CONCISE COMMUNICATION

Plasma Concentrations and Role of Macrophage Inflammatory Protein–1α during Chronic Schistosoma mansoni Infection in Humans

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Chemokines play an important role during granulomatous inflammation in murine models of Schistosoma mansoni infection. Here, the expression and possible roles of chemokines during human S. mansoni infection were examined. Compared with uninfected individuals, infected patients had elevated plasma concentrations of macrophage inflammatory protein (MIP)-1α, RANTES (regulated on activation, normally T cell–expressed and secreted), and eotaxin. Concentrations of macrophage-derived chemokine, eotaxin-2, monocyte chemotactic protein–1, growth-related oncogene, and interleukin-8 were similar between the 2 groups. When subjects were grouped according to disease severity, individuals with a plasma MIP-1α concentration >400 pM had a 10-times greater risk of having the more severe hepatosplenic form of disease. In the in vitro granuloma reaction, greater concentrations of MIP-1α were produced by cells of patients with hepatosplenic disease than cells of patients with intestinal disease. Pretreatment with a chemokine receptor antagonist attenuated the enhanced in vitro reaction seen with cells derived from patients with hepatosplenic disease. MIP-1α may not only mark a subset of patients with a greater risk of having more severe disease but also play a relevant pathophysiological role in human schistosomiasis.
ing *S. mansoni* infection. Because biopsy specimens from livers of infected patients are not freely available because of ethical reasons, one may obtain further insights into the disease by probing the plasma concentrations of inflammatory mediators. Here, the concentrations of chemokines were evaluated by ELISA in plasma from uninfected individuals and individuals with different clinical forms of chronic schistosomiasis. Further studies to evaluate the putative role of chemokines were then carried out using an in vitro granuloma assay.

**Subjects, Materials, and Methods**

**Study population.** All individuals included in this study were from an area where *S. mansoni* is endemic (Patiromônia Velho, Minas Gerais, Brazil). Measurement of chemokines was carried out in plasma from 58 individuals who had at least one positive Kato-Katz thick smear stool test for *S. mansoni* eggs and 11 uninfected individuals (defined as subjects with at least 3 negative stool tests) with a similar age range. A trained physician performed a clinical examination, and individuals were classified on the basis of the physical examination as having the intestinal (INT) or HS form of the disease [6, 7]. After clinical examination, 16 infected individuals were considered to have the HS form, and 42 were considered to have the INT form of the disease. Summarized characteristics of the 58 infected and 11 uninfected individuals whose blood was analyzed for chemokine concentrations are shown in table 1. For the in vitro experiments evaluating macrophage inflammatory protein (MIP)-1α production and chemokine receptor blockade, 15 other infected subjects (9 with INT disease and 6 with HS disease) from the same area were recruited at a later stage. These patients were recruited at the time of diagnosis, and blood samples were taken. Antischistosomical treatment and follow-up was offered to all patients, regardless of their participation in the study.

**Plasma processing.** Blood collected in heparin was used to prepare plasma that was stored at −70°C. For analysis, samples were thawed, and excess proteins were removed by acid/salt precipitation. The supernatants were then adjusted for salt content (0.14 M sodium chloride and 0.01 M sodium phosphate) and pH (7.4), for the determination of chemokine concentrations.

**Quantitation of chemokines.** The concentration of chemokines in plasma of infected and uninfected individuals and in tissue culture supernatant (see below) after the in vitro granuloma reaction was measured using sandwich ELISAs with matched antibody pairs (R&D Systems).

**In vitro granuloma assay.** Soluble *S. mansoni* egg antigens (SEAs) were prepared and conjugated to polyacrylamide beads (PBs), and the in vitro granuloma assay was done as described elsewhere [7]. Peripheral blood mononuclear cells (PBMC; 3 × 10⁵) and 200 PB-SEA beads were cultured in the presence or absence of the amino-terminus-modified chemokine receptor antagonist methionine-RANTES (Met-RANTES; 1 μM) [10]. Cultures were set up in triplicate and maintained at 37°C in a CO₂ incubator. In vitro granuloma reactivity was evaluated by morphologic quantification of cellular reactivity, as described elsewhere [7]. In these experiments, culture supernatants were collected for the evaluation of MIP-1α concentrations.

**Statistical analysis.** Differences between 2 groups were evaluated using Student’s *t* test or the Mann-Whitney *U* test in normally and nonnormally distributed data, respectively. Differences among 3 groups were evaluated using analysis of variance, followed by Student-Newman-Keuls’ posttest, in normally distributed data. Two-by-two contingency tables were analyzed using Fisher’s exact test. All calculations were performed using InStat software (GraphPad).

**Results**

**Concentration of chemokines in plasma.** There were no significant differences between infected and uninfected individuals in the plasma concentrations of the CC chemokines eotaxin-2, monocyte chemotactic protein (MCP)-1, and macrophage-derived chemokine (MDC) and of the CXC chemokines interleukin (IL)–8 and growth-related onocogene (GRO) (data not shown). In contrast, there were significantly greater concentrations of the CC chemokines eotaxin, RANTES, and MIP-1α in the plasma of chronically infected individuals, compared with uninfected individuals (figure 1).

The group of chronically infected individuals was then subdivided into 2 groups on the basis of their clinical presentation with either the HS or the INT form of disease. There were no significant differences between the 2 groups in the concentrations of RANTES or eotaxin (figure 1B and 1D). The concentration of MIP-1α was apparently greater in the HS than the INT group, but this did not reach statistical significance (figure 1F). However, there was a significantly greater risk of more-severe disease in patients with higher concentrations of MIP-1α when the 2 groups were compared with respect to the number of patients possessing an MIP-1α concentration >400 pM (81% of patients with HS disease and 31% of patients with INT disease; OR, 9.7; *P* = .0009) or >1000 pM (69% of patients with HS disease and 31% of patients with INT disease; OR, 4.9; *P* = .016). None of the uninfected patients had elevated concentrations of MIP-1α in plasma (figure 1E), and 31% of patients with INT disease had MIP-1α concentrations similar to or greater than those of patients with HS disease (figure 1F). The concentration of MIP-1α in plasma did not correlate with

**Table 1.** Epidemiological data of *Schistosoma mansoni*-infected and –uninfected individuals selected for the evaluation of the concentration of chemokines in plasma.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Uninfected subjects (n = 11)</th>
<th>Intestinal subjects (n = 42)</th>
<th>Hepatosplenic subjects (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>7:4</td>
<td>25:17</td>
<td>3:13</td>
</tr>
<tr>
<td>Age, years</td>
<td>30.6 ± 3.4</td>
<td>27.8 ± 1.7</td>
<td>29.8 ± 5.1</td>
</tr>
<tr>
<td>No. of eggs/g of feces</td>
<td>176 ± 45</td>
<td>280 ± 93</td>
<td></td>
</tr>
<tr>
<td>Granuloma index</td>
<td>1.35 ± 0.07</td>
<td>2.69 ± 0.07b</td>
<td>3.80 ± 0.17b,c</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD, except for ratio of male-to-female.  

* Uninfected individuals were those who had at least 3 negative stool examinations.  

* P < .001, vs. uninfected individuals.  

* P < .001, vs. patients with the intestinal form of chronic *S. mansoni* infection.
Concentrations of chemokines in plasma. A, C, and E, The concentration of chemokines in the plasma of chronically *Schistosoma mansoni*-infected individuals is compared with that in uninfected individuals. B, D, and F, Comparison of the concentration of chemokines in the plasma of *S. mansoni*-infected patients with either the hepatosplenic or intestinal form of the disease. Chemokines were measured using sandwich ELISAs specific for eotaxin (CCL11), RANTES (CCL5), and macrophage inflammatory protein (MIP)-1α (CCL3). Results are shown as individual values and median (horizontal line) for 58 infected individuals (16 with hepatosplenic disease and 42 with intestinal disease) and 11 uninfected individuals. * and **, infected vs. uninfected individuals (Mann-Whitney U test).

In vitro granuloma model. Stimulation of PBMC with PB-SEA induced a cellular reaction characteristic of the in vitro granuloma reaction. This reaction was accompanied by the production of MIP-1α that was already noticeable on the first day of culture and peaked at day 5 (data not shown), at a time when the in vitro granuloma reaction reaches its peak [7, 8]. When PBMC obtained from patients with HS and INT disease were compared, there was greater baseline MIP-1α production by PBMC from patients with HS disease (figure 2A). The activation of PBMC with beads coupled to antigen induced further production of MIP-1α in both groups of patients. However, the production of MIP-1α by PBMC from patients in both the HS and INT groups after PB-SEA stimulation was not significantly different (figure 2A).

To assess the effects of the antagonism of chemokine receptors to which MIP-1α binds, PBMC were coincubated with PB-SEA in the presence or absence of the chemokine receptor antagonist Met-RANTES, and the granuloma index was evaluated. Pretreatment with Met-RANTES failed to affect the development of the in vitro granuloma reactivity of PBMC obtained from patients with INT disease (figure 2B). In contrast, treatment with Met-RANTES markedly attenuated the in vitro granuloma reaction of PBMC from patients with HS disease (figure 2C).

Discussion

The granulomatous inflammation that occurs around eggs deposited in tissues is central for the pathogenesis of chronic human *S. mansoni* infection. The existence of a role for cyto-
In an attempt to examine the function of the chemokine system during chronic human schistosomiasis, the concentration of these mediators was measured in plasma. Although detection of plasma concentration of mediators does not necessarily signify pathophysiological importance for the disease, plasma is easily accessible and allows for initial incursions into understanding the role of novel mediator systems. Compared with age- and area-matched uninfected individuals, infected patients had elevated plasma concentrations of the chemokines MIP-1\(\alpha\), RANTES, and eotaxin. Concentrations of MDC, eotaxin-2, MCP-1, GRO, and IL-8 were similar between the 2 groups. Moreover, when chronically infected individuals were grouped according to disease severity, individuals with a plasma MIP-1\(\alpha\) concentration >400 \(\mu M\) had a 10-times greater risk of having the more severe HS form of disease than did those with lower concentrations of the chemokine. Of note, ~30% of patients with INT disease had elevated plasma concentrations of MIP-1\(\alpha\). The reasons for the latter finding are not immediately apparent, but it could be due to individual variation within the population or to the presence of more-severe schistosomiasis not detected by physical examination. Although we did not find a correlation between MIP-1\(\alpha\) concentration and the in vitro granuloma reaction, future studies using abdominal ultrasound should help define the issue in more detail.

Because murine models suggest that there is an important role for MIP-1\(\alpha\) in \textit{S. mansoni}-induced granulomatous inflammation [12], it was possible that MIP-1\(\alpha\) might also play a role during human infection. An in vitro granuloma model was used to gain further insight into the role of MIP-1\(\alpha\) during chronic human \textit{S. mansoni} infection. Although the in vitro granuloma assay does not mimic all pathological aspects of the granulomatous inflammation observed in tissue, it is useful for evaluation of the proliferation and migration of PBMC in response to antigen given in particulate form [7, 8]. In patients with HS disease, the agglomeration of cells around the antigen bead is much larger than that in patients with INT disease, an effect that appears to be dependent on the lack of production and/ or action of IL-10 [7]. A previous study has shown that MIP-1\(\alpha\) is produced during the in vitro granuloma reaction [13]. In our experiments, greater concentrations of MIP-1\(\alpha\) were produced by PBMC from patients with HS disease than PBMC from patients with INT disease. Moreover, and more important, pretreatment of cells with Met-RANTES, an antagonist of chemokine receptors (especially CCR1 and CCR5), markedly attenuated the enhanced in vitro reaction seen with cells obtained from patients with HS disease [10]. In contrast, Met-RANTES failed to affect the reaction of cells from patients with INT disease. These results suggest that, in addition to IL-10, MIP-1\(\alpha\) may be a factor that underlies the enhanced responsiveness of patients with HS disease. Of interest, these results are qualitatively similar to those obtained in the murine model, in which anti–MIP-1\(\alpha\) pretreatment affected primary and vigorous (isolated from 8-week-infected mice), but not modulated (isolated from 20-week-infected mice), granulomas [12].

In conclusion, our results suggest that elevated plasma con-

![Figure 2. Secretion of macrophage inflammatory protein (MIP)-1\(\alpha\) by peripheral blood mononuclear cells (PBMC) and effect of a chemokine receptor antagonist in the in vitro granuloma assay. A. Production of MIP-1\(\alpha\) by PBMC from patients with the hepatosplenic or intestinal forms of the disease in the presence (black bars) or absence (white bars) of antigen-coupled beads. MIP-1\(\alpha\) concentrations were determined by ELISA, and results are shown as the mean \pm SEM for 5 subjects in each group. * \(P<.05\), presence vs. absence of antigen; ** \(P<.01\), patients with the hepatosplenic vs. intestinal forms of the disease. PBMC from patients with the intestinal (B) or hepatosplenic (C) form of the disease were preincubated with methionine-RANTES (Met-RANTES; 1 \(\mu M\)) or vehicle and cultured in the presence (black bars) or absence (white bars) of antigen-coupled beads. In vitro granuloma reactivity (granuloma index) was evaluated on day 5 after culture initiation by quantification of cellular reactivity around the beads. Results are shown as the mean \pm SEM for 6 subjects in each group. * \(P<.05\) and ** \(P<.01\), presence vs. absence of antigen; * \(P<.05\), patients treated with Met-RANTES vs. those treated with vehicle.](https://academic.oup.com/jid/article-abstract/186/11/1696/853937/Plasma-Concentrations-and-Role-of-Macrophage/fig-2)
centrations of MIP-1α may not only mark a subset of patients with a greater risk of having more severe disease but also play a relevant pathophysiological role in human schistosomiasis by acting on chemokine receptors on mononuclear cells. Further studies will be necessary to identify the subtypes of chemokine receptors involved and to investigate the mechanism of action of MIP-1α in our in vitro system (i.e., the effects of MIP-1α/ MIP-1α receptor blockade on local cytokine production and recruitment of various cell types around the particulate antigen). Moreover, there is a need for studies in tissue using historical samples to confirm the expression of chemokines and their receptors in the human disease. An understanding of the molecular mechanisms that explain why a few patients go on to develop more severe disease may aid in the definition of markers of severity and define novel immunological or pharmacological targets to prevent severe disease.

References