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Monocyte Chemotactic Protein Expression during Schistosome Egg Granuloma Formation

Sequence of Production, Localization, Contribution, and Regulation

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The present study explored the role of murine monocyte chemotactic protein (MCP) in the T cell-mediated hypersensitive granulomatous response to* Schistosoma mansoni *eggs. The study examined the time course of local production, contribution to cellular infiltration, and the role of T cells in endogenous regulation. Synchronized pulmonary granulomas were induced under conditions of primary and secondary states of immunity. Primer-directed polymerase chain reaction analysis showed increased MCP mRNA expression in granulomatous lungs, mainly in the secondary response. Levels of MCP were measured by enzyme-linked immunosorbent assay in cultures of intact granulomas. Spontaneous MCP production was modest in primary granuloma cultures, reaching a maximum of 5.7 ± 0.9 ng/ml by 16 days. In contrast, the secondary response showed augmented and accelerated production, achieving 13 ± 2.0 ng/ml by 2 days. Immunohistochemical staining revealed the strongest MCP expression within microvascular adventitial cells or pericytes as well as in scattered mononuclear cells associated with granulomas. Staining was not detected in normal lungs. Passive immunization with anti-MCP-1 antibodies caused a 40% reduction in the secondary granuloma area but did not significantly affect the primary response. With adoptive cell transfer and T cell subset depletion, it was shown that Thy-1$^+$ and CD5$^+$ cells augmented, whereas CD8$^+$ cells appeared to impair, MCP production. This provides direct evidence that MCP is involved in secondary Th2-mediated response to schistosome eggs and is subject to regulation by T cells. (Am J Pathol 1995, 146:130–138)

Monocyte chemotactic protein (MCP) is a member of a family of chemotactic polypeptides that are thought to be associated with immunity and inflammation.[1] In the mouse, MCP, originally called JE, is a 13- to 15-kd glycosylated protein that was identified as an early response gene expressed by stimulated fibroblasts.[2] Human and murine MCP-1 are 65% homologous and, as their name indicates, both are chemotactic for monocytes.[2–4] Not surprisingly, MCP has been predicted to be an important cytokine in delayed-type and chronic inflammatory reactions that require participation of mononuclear phagocytes, although there are few reports directly supporting this notion.

Granulomas are a form of chronic inflammation observed in a wide variety of diseases[5] in which the defining element is the mononuclear phagocyte. Using the murine model of schistosomiasis mansoni, we reported that the hypersensitive-type granulomatous response induced by tissue-deposited parasite eggs involves an orchestrated production of cytokines and acquisition of macrophage effector functions.[6,7] Those studies suggested that the recruitment stage of the inflammatory response was critical to the final establishment of the lesion. The present study

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was undertaken to determine whether MCP contributed to the granulomatous response to schistosome eggs. To this end, we asked four primary questions: 1), Is MCP produced at the sites of granuloma formation? 2), What are potential cellular sources of MCP? 3), What is the relative contribution of MCP to the primary and secondary granulomatous response to schistosome eggs? and 4), Do antigen-specific T cells regulate MCP production?

Indeed, the present study revealed significant spontaneous MCP production primarily during secondary egg granuloma formation that correlated with levels of MCP-specific mRNA transcripts. Furthermore, *in vivo* depletion of MCP caused significant abrogation of the secondary response. Immunohistochemical localization of MCP in granulomatous lungs indicated that microvascular-associated stromal cells were potentially important sources of MCP. Finally, T cell subset depletion studies indicated that MCP production in the secondary granulomatous response was regulated by T cells.

Materials and Methods

Animals and Infection

Female, CBA mice obtained from the Jackson Laboratories (Bar Harbor, ME) were maintained under specific pathogen-free conditions and given food and water *ad libitum*. Chronic infection was achieved by percutaneous tail infection by 25 to 30 cercaria of the Puerto Rican strain of *Schistosoma mansoni*.

Induction of Synchronous Pulmonary Granulomas and Adoptive Transfer

S. mansoni eggs were isolated aseptically from granulomatous livers of infected mice by the method of Coker and von Lichtenberg.[8] Synchronous pulmonary granulomas were induced by intravenous injection of 3000 eggs into naive mice or infected mice at the secondary acute stage of granuloma formation (8 weeks). The embolized eggs elicit individual granulomas that can be measured in histological sections or isolated for culture as described below.

In T cell subset depletion experiments, spleen and mesenteric lymph node cells (mixed 3:1) from 8-week-infected donor mice were pretreated with antibodies and complement, after which 5×10^6 viable cells were transferred intravenously to syngeneic recipients. At the time of cell transfer, mice were challenged with eggs as described above. These mice develop lesions similar to those of actively infected donors and show a similar time course of MCP production.

Isolation and Culture of Pulmonary Granulomas

Granulomas were isolated at 1, 2, 4, 8, and 16 days after embolization of eggs as previously described.[7] Briefly, mice were sacrificed and lungs were perfused, lavaged and inflated with cold RPMI culture media, excised, and then homogenized in a Waring blender. The intact granulomas were collected over a stainless steel mesh, washed, and suspended to 700 lesions per ml of RPMI supplemented with 10% fetal bovine serum, 100 mmol/L glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml). A total of 2 ml of suspension was incubated in 60-mm culture dishes in the presence or absence of 5 μg/ml schistosome egg antigen (provided by the World Health Organization, Geneva, Switzerland) at 37 C in a 5% CO_2, humidified atmosphere. Supernatants were collected from cultures at 24 and 48 hours and frozen at −80 C.

Antibodies and Depletion Protocols

Rabbit anti-murine MCP was prepared by multiple site immunization of New Zealand White rabbits with a recombinant murine MCP-1/JE (provided by Dr. B. Rollins, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA) incorporated into complete Freund's adjuvant and boosted biweekly with MCP-1 in incomplete adjuvant. Antibody specificity and titer was determined by direct enzyme-linked immunosorbent assay (ELISA) with a battery of target recombinant rat and murine cytokines including mIL-1β, mIL-1ra, mTNFα, mMIP-1α, mMIP-1β, mMIP-2, rat-GROα, rat-MCP-1, mIL-2, mIL-4, mIL-5, mIFN-γ, mIL-6, and mIL-10. The anti-mMCP-1 titer was determined to be $1{:}10^5$ with no significant cross-reactivity with the above tested proteins. This preparation was used for sandwich ELISA. A second preparation of rabbit anti-rat MCP-1 was similarly prepared with recombinant rat-MCP-1 (kindly donated by Dr. R. Newton, Dupont, Bloomington, DL) and was found to be cross-reactive with murine MCP-1/JE and, other than rat-MCP-1, was unreactive with the above described cytokine battery. The anti-mMCP-1/JE titer of this preparation was $1{:}10^6$ and was used for *in vivo* depletion studies and immunohistochemical staining. In addition, a third independently prepared nonneutralizing rabbit anti-murine MCP-1/JE, kindly provided by Dr. B. Rollins, was used to confirm immunohistochemical staining

patterns. Staining specificity was demonstrated by solid phase immunoabsorption with recombinant MCP, which virtually eliminated staining capacity (Table 1).

For *in vivo* anti-MCP treatment, naive or adoptively sensitized mice were given a single intraperitoneal injection of 5 mg of protein A purified anti-MCP or control rabbit IgG, 1 to 2 hours before intravenous egg challenge. Four days later, lungs were removed and prepared for histological evaluation.

In some experiments, adoptively transferred cells were pretreated to deplete all T cells, Lyt-1$^+$ (CD5$^+$) and Lyt-2$^+$ (CD8$^+$) by using, respectively, monoclonal anti-Thy-1.2, anti-Lyt-1.1, and anti-Lyt-2.1 diluted 1:500 (clones 5a-8, 7–20.6/3, and 49–31.1, respectively; Accurate Chemicals, Westbury, NY) followed by rabbit complement diluted 1:20 (Accurate Chemicals). These antibodies were pretested for specificity and were shown to kill the appropriate target populations by flow cytometric analysis with independent fluorochrome-labeled monoclonal antibodies.

Immunohistochemistry

Paraffin-embedded tissue sections mounted on poly-l-lysine coated slides were first deparaffinized with xylene followed by stepwise hydration in 100, 95, 70, and 50% ethanol. Final hydration was performed by a 10-minute incubation in phosphate-buffered saline (PBS). All sections were blocked with avidin, biotin, and 2% normal goat serum. The sections were covered with 1:500 dilutions of anti-MCP antiserum or control nonimmune rabbit serum and incubated for 20 minutes at 37 C. After rinsing, the sections were incubated for 10 minutes in PBS at room temperature, then overlaid with biotinylated goat anti-rabbit IgG (supersensitive reagent 1:10, Biogenex, San Ramon, CA). After a 20-minute incubation at 37 C, the slides were rinsed and incubated for 10 minutes in PBS, then overlaid with streptavidin alkaline phosphatase (1:20, Biogenex) and incubated for 20 minutes at 37 C. The slides were rinsed again in PBS, then overlaid with alkaline phosphatase substrate solution (Vector Laboratories, Burlingame, CA) for 30 minutes at room temperature. Finally, the sections were rinsed and counterstained with Mayer's hematoxylin.

Table 1. *Demonstration of Staining Specificity of Rabbit Anti-Rat MCP-1 Antiserum by Immunoabsorption**

	Percent of maximum staining at:			
Absorbent	1:500	1:1000	1:2000	1:4000
BSA column	92%	46%	45%	15%
MCP column	2%	0%	0%	0%

*Samples of antiserum were preabsorbed on 1-ml Sepharose 4B columns bearing either covalently bound bovine serum albumin (BSA; 10 mg) only or bovine serum albumin (10 mg) plus 10 μg of recombinant rat MCP-1. The antisera were then tested for their capacity to stain BC3H1 smooth muscle-like cells stimulated for 24 hours with 5 ng/ml IL-1β. Staining intensity was determined spectrophotometrically with a microwell system and ELISA reader. Staining intensity was normalized to the maximum staining achieved with a 1:500 dilution of unabsorbed anti-MCP serum. Control rabbit serum showed no staining.

Cytokine Measurement

Levels of MCP in culture fluids were determined by an ELISA polyclonal sandwich method. Briefly, anti-MCP IgG preparations were split and a portion was biotinylated. Next, 96-well polystyrene culture dishes were coated overnight at 4 C with 10 μg/ml of nonbiotinylated IgG then washed with PBS-0.05% Tween. After a blocking step with 0.5% bovine serum albumin, standards and serial dilutions of test supernatant in 0.1-ml volumes were added to the wells and incubated at 37 C for 1 hour. After washing, biotinylated anti-MCP IgG (10 μg/ml) was added for 1 hour at 37 C. After additional washing, peroxidase-labeled avidin (1 μg/ml) was added to each well for 45 minutes followed by washing and substrate (1,2-phenylenediamine) addition. The reaction was stopped by acid treatment and development was measured with a microELISA reader. Sensitivity was to 100 pg/ml.

RNA Isolation

Lungs were excised and snap frozen in liquid N_2. Total cellular RNA was then extracted by a modified method of Chirgwin et al[9] and Jonas et al.[10] The frozen lungs were suspended in extraction buffer (25 mmol/L Tris, pH 8.0, 4.21 mol/L guanidine isothiocyanate, 0.5% Sarkosyl, and 0.1 mol/L 2-mercaptoethanol), homogenized, then added to an equal volume of 100 mmol/L Tris, pH 8.0, containing 10 mmol/L EDTA and 1% sodium dodecyl sulfate. This mixture was extracted with chloroform-phenol and chloroform-isoamyl alcohol. The RNA was precipitated in ethanol, dissolved in buffer (10 mmol/L Tris, pH 8.0, 0.1 mol/L EDTA, and 0.1% Sarkosyl) and stored at −20 C before use.

mRNA Analysis by Polymerase Chain Reaction Amplification

Samples of RNA (5 μg), each pooled from three mice per group, were subjected to reverse transcription in

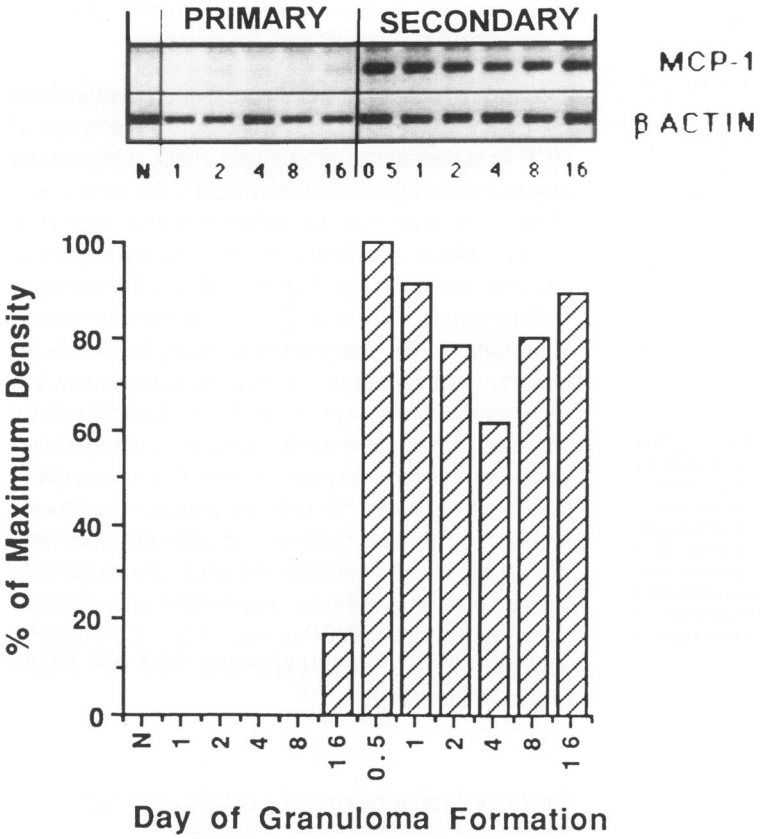

Figure 1. *Expression of MCP-1/JE mRNA transcripts during synchronous pulmonary granuloma formation induced by schistosome eggs under conditions of primary and secondary vigorous immunity. Total RNA was extracted from granulomatous or normal (N) lungs (three per group) and subjected to reverse transcription and primer-directed PCR amplification. Shown is a representative ethidium bromide-stained gel after 26 cycles of amplification. β-Actin mRNA expression is shown as positive control.*

Tris-buffered DEPC water, pH 8.3, containing 0.5 mg/ml oligo(dT)12–18 primers, 0.1 mol/L dithiothreitol, 40 U/ml RNAsin, 100 mmol/L (each) dNTPs, and 300 U/ml reverse transcriptase.[11] After a 1-hour incubation at 38 C the temperature was increased to 90 C for 10 minutes. The resulting cDNA was then stored at −20 C before PCR. In the next stage, 2 μl of cDNA solution was subjected to primer-directed PCR amplification[12] in Tris-buffered DEPC, pH 8.3, containing 5 U/ml Taq polymerase, 100 mmol/L (each) dNTPs, 300 ng of IRAP or β-actin (housekeeping gene) sense and antisense oligonucleotide primers (prepared by University of Michigan DNA Core Research Facility), having the following sequences; MCP-1 sense, 5′ CTC ACC TGC TGC TAC TCA TTC 3′; MCP-1 antisense, 5′ GCA TGA GGT GGT TGT GAA AAA 3′; actin sense primer, 5′ GTG GGG CGC CCC AGG CAC CA-3′; and actin antisense primer, 5′ GCT CGG CCG TGG TGG TGA AGC 3′. The mixture was overlaid with mineral oil, placed into a thermal cycler, and preheated to 90 C for 5 minutes followed by 26 cycles of the following temperatures: 1), 30 seconds at 95 C; 2), 1 minute at 58 C; and 3), 2 minutes at 72 C. The DNA was then collected from under the mineral oil and stored at −20 C before standard gel electrophoresis and visualization under UV light in ethidium bromide-stained gels. The sizes of the actin and MCP PCR products were 550 and 350 bp, respectively. Quantitation was performed by densitometric scanning of gel photos.

Granuloma Measurement

In some experiments, granulomatous lungs were inflated with buffered formalin, assigned a code number, then submitted for tissue processing and paraffin embedding. Granuloma area was measured blindly in stained histological sections by a computerized morphometer (The Morphometer; Woods Hole Educational Associates, Woodshole, MA). Only lesions cut in the plane of the central egg nidus were measured and a minimum of 20 lesions were measured per lung.

Statistics

The Student's *t*-test was used to compare control and experimental groups. Values of $P > 0.05$ were considered to indicate lack of significance.

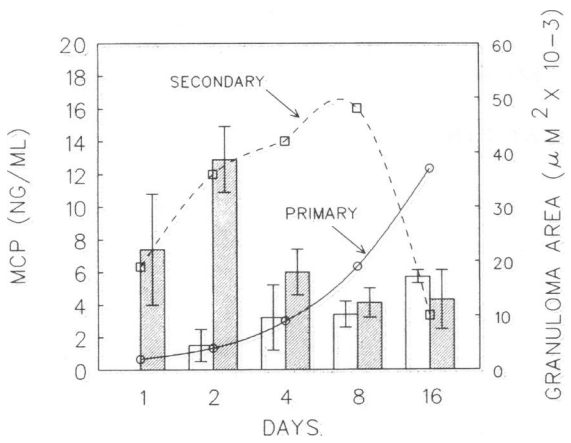

Figure 2. *Production of MCP by cultured primary and secondary schistosome egg pulmonary granulomas as related to granuloma growth. Granulomas (700/ml) were isolated intact and cultured. Bars are means ± SE derived from three separate experiments with a minimum of three mice per group in each experiment. Solid bars, primary granuloma MCP; hatched bars, secondary granuloma MCP. Granulomas from control lungs injected with carbohydrate beads produced less than 2 ng/ml. Lines indicate corresponding granuloma growth curves derived from measurements of 5 to 6 mice per point. A minimum of 20 lesions were measured per mouse. SE were no greater than 5% of mean.*

Results

Detection of MCP mRNA in Granulomatous Lungs

We initially screened lungs bearing synchronously developing granulomas for the presence of MCP mRNA by using the method of primer-directed PCR amplification. Granulomas were induced in naive and 8-week *S. mansoni*-infected mice to examine the primary and secondary granulomatous response, respectively. The primary response is initiated by interferon-producing cells whereas the accelerated secondary response involves Th2-derived cytokines, IL-4 and IL-5.[7,13,14] Figure 1 shows the results of the RNA analysis of these groups and control mice. After 26 cycles of amplification, MCP-specific mRNA was weakly detectable in lungs with primary granulomas at 16 days, but a strong signal was present in the lungs from mice at the acute stage of infection appearing as early as 12 hours and persisting during the study period. Normal lungs had no significant activity. However, by increasing amplification cycles to 30 or greater, stronger signals were detected in the primary lesions and to a lesser extent in normal lungs (data not shown). Overall, these findings suggested that MCP was expressed mainly during the secondary response to *S. mansoni* eggs, but they provide no information regarding quantities or sources of MCP production.

Production of MCP during Primary and Secondary Granuloma Formation

To determine whether MCP was indeed synthesized by inflammatory tissues, we measured the levels of MCP in supernatants of cultured intact schistosome egg pulmonary granulomas by ELISA. As above, synchronously developing granulomas were induced under conditions of primary and secondary immunity, isolated, and cultured. Figure 2 shows the levels of spontaneously released MCP in the 24-hour culture supernatants of primary and secondary lesions as related to granuloma size. As reported previously, the granulomas of primary mice reached maximal size by 16 days, whereas in 8-week-infected mice there is an accelerated secondary response that achieves maximal size by 8 days.[15] In primary granuloma cultures, levels of MCP were modest and seemed to parallel granuloma size. In contrast, the secondary response was characterized by an augmented and accelerated appearance of MCP on days 1 and 2 correlating with the greater mRNA expression and the growth phase of the lesion.

Immunohistochemical Localization of MCP in Granulomatous Lungs

The above studies suggested that secondary granulomas were producing MCP in significant amounts but provided no information regarding the cell(s) of origin. To approach this question, we performed immunohistochemical staining for MCP in sections of lungs with synchronously developing lesions.

As shown in Figure 3, two major distributions of staining were observed. Lungs with secondary granulomas showed strong perivascular staining (Figure 3C), primarily involving terminal arterioles near sites of egg deposition and associated with cellular infiltrates. This staining persisted over the study period and the positive cells within vessels appeared to be pericytes or adventitial stromal cells. Endothelial cells were generally negative. The other staining distribution was observed within the granuloma (Figure 3A, E). Scattered, largely mononuclear cells within the lesions (especially on day 2 in secondary granulomas) were positive but their precise identity was unclear. It was also noted that staining was sometimes observed within bronchoepithelium in areas of inflammation. Interestingly, by day 16 primary granulomas contained positively staining cells but the vascular-associated pattern was not present (Figure 3F). The addition of vascular-associated MCP in the secondary response would explain the stronger MCP mRNA

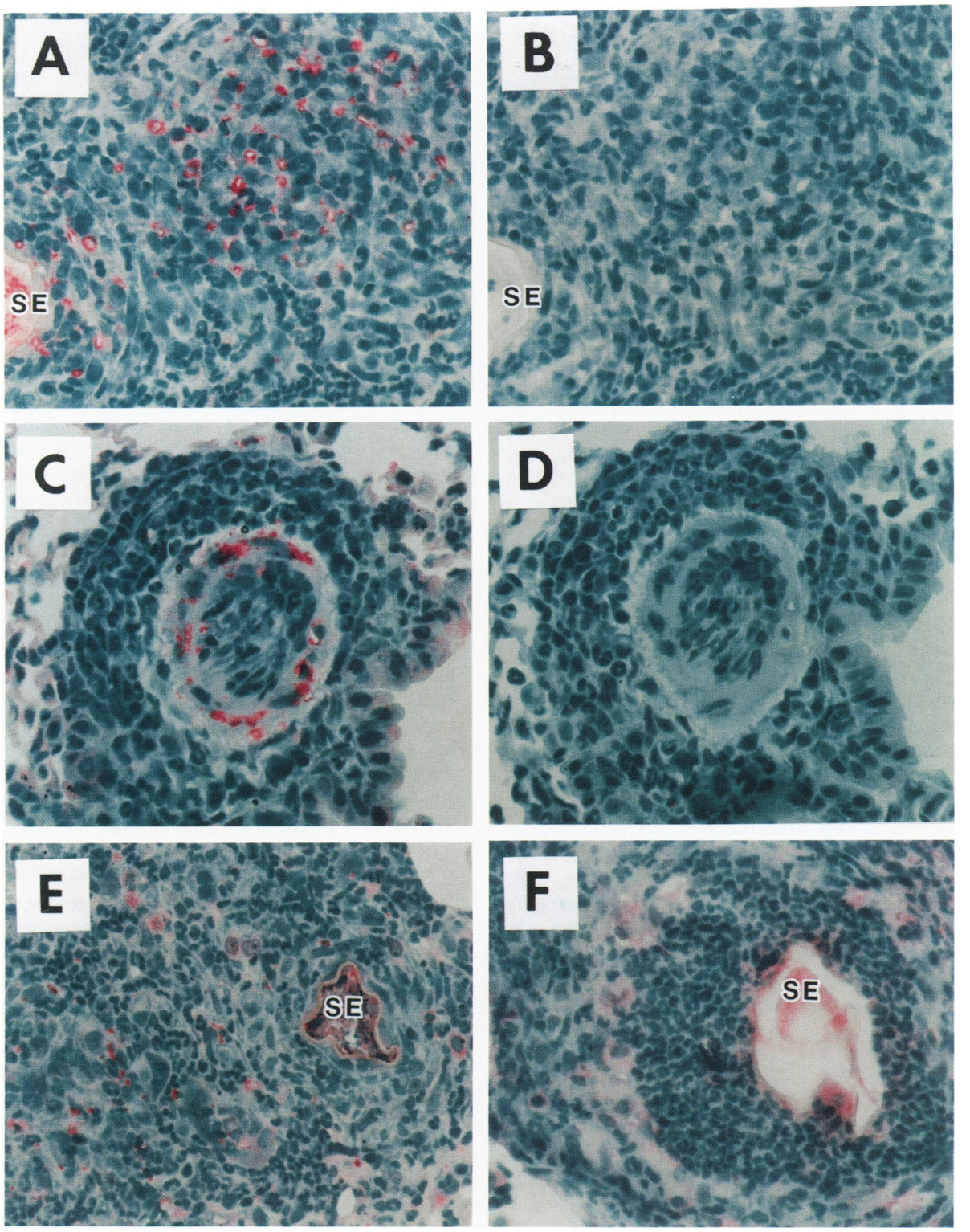

Figure 3. *Immunohistochemical detection of MCP in granulomatous lungs.* **A**: *MCP stain of day 4 secondary schistosome egg (SE) granuloma. Note staining predominates in large mononuclears cells.* **B**: *Serial section of* **A** *stained with control serum.* **C**: *MCP stain of vessel with perivascular infiltrate. Note intense staining of vessel wall.* **D**: *Serial section of* **C** *stained with control serum.* **E**: *MCP stain of day 2 secondary schistosome egg granuloma. Note stain is predominant in large mononuclears cells.* **F**: *MCP stain of day 16 primary schistosome egg granuloma. Note that granulocytes, mostly eosinophils, are weakly positive to negative. Counterstained with Mayer's hematoxylin. Magnification, ×400.*

expression observed during the study period. Unchallenged control lungs showed no staining.

Effect of In Vivo MCP-1 Depletion on Primary and Secondary Granuloma Formation

To examine the relative contribution of MCP to the granulomatous response, naive and adoptively sensitized mice were passively immunized with anti-MCP antibodies before granuloma induction. As shown in Figure 4, the primary lesions were not affected by MCP depletion but the secondary response was abrogated by nearly 40%. This finding provided direct evidence that MCP contributed to the cellularity of the secondary egg granuloma and was less important in the early stages of the primary lesion.

Endogenous Regulation of MCP Production

The findings suggested that levels of MCP were related to the degree of immunity and presumably T helper cell activity. Currently, it is not clear whether T cells regulate MCP expression *in vivo*. To test this possibility, we used a model in which granulomas were induced in groups of mice that were adoptively sensitized with lymphoid cells depleted of T cell subsets.

As shown in Table 2, depletion of Thy-1$^+$ (pan-T cell marker) and Lyt-1$^+$ (CD5$^+$, predominantly expressed by T helper cells in the mouse[16]) cells reduced levels of local MCP production by up to 70 to 80%. This was associated with a reduction in granuloma size; thus, the lower amounts of MCP could simply reflect the decreased lesion cellularity. However, depletion of Lyt-2$^+$ (CD8$^+$, T suppressor/cytotoxic) cells doubled spontaneous MCP production and egg antigen-elicited MCP production by nearly six-fold, outstripping the associated increase in granuloma size. Taken together, these findings suggest that MCP production was regulated directly or indirectly by T cell subsets.

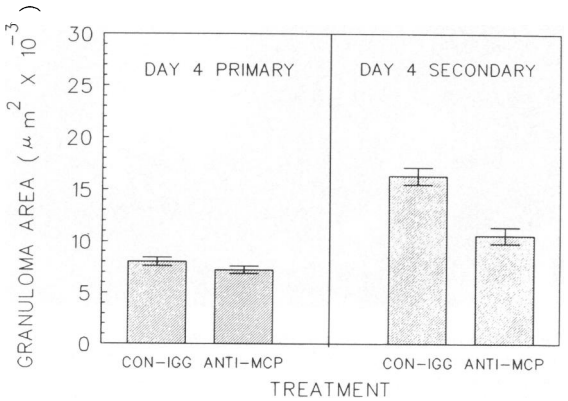

Figure 4. *Effect of MCP-1* in vivo *depletion on primary and secondary schistosome egg granuloma formation. Naive or sensitized mice were given 5 mg of control or anti-rat MCP-1 IgG before egg challenge. Bars are means ± SE derived from six to eight mice. A minimum of 20 lesions were measured per mouse.*

Table 2. *Effect of T Cell Subset Depletion on Granuloma Formation and Local MCP-1 Production*

Treatment	Granuloma area ($\mu m^2 \times 10^{-3}$)	MCP (ng/ml)	
		No Ag	Plus Ag
Control Ig	24 ± 2.0*	4.3 ± 2.6	3.5 ± 1.4
Anti-Thy 1	11 ± 0.5	1.3 ± 1.0	0.6 ± 0.5
Anti-CD5	16 ± 3.5	1.0 ± 0.2	1.5 ± 0.7
Anti-CD8	36 ± 2.0	8.2 ± 0.2	24 ± 9.0

Ag, antigen.
*Values are means ± SD derived from two separate experiments.

Discussion

Recently, a group of novel polypeptides with chemotactic activity has been described and is currently under intense investigation. These proteins show a large degree of homology between mice and humans, are generally less than 15 kd and some are highly positively charged molecules. They have been classified into C-C and C-X-C families on the basis whether or not the first cysteine residues are separated by an amino acid.[1] MCP-1 is a member of the C-C family and was shown to be chemotactic for mononuclear cells *in vivo* and *in vitro* and is suspected to participate in inflammation.[1–4] The present study was undertaken to determine the potential participation of MCP in a model of granuloma formation induced by eggs of the parasite *Schistosoma mansoni* and to provide information regarding the kinetics, cellular sources, contribution to inflammation, and regulation of MCP production.

The schistosome egg granuloma represents a well characterized T helper cell-mediated hypersensitivity response involving local production of IL-4 during the vigorous stage of infection.[7,17] Using this model, we were able to examine egg granuloma formation under conditions of primary and secondary immunity. Compared with the primary response, the secondary vigorous response was associated with accelerated and augmented MCP production. Our time course studies demonstrated enhanced MCP production during the growth phase of the secondary lesions indicating an association with cell recruitment. Our *in vivo* depletion

studies provided direct evidence that MCP contributes to the secondary inflammatory response. In contrast, we recently reported that a related chemokine, macrophage inflammatory protein-1α (MIP-1α), is also produced by the schistosome egg granuloma and contributes principally to the primary response.[18] Furthermore, the differential expression of these chemoattractants reflects the observed shift from a Th1-like cytokine profile in the primary response to a dominant Th2 profile in the secondary response.[13] Thus, different chemoattractants may be mobilized depending the nature and degree of T cell involvement.

Indeed, our study revealed that MCP production was regulated by T cells. Specifically, T helper cells were required for augmented MCP production whereas $CD8^+$ T cells suppressed maximal MCP synthesis. Although this suppression may represent a direct effect on MCP-producing cells, it more likely reflects indirect regulation through effects on T helper cells. Specifically, $CD8^+$ T cells appear to regulate the activity or generation of Th2 cells in the secondary response to schistosome eggs.[13,17] It is noteworthy that we previously reported that IL-4, a product of Th2 cells, is maximally produced on days 1 and 2 in the secondary vigorous response, corresponding to the time of maximal MCP production.[7] Moreover, IL-4 depletion profoundly abrogated secondary granuloma formation.[7] Rollins and Pober[19] reported that IL-4 is a potent inducer of MCP-1 production by endothelial cells *in vitro*. Studies in our laboratory have shown that IL-4 also induces MCP-1 production in the mouse smooth muscle cell line BC3H1 (manuscript in preparation). Thus, we may be observing an *in vivo* counterpart of this cytokine cascade. Inasmuch as IL-4 also induces vascular adhesion molecule-1 (VCAM-1) on endothelial cells,[20–22] it would initiate coordinated events leading to mononuclear cell recruitment. Interestingly, the primary granulomatous response shows a delayed onset of IL-4 expression[13] that corresponds to our observed delayed expression of MCP, lending further circumstancial evidence for a relationship between IL-4 and MCP. Thus, MCP-1, a potent chemotactin and histamine-releasing factor,[23] may have an important role in hypersensitive inflammatory responses mediated by Th2 cells.

Interesting findings were revealed by our immunohistochemical studies. These studies localized MCP largely to vascular-associated cells in terminal pulmonary arterioles in which eggs were embolized. The positively staining cells appeared to be pericytes (a smooth muscle-like cell) or adventitial fibroblasts. Positively staining vessels were consistently associated with infiltrating inflammatory cells. Teleologically, such cells would be ideal sources of chemokines as they are positioned beneath the endothelium and could establish a chemotactic gradient within the interstitium to attract marginated leukocytes. Our observations are consistent with *in vitro* studies demonstrating MCP production by stromal cells, including fibroblasts and smooth muscle cells[2,22] MCP-positive cells were also noted within granulomas, but their precise identity was unclear, although these were largely mononuclear cells. We recently reported that granuloma fibroblasts are a potential source of MCP-1,[24] but our results suggest other cells are also involved. It is likely that a variety of cells contribute to sustaining cell recruitment once lesions are established. One can speculate that vascular-associated cells may provide one source of MCP and then recruited cells provide additional chemoattractants for accelerating lesion growth and cellular activation. Studies to identify the specific MCP-1-producing cells are currently underway.

The present study provides evidence supporting a role for MCP-1 in the hypersensitivity granulomatous response to *Schistosoma mansoni* eggs. Future studies will need to focus on the contribution of MCP in responses to other pathogens as it represents one of several potential chemotactic agents that may contribute to chronic inflammatory responses.

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