EVIDENCE OF ALTERED SECONDARY LYMPHOID-TISSUE CHEMOKINE RESPONSIVENESS IN BALB/C MICE INFECTED WITH SCHISTOSOMA MANSONI

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ABSTRACT: To determine the extent to which splenic T cells were affected by Schistosoma mansoni infection, we investigated the ability of the T cells to produce interferon (IFN)-γ, as well as their chemotactic ability 7 wk PI. In this study, we report that splenic T cells from Balb/c mice with S. mansoni infections were capable of producing levels of IFN-γ comparable with splenic T cells from naive mice. However, the T cells exhibited altered chemotactic activity, as evidenced by an inability to respond to secondary lymphoid-tissue chemokine (SLC/CCL21). Although no difference in chemokine expression was found between the spleens of infected versus control mice, chemokine production was greater in the livers of infected versus control mice. Collectively, these data indicate that Balb/c mice with 7-wk S. mansoni infection possess splenic T cells with altered chemotactic activity and that the alterations may be a consequence of the granulomatous response in the liver.

Species of Schistosoma infect approximately 200 million people worldwide (Bergquist and Colley, 1998). Because of this, Schistosoma spp. are widely used in biological research. Many aspects of the schistosome's anatomy, physiology, and life cycle, as well as treatments and immunological responses, are under investigation. A relatively new field of interest is the role of chemokines in schistosome infections.

Chemokines are small molecules that are capable of mediating recruitment of hematopoietic and nonhematopoietic cells. Members of the chemokine family are characterized by the presence of 4 conserved cysteine motifs in the amino terminal of the protein (Rollins, 1997). Currently, there are 4 known chemokine subfamilies. The CC, CXC, CX3C, and C chemokines include members such as monocyte chemoattractant protein-1 (MCP1, CCL2), interferon (IFN)-γ-induced monokine (MIG, CXCL9), fractalkine (CX3CL1), and lymphotoxin (XCL1), respectively (Rollins, 1997). These compounds are essential for virtually every component of an immune response, such as maturation of leukocytes (Campbell et al., 1999), peripheral leukocyte trafficking (Wang et al., 1998), and initiation of an immune response (Saeki et al., 1999).

The role of chemokines in Schistosoma mansoni infections for the most part has focused on granuloma formation induced by eggs released from the female worms (Lukacs et al., 1994). These studies have revealed that chemokines control much of the granulomatous response. For instance, Chensue et al. (1996, 1999) reported that CCL2 contributed to a Th2 granulomatous response to soluble antigens from S. mansoni eggs, whereas Regulated Upon Activation Normal T cell Expressed and Secreted (RANTES, CCL5) favored a Th1 response. Lu et al. (1998) reported that CCL2 contributed to pulmonary granuloma in response to S. mansoni. Macrophage inflammatory protein-1α (MIP-1α, CCL3) has also been implicated in the granulomatous reaction. El-Ahwany et al. (2000) reported that CCL3 was associated with the early granulomatous response, and Gao et al. (1997) reported that CCR1 −/− mice exhibited a 40% reduction in granuloma size, therefore implicating CCL3, CCL5, or CCL7 as important mediators of the granulomatous reaction. Despite these studies there is a paucity of information on the impact of elevated chemokine production on peripheral T lymphocytes.

Previously we reported that elevated chemokine production, associated with progressing tumors, adversely affected T-cell function, as evidenced by the abnormal chemotactic ability of T cells located distal from the tumor, splenic T cells (Kurt et al., 2001). In the present study, we determined whether elevated chemokine production in mice infected with S. mansoni similarly possessed splenic T cells with altered chemotactic activity. We report that Balb/c mice infected with S. mansoni for 7 wk possess splenic T cells that exhibit altered chemotactic activity and that this is associated with increased levels of chemokine production in the livers but not the spleens of infected mice.

MATERIALS AND METHODS

Mice and infection

For each experiment, three 6- to 8-wk-old Balb/c mice (male and female) were exposed in a finger bowl for 1 hr at 22–24°C to a total of 150 freshly emerged cercariae of S. mansoni (Puerto Rican strain) in 100 ml of artificial spring water, as described by MacInnis (1970). These mice were killed by cervical dislocation 42 days postexposure (PE), and the livers, spleens, and mesenteric veins were examined in saline. Three additional uninfected mice (controls) were maintained identically as the experimental mice, but were not exposed to S. mansoni cercariae. The livers and spleens of these mice were used for analysis of control mice.

At necropsy, the livers and spleens of all the exposed mice were markedly enlarged compared with those of the controls, and these organs contained the characteristic granuloma associated with S. mansoni infection. Examination of the liver washings and dissection of the mesenteric veins revealed that the infected mice contained at least 2–6 worm pairs in addition to unpaired male worms. The livers and spleens of these mice were used for analysis as described below.

T-cell isolation

Splenic T cells were removed by placing the spleens in a petri dish and using the flat end of a syringe plunger to press out the cells. The cell suspension was passed through a 40-μm cell strainer (BD Falcon, Franklin Lakes, New Jersey). Red blood cells were lysed by hypotonic shock, washed twice in Roswell Park Memorial Institute medium (RPMI) (BioWhittaker, Walkersville, Maryland) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO BRL, Gaithersburg, Maryland), glutamine (2 mM, BioWhittaker), penicillin (100 U/ml, BioWhittaker), streptomycin (100 μg/ml, BioWhittaker), 1× nonessential amino acids (Sigma, St. Louis, Missouri), 2-ME (5 × 10−3 M, Sigma), and sodium pyruvate (1 mM, BioWhittaker) (cRPMI), and counted. Next, 1 × 106 cells resuspended in 2 ml of cRPMI were added to a pre-equilibrated nylon wool column and incubated for 45 min at 37°C. The T cells were recovered by passing 20 ml of cRPMI over the column and collecting the eluent. The resulting cell population was 85–95% lymphocytes, which were presumed to be T cells. Differential cell counts were performed by centrifuging 5 × 106 cells onto a slide using a Cytospin (Thermo-Shandon, Pittsburgh, Pennsylvania) and staining the cells with the Hema 3 stain set (Fisher Scientific, Pittsburgh, Penn-
sylva). The percentage of lymphocytes, monocytes, and neutrophils was determined by morphology of 3 fields of view per slide.

T-cell cytokine production

To measure the ability of T cells to produce cytokines, spleens from control or infected mice were removed, and the T cells were enriched by passage over a nylon wool column. These cells were plated at 1 x 10^6 cells per well in a 24-well culture cluster plate (Costar Corp., Cambridge, Massachusetts) precoated with 5 μg/ml of anti-CD3 (BD Pharmingen, San Diego, California). The supernatants were harvested after 24 hr, centrifuged for 5 min at 350 g, transferred to clean polypyrrole microtubes, and stored at -20°C. The supernatants were tested for IFN-γ, using a specific enzyme-linked immunosorbant assay, according to the manufacturer's instructions (R&D Systems, Minneapolis, Minnesota).

Chemotaxis assay

Spleens from control or infected mice were removed, and T cells were enriched by passage over nylon wool. A 96-well chemotaxis chamber (Neuro Probe, Inc., Gaithersburg, Maryland) was used for the chemotaxis assay as described previously (Kurt et al., 2001). Briefly, the bottom wells of the chamber were loaded with CCL21 (R&D Systems) resuspended in Hanks' balanced salt solution (HBSS) + 0.1% bovine serum albumin (Sigma) or with HBSS + 0.1% bovine serum albumin alone. The upper well of the chemotaxis chamber, containing 1 x 10^6 effector cells, was separated from the lower well by a 5-μm polycarbonate filter with adhesive precoated with murine laminin (Invitrogen, Carlsbad, California). After 1 hr of incubation at 37°C, the filter and plate were centrifuged at 400 g for 5 min. The cells that had migrated were removed from the lower wells and counted with a hemacytometer. Random T-cell movement, i.e., chemokinesis as the mechanism of cell migration, was ruled out by placing chemokines in the top and bottom wells of the chemotaxis chamber.

Reverse transcriptase–polymerase chain reaction

To evaluate chemokine expression by the livers and spleens and chemokine receptor expression by the splenic T cells, messenger RNA (mRNA) was extracted from 0.1 g of liver or 1 x 10^6 T cells by using an mRNA isolation kit (Qiagen, Chatsworth, California). The absence of DNA contamination was confirmed by polymerase chain reaction (PCR) with mRNA before the reverse transcription reaction. Complementary DNA (cDNA) was generated using random hexamer primers (Promega, Madison, Wisconsin) and M-MLV reverse transcriptase (Promega). A 3-μl aliquot of the resulting cDNA was subjected to semi-quantitative PCR (94°C, 15 sec; 59°C, 30 sec; 72°C, 45 sec) for 30 cycles using chemokine and chemokine receptor–specific primers on an MJ Research Thermocycler (MJ Research, Waltham, Massachusetts). The primers were synthesized by Integrated DNA Technologies (Corvaliville, Iowa): reduced form of guanosine adenine dinucleotide phosphate (GAPDH) (212 bp); sense CAG GGT GCT TTC TGG GAC TT; antisense CTT GCT CAG TGG CCT TGC TG; CCR7 (453 bp); sense GAC GGA TAC CTA CCT GCT CAA C; antisense TAG CAG AAA CTA ATG ACC AGCA; CXCL1 (302 bp, KC); sense AAC CGA AGT CAT AGC CAC ACT; antisense GAA GAA CAA GAA GAA CTG AAC TACC; CXCL9 (350 bp, Mig); sense CCT GGA GCA GTG TGG AGT TC; antisense TGG TCT CTT ATG TAG TCT TTC AG; CCL2 (288 bp, SDF-α); sense AAA CTG TGC CCT TCA GAT GTG TT; antisense ATA TGA TGT GGC GGA GTG TCT; CCL3 (345 bp, MCP-1); sense CAC TCA CCT GCT GCT ACT CAT T; antisense TCA CAC TGG TCA CTC CTA CAG AA; CCL7 (283 bp, MCP-3); sense GCC AGT CTC CTC ACT CTC TTT; antisense TAT AGC CTC CTC GAC CCA CT; CCL11 (255 bp, eotaxin); sense TCC AAA ACC ATA AAC AAG CTC; antisense TCC TCA ATA ATC CCA CAT CTC; CCL22 (268 bp, MDC); sense CTA TGG TGC CAA TGT GGA AGA; antisense CAG AAG AAT AGG GCT TGC TGA.

PCR products were analyzed on a 2% agarose gel with ethidium bromide (Sigma) and also with the Alpha Innotech gel documentation system (Alpha Innotech Corp., San Leandro, California).

RESULTS

Splenomegaly in mice infected with Schistosoma mansoni

Seven weeks PE, the spleens from mice infected with S. mansoni and control mice were collected and used to determine the number of splenocytes. The cell counts revealed that there were 3 times more cells found in the spleens of infected mice compared with control mice (Fig. 1A). Typically, 1 x 10^8 and 3 x 10^8 cells were recovered from the spleens of control and infected mice, respectively. There was no difference in viability between the splenocytes of these mice. Differential cell counts revealed a large increase in macrophages in infected mice (Fig. 1B), which is consistent with a granulomatous response. In addition, at 7 wk PE, the livers were hypertrophied and granulomatous. These data indicate that at 7 wk PE, Balb/c mice with S. mansoni infections have splenomegaly and an ongoing granulomatous response.

T-cell cytokine production

To evaluate the ability of spleen T cells of mice infected with S. mansoni to produce cytokines, we isolated T cells from control and infected mice. For this purpose, an equal number of T cells were activated for 24 hr with immobilized anti-CD3, and supernatants were evaluated for IFN-γ production (Fig. 2). Although the S. mansoni–induced immune response is typically characterized by a type 2 cytokine response (Pearce and MacDonald, 2002), we found high levels of IFN-γ produced from control and infected mice. The data revealed that T cells from Balb/c mice infected with S. mansoni produced levels of IFN-γ comparable with the levels produced by T cells from control mice. Splenocytes from control and infected mice typically pro-
produced 2 ng/ml of IFN-γ. These data indicated that splenic T cells from Balb/c mice with 7-wk *S. mansoni* infection produce normal levels of IFN-γ.

**Chemotactic activity and receptor expression**

To evaluate the chemotactic ability of splenic T cells of mice infected with *S. mansoni*, we isolated T cells from control and infected mice. For this purpose, an equal number of T cells were assayed in a 1-hr chemotaxis assay to CCL21. CCL21 was chosen because the receptor for CCL21 (CCR7) was expressed at high levels in both T-cell populations. The data revealed that splenic T cells from mice with 7-wk *S. mansoni* infection were not as efficient at migrating toward CCL21 as splenic T cells from control mice (Fig. 3). T cells from control mice consistently showed the highest level of migration toward 1.0 μg/ml CCL21, whereas the T cells from infected mice showed little to no migration in response to 0.01, 0.1, or 1.0 μg/ml of CCL21. Because no antibody was available, we used semiquantitative reverse transcriptase (RT)-PCR to determine whether the decreased responsiveness was due to a decrease in the CCL21 receptor expression. The data revealed that CCR7 was expressed at similar levels in T cells from control and infected mice (Fig. 4). These data indicate that Balb/c mice infected with *S. mansoni* possess splenic T cells with altered chemotactic activity, and that a decrease in gene expression of the CCL21 receptor was not likely the cause.

**Chemokine expression in the liver and spleen**

Because elevated chemokine production can impair chemokine receptor function (Morris and Malbon, 1999), we assayed the livers and spleens of infected and control mice in an attempt to find out whether chemokine production was present. The data revealed that chemokine expression was greater in livers of infected mice than in those of control mice (Fig. 5). The infected mice showed greater levels of CXCL1, CXCL9, CXCL12, CCL7, and CCL22, whereas CCL2 and CCL11 were undetectable in the control and infected livers (Fig. 5). Analysis of the spleens revealed similar levels of chemokines expressed in the infected and control mice (Fig. 5). These data indicated that livers of Balb/c mice infected with *S. mansoni* have elevated chemokine production.

**DISCUSSION**

Chemokines are small molecules that are essential for virtually every component of an immune response, such as maturation of leukocytes (Campbell et al., 1999), peripheral leukocyte trafficking (Wang et al., 1998), and initiation of an immune response (Saeki et al., 1999). Chemokine receptors belong to the family of G protein-coupled receptors (GPCR) (Rossi and Zlotnik, 2000). The GPCR are desensitized by exposure to high concentrations of ligand or prolonged stimulation (Morris and Malbon, 1999). Previously, we reported that the murine mammary carcinoma, 4T1, expresses a number of chemokines (Kurt et al., 2001). Moreover, mice bearing these tumors possessed splenic T cells that exhibited altered chemotactic activity, despite the fact that the tumors were located distal from the spleens. In the present study, we were interested in investigating whether another disease with elevated chemokine production would similarly reveal abnormal splenic chemotactic activity. For this purpose, we used Balb/c mice infected with *S. mansoni* for 7 wk.
A hallmark characteristic of an *S. mansoni* infection is the granulomatous response. A number of investigators have suggested that the granulomatous response is controlled by chemokine expression. In response to *S. mansoni* eggs, Chensue et al. (1996, 1999) reported that chemokine expression could determine whether the granulomatous reaction was characterized as Th1 or Th2 response in CBA/J mice. CCL2 was associated with a Th2 response, whereas CCL5 was associated with the Th1 response (Chensue et al., 1996, 1999). In addition, Park et al. (2001) demonstrated recently that the chemokine expression reflected the cytokine response in *Schistosoma*-infected mice. Collectively, these data indicate that the immune response could be modulated through the administration of a recombinant chemokine or by blocking a chemokine, which results in the generation of an unfavorable response. Additional evidence that supports a role for CCL2 in the granulomatous reaction was provided by Lu et al. (1998), who reported that CCL2 deficient 129Sv/J × C57Bl/6 mice develop fewer granulomas than control wild-type mice. Despite the prominent role of CCL2 in the granulomatous response, CCL3 has also been implicated in the granulomatous reaction. El-Alwany et al. (2000) reported that CCL3 was expressed early in the granulomatous response in C57Bl/6 mice and, thus, may be involved in the initiation of the granuloma.

In addition to the role of chemokines in controlling the granulomatous reaction, Lukacs et al. (1994) suggested that chronic chemokine production might lead to tissue damage and potential organ dysfunction. Additionally, reports have indicated that the aberrant expression of chemokines may have unfavorable effects on bacterial immunity. For example, CCL2 transgenic mice exhibit increased sensitivity to bacterial infections such as *Listeria monocytogenes* and *Mycobacterium tuberculosis* (Rutledge et al., 1995). The elevated expression of the chemokine results in either receptor desensitization or disruption of the chemotactic gradient. As a result, monocytes exhibited an impaired ability to respond to CCL2 produced at the site of infection and, therefore, could not assist in antigen clearance. CCL2 has also been reported to inhibit the generation of tumor-reactive T cells (Peng et al., 1997). Neutralization of tumor-derived CCL2 during in vitro sensitization of T cells resulted in a T-cell population with enhanced therapeutic efficacy. These data suggested that prolonged chemokine receptor signaling in T cells adversely affected the T-cell effector function. Additionally, CCL2 expression was reported to contribute to a more malignant phenotype of the mammary carcinoma line Ly-6hi DA3, and CCL5 has been reported to increase the tumorigenicity of human melanoma cells in nude mice (Morowietz et al., 1999; Neumark et al., 1999). Thus, there is increasing evidence that T cells can be influenced by elevated chemokine production.

We report that Balb/c mice with granuloma possess splenic T cells with altered chemotactic activity compared with T cells from control mice. The presence of T cells with altered chemotactic activity correlated with an increase in chemokine expression from the livers but not the spleens. Further investigations are necessary to find out whether chemokine expression in the liver is directly responsible for the altered chemotactic ability. In particular, it is necessary to determine whether CCL21 expression is responsible for the abnormal chemotactic activity or whether the lack of reactivity to CCL21 was due to heterologous receptor desensitization. Additionally, this alteration could adversely affect the ability of mice infected with *S. mansoni* to respond to antigen because the T cells would be incapable of proper chemotaxis. Finally, these data suggest that the altered chemotactic ability of splenic T cells may be indicative of elevated chemokine production during an ongoing inflammatory response.
ACKNOWLEDGMENT

We are grateful to Fred A. Lewis, Schistosomiasis Laboratory, Biomedical Research Institute, Rockville, Maryland, for supplying snails (Biomphalaria glabrata) infected with Schistosoma mansoni cercariae used in this work through NIH-NIAID contract 1-AI-55270.

LITERATURE CITED


