basis of previously defined criteria, in accordance with World Health Organization guidelines, which range from absence of fibrosis (grade F-0) to severe fibrosis (grade F-3) (14, 20).

Blood samples were collected by intravenous puncture without anticoagulant. Sera were collected after centrifugation, aliquoted, and stored frozen at -80°C until tested.

Levels of IL-1 β , IL-4, IL-5, IL-10, IL-13, IFN- γ , and TNF- α in serum were determined by enzyme-linked immunosorbent assay (ELISA) with specific monoclonal antibody (MAb) pairs.

* Corresponding author. Mailing address: Laboratory of Immunology, Faculdade de Medicina do Triângulo Mineiro, Rua Frei Paulino 30, Uberaba (MG) CEP 38025-180, Brazil. Phone: 55-34-3318 5289. Fax: 55-34-3318 5651. E-mail: vrodrigues@mednet.com.br.

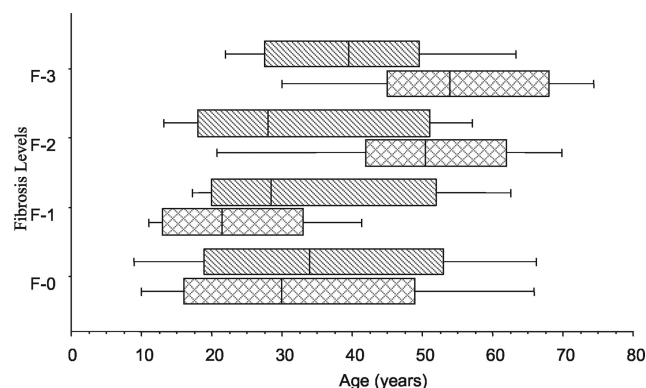


FIG. 1. Distribution of schistosomiasis patients by fibrosis intensity, age, and gender. Patients were grouped by gender (males, striped; females, crosshatched), and fibrosis was graded by echography. Vertical lines represent the median values, boxes represent the 25th to the 75th percentiles, and horizontal lines represent the 10th to the 90th percentiles.

Microplates (Nunc, Roskilde, Denmark) were sensitized overnight with an anti-IL-1 β MAb (Genzyme, Cambridge, Mass.), an anti-TNF- α MAb (Genzyme), an anti-IL-4 MAb (Mabtech, Nacka, Sweden), an anti-IL-5 MAb (Pharmingen, San Diego, Calif.), an anti-IL-10 MAb (Pharmingen), an anti-IL-13 MAb (Mabtech), or an anti-IFN- γ MAb (Mabtech). Nonspecific binding was prevented by incubating the plates with 3% bovine serum albumin (Sigma, St. Louis, Mo.) in phosphate-buffered saline (PBS). Plates were incubated overnight with 100 μ l of a 1:2 dilution of serum samples in PBS, 2% bovine serum albumin, and standard cytokines (Pharmingen and R&D, Minneapolis, Minn.). Plates were then washed four times with 0.05% Tween in PBS and incubated with a rabbit anti-IL-1 β antibody (Genzyme), a rabbit anti-TNF- α antibody (Genzyme), a biotinylated anti-IL-4 MAb (Mabtech), a biotinylated anti-IL-5 MAb (Pharmingen), a biotinylated anti-IL-10 MAb (Pharmingen), a biotinylated anti-IL-13 antibody (Mabtech), or a biotinylated anti-IFN- γ MAb (Mabtech) for 4 h. Plates were washed and incubated for 2 h with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Pierce) or alkaline phosphatase conjugated to streptavidin. Finally, plates were washed four times and incubated with *p*-nitrophenyl phosphate (Sigma). The A_{405} was read in a microplate reader (Bio-Rad, Hercules, Calif.). The sensitivity of the tests was 10 to 20 pg/ml.

Data were analyzed statistically by the Mann-Whitney test or the Kruskal-Wallis test, with the level of significance set at $P \leq 0.05$.

Although many studies have revealed the involvement of T cells and effector cells in the human immune response against *S. mansoni* (1, 7–9, 28, 30), the correlation between the outcomes of different clinical forms of this parasitic disease and the establishment of distinct cytokine secretion patterns has not been completely defined. Analysis of cytokines in sera from a great number of individuals with different degrees of hepatic fibrosis, related to distinct fibrosis levels determined by echography, is one way to address this issue. In the present paper, involvement of the immune response in fibrosis development and the cytokine patterns presented by patients with no hepatic fibrosis (F-0) were compared to those of patients with more severe fibrosis (F-3).

We focused our interest especially on polar groups, i.e., the F-3 group ($n = 34$) on the one hand and the F-0 group ($n = 114$) on the other hand. The other patients were characterized as F-1 ($n = 36$) and F-2 ($n = 31$). Figure 1 shows the distribution of the individuals analyzed in this study by age, by Symmers' fibrosis grade, and by gender. In the F-2 and F-3 grades, the mean age of fibrosis development is clearly divergent between the genders ($P \leq 0.05$, Kruskal-Wallis test), as females had moderate (F-2) or intense (F-3) fibrosis, especially at more advanced ages, while males had the same frequency of fibrosis grades independent of age. This observation is similar to other studies of Brazilian and Sudanese populations (20, 24).

Results shown in Fig. 2 demonstrate different patterns of serum cytokine expression between individuals not affected (F-0) and those with extensive fibrosis (F-3). Levels of IL-4, IL-5, and TNF- α in sera from F-3 patients were statistically significantly higher ($P \leq 0.05$, Mann-Whitney test) than those in sera from F-0 patients. Conversely, IL-13 levels were significantly lower in patients with advanced fibrosis.

Previous studies have revealed that Th2 cells, and their cytokines, are required for fibrosis development in an experimental model (19) and that TNF- α and IL-4 displayed profibrotic activities (2). Studies with human T-cell clones revealed that TNF- α is necessary to stimulate in vitro granuloma formation (30) and that low IFN- γ production and high TNF- α production are associated with severe fibrosis (17). The results presented here point out the association of high levels of IL-4, IL-5, and TNF- α with the development of Symmers' fibrosis. On the other hand, IL-13 levels were significantly lower in patients with extensive fibrosis. Our results also show that no significant differences appeared for IL-1 β , IL-10, and IFN- γ .

As we found significant differences among males and females with higher fibrosis levels (Fig. 1), we also analyzed serum cytokine patterns by gender (Fig. 3). These results revealed completely different patterns among males and females, based on fibrosis grades. Significantly high levels of IL-4, IL-5, and TNF- α were observed in F-3 male patients, while the levels

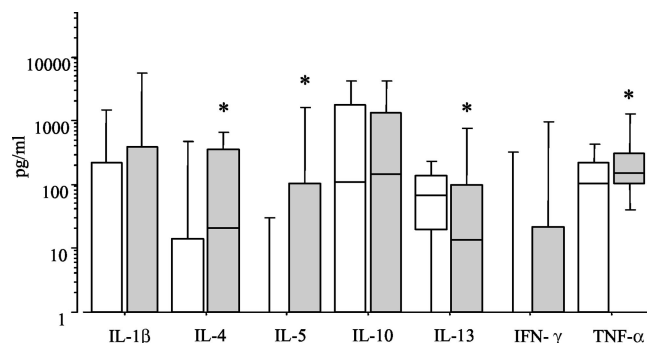


FIG. 2. Serum cytokine profiles related to fibrosis intensity. Levels of IL-1 β , IL-4, IL-5, IL-10, IL-13, IFN- γ , and TNF- α were determined by ELISA in serum samples from individuals with schistosomiasis and intense Symmers' fibrosis (F-3) (grey bars) or nonaffected individuals (F-0) (open bars). Horizontal lines represent the median values, boxes represent the 25th to the 75th percentiles, and vertical lines represent the 10th to the 90th percentiles. Asterisks indicate statistically significant differences ($P \leq 0.05$).

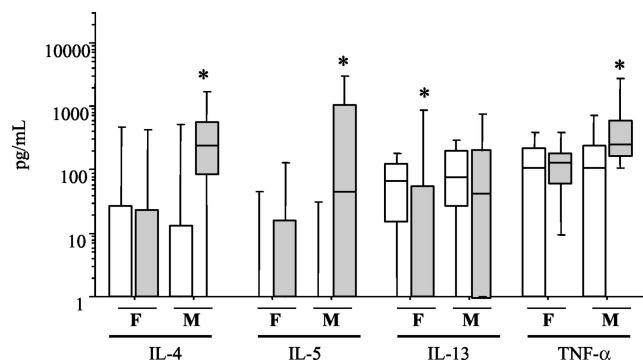


FIG. 3. Male and female serum cytokine profiles related to fibrosis intensity. Levels of IL-4, IL-5, IL-13, and TNF- α were determined by ELISA in serum samples from males (M) and females (F) with schistosomiasis and intense Symmers' fibrosis (F-3) (grey bars) or nonaffected persons (F-0) (open bars). Horizontal lines represent the median values, boxes represent the 25th to the 75th percentiles, and vertical lines represent the 10th to the 90th percentiles. Asterisks indicate statistically significant differences ($P \leq 0.05$).

of these cytokines in F-3 females were equivalent to those in nonaffected patients.

In fact, previous epidemiological studies described differences in fibrosis development between the genders (20, 24). Nonimmune physiological factors, such as steroid hormones, could have an important role in the orientation of immune response in females, leading to modulation of cytokine production and consequently to slow fibrosis development. It was demonstrated that progesterone favors the switch to Th2 to the detriment of Th1 responses (6). Estradiol seems to modulate TNF- α production and increase IL-10 production, in contrast to our results (11). A gender-dependent specific immune response during chronic human schistosomiasis haematobia was also described with high IL-10 and TGF- β production and low TNF- α and IFN- γ production by females (27). Our results demonstrated that *S. mansoni*-infected females with intense fibrosis have lower IL-4, IL-5, and TNF- α levels in serum; this finding suggested that other mediators may be involved in the development of Symmers' fibrosis in females. It is interesting that males are affected by severe fibrosis predominantly and early and that high levels of profibrotic cytokines were observed in F-3 males. It is well documented in the literature that TNF- α and IL-4 are able to stimulate collagen synthesis (15, 31), leading us to suggest that these cytokines are involved in fibrosis development in *S. mansoni*-infected male patients. It also seems that these cytokines do not contribute to fibrosis development in females. Other mediators may account for fibrosis in females, who are affected at a lower intensity and frequency than are males, perhaps owing to lower levels of profibrotic cytokines.

Conversely, the level of IL-13 was significantly lower in F-3 females, while F-3 males had levels equivalent to those of nonaffected individuals.

The gender-related cytokine patterns associated with Symmers' fibrosis grades, shown in this work, may lead to a better understanding of the immunological mechanisms involved in fibrosis development in schistosomiasis and a variety of other chronic diseases. The localization of cytokine receptor genes such as those for IL-13R and IL-2R γ in the Xq24 and Xq13

regions of chromosome X (13, 26) may help us to explain the observed gender-dependent differences in immune mediators associated with fibrosis revealed in this work.

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