REGULATION OF GRANULOMATOUS INFLAMMATION IN MURINE SCHISTOSOMIASIS

In Vitro Characterization of T Lymphocyte Subsets Involved in the Production and Suppression of Migration Inhibition Factor*

BY STEPHEN W. CHENSUE, DOV L. BOROS, AND CHELLA S. DAVID

From the Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201; and the Department of Immunology, Mayo Clinic and Medical School, Rochester, Minnesota 55901

In hepatosplenic schistosomiasis mansoni, the pathological effects can be largely attributed to the T lymphocyte-mediated granulomatous host response (1, 2). The chronic granulomatous inflammations terminate in reparative fibrosis, which can lead to portal hypertension, esophageal varices, bleeding, and death (3). Andrade and Warren (4, 5) first showed, that mice with chronic schistosomiasis at the late stage of the infection displayed a spontaneous diminution in granuloma size, with a corresponding improvement in the clinico-pathological parameters of the disease. Periodic injection of eggs has shown that whereas at 8 wk of the infection mice mounted large pulmonary granulomatous reactions, beyond 16 wk granuloma formation decreased significantly, thus indicating a desensitization process (6). Further analysis showed that this process entailed complex changes in the humoral and cell-mediated egg-specific immune responses as well as the lymphocyte dynamics of the infected mice. Thus, passive hemagglutination and passive cutaneous anaphylaxis tests showed an increase in the level of circulating anti-soluble egg antigen (SEA) antibodies. In contrast, delayed footpad reaction of infected mice was abrogated, and no migration inhibition factor (MIF)- or eosinophil stimulation promoter (ESP)-active lymphokines were produced by cultured lymphoid cells (7, 8). A change in the ratios of T:B lymphocytes was also demonstrable in the blood, lymphoid organs, and granulomas of the chronically infected mice (9). So far both humoral- and cell-mediated regulatory mechanism have been considered to act in the modulation of the granulomatous response. Antibody-mediated afferent immunologic blockade of granuloma formation has been described by Pelley and Warren (10), and the presence and active synthesis of immunoglobulins and anti-SEA antibodies within liver granulomas has been reported (11, 12). On the cellular side, adoptive transfer of lymphoid cells from mice

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1 Abbreviations and nomenclature used in this paper: ESP, eosinophil stimulation promoter; MIF, migration inhibition factor; MLC, mixed lymphocyte cultures; NHS, normal human serum; NMS, normal mouse serum; NPEC, normal peritoneal exudate cells; NSP, normal spleen cells; PBS, phosphate-buffered saline; PPD, purified protein derivative; RAMab, rabbit anti-mouse IgG antibodies; SEA, soluble egg antigen(s); SPEC, peritoneal exudate cells of mice sensitized with complete Freund's adjuvant and SEA; T~, T suppressor; 8-wk SP, spleen cells of 8-wk infected mice; 20-wk SP, spleen cells of 20-wk infected mice.
undergoing modulation successfully suppressed the granulomatous response in recipient mice (13). Characterization of the suppressor lymphocytes suggested that they are cyclophosphamide-sensitive T cells (14). Further adoptive transfer studies have shown that Ia antigen-bearing T lymphocytes are required for the suppression of the egg-specific inflammatory response. Because a significant increase occurred in the numbers of Thy-1.2+, Ia+ lymphocytes of the suppressed granuloma after adoptive transfer of T suppressor cells, the conclusion was reached that these cells may be involved in the regulation of the granulomatous response (15). Isolated, cultured liver granulomas of infected mice were shown to produce MIF and ESP-active lymphokines (16, 17). Thus, such mediators are considered to be responsible for the initiation and maintenance of the granulomatous response. To further explore the role of suppressor T lymphocytes in the regulation of the granulomatous response, we characterized the splenic lymphocytes of infected mice active in lymphokine production and suppression. The MIF-producing T lymphocytes found at 8 wk in the spleens of infected mice belonged to the Lyt-1+ subclass of cells, and did not express Ia antigens. In contrast, the suppressor cells detectable at 20 wk of the infection were Lyt-2+ and also expressed I-J and I-C subregion-encoded determinants. Because the latter cells mixed with Ly-1+ cells in vitro abrogated MIF production, it is postulated that the modulation of granulomatous hypersensitivity may be the result of T-T cell interactions that regulates lymphokine production.

Materials and Methods

**Animals and Infection.** Female, CBA/J(H-2k) mice obtained from The Jackson Laboratory, Bar Harbor, Maine were used in all experiments. Mice were maintained under standard laboratory care. 7- to 8-wk-old mice were injected subcutaneously with 25 cercariae of the Puerto Rican strain of *Schistosoma mansoni.*

**Preparation of SEA.** SEA were prepared from eggs isolated from the livers of infected mice (18) as described previously (2).

**Preparation of Cells.** Splenic T cells were isolated by the method of Mage et al. (19). Briefly, specific rabbit anti-mouse IgG antibodies (RAMab) directed against light and heavy chains were prepared from 25 ml of rabbit anti-mouse IgG serum (Miles Laboratories Inc., Elkhart, Ind.) with standard immunoaffinity techniques. 5 ml of a 25 μg/ml solution of RAMab in phosphate-buffered saline (PBS) (pH 7.4) was distributed onto 100-mm tissue culture dishes (BioQuest, Becton, Dickinson & Co., Oxnard, Calif.) and incubated at 4°C overnight to allow for passive binding of antibodies to the plastic. After rinsing the dishes five times with 5 ml of PBS, 5 × 10⁷ spleen cells suspended in 5 ml of RPMI-1640 medium that contained 20 mM Hepes buffer (Grand Island Biological Co., Grand Island, N. Y.) were added to each dish. The dishes were then incubated for a total of 1 h at room temperature with a single agitation at 30 min. After B cell adherence was complete, the nonadherent T cell-enriched fraction was collected by gentle rinsing of the dishes with RPMI-Hepes. This preparation contained >80% Thy-1-bearing cells with a total recovery of >90%.

**Antisera and Complement Treatments.** Anti-Thy-1.2 serum was obtained from Bionetics Laboratory Products, Litton Bionetics Inc., Kensington, Md. This preparation killed >95% of normal CBA thymocytes and <3% of normal bone marrow cells. Anti-Lyt-1.1 and anti-Lyt-2.1 sera were the generous gifts of Dr. F.-W. Shen (Sloan-Kettering Cancer Institute, New York). Anti-I(AB)k [(A × B10) anti-A.TL], anti-I(E)k [(B10.D2 × A.BY) anti-B10.A(3R)], anti-I(A)k [ATFR.5 × B10.S(9R) anti-A.TL], anti-I(J)k [B10.HTT anti-B10.S(9R)], anti-I(C)k [(A × B10 anti-B10.AM), and anti-I(EC)k [(B10.S(7R) anti-B10.HTT] sera were prepared at the Mayo Medical School, Rochester, Minn.

Rabbit serum absorbed with mouse tissues (Cedarlane Laboratories, London, Ontario) served as a source of complement. The antisera and complement were used for cell lysis as follows: Spleen cells (1 × 10⁷/ml) were incubated for 30 min at 4°C in antiserum diluted in
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RPMI (anti-Lyt-1.1, 1:100; anti-Lyt-2.1, 1:50; all others, 1:10). Control sera consisted of mock anti-Thy-1.2 (Bionetics Laboratory Products, Litton Bionetics Inc.) serum or normal (BALB/c x C57BL/6)F1 serum. After incubation, cells were washed by centrifugation at 4°C, suspended to their original volume in a 1:10 dilution of complement, and then incubated for 30 min at 37°C. After incubation, cells were washed from two to three times. Viability was determined by trypan blue staining, and cell concentrations were adjusted according to the number of viable cells.

Preparation of Supernates That Contained MIF. Spleen cells were suspended to a concentration of 1 x 10^7 cell/ml in RPMI-1640 medium that contained 10% heat-inactivated normal human serum (NHS) (Grand Island Biological Co.) (adsorbed with 1:5 vol of a mixture of mouse spleen and peripheral blood cells for 30 min at room temperature), 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin (Grand Island Biological Co.), and 1 µg/ml of SEA (RPMI-NHS). 2 ml of suspension were distributed into 16- x 125-mm tissue culture tubes (BioQuest, Becton, Dickinson & Co.) and incubated at 37°C for 24 h in a humidified 5% CO2 atmosphere. After incubation, the tubes were centrifuged at 300 g for 10 min, and the supernates were collected from above the cell pellet and then stored at -70°C. Cultures were usually prepared in triplicate or quadruplicate. The MIF activity of the supernates was detected with the standard capillary migration inhibition assay described below.

Assays for MIF Activity. Normal peritoneal exudate cells (NPEC) were induced in virgin CBA mice by the injection of 2.0 ml i.p. of light mineral oil (Marcol 52, Exxon Chemical Co., Houston, Tex.). 4-5 d later, the NPEC were harvested aseptically with siliconized glassware. The cells were washed four times in RPMI, counted, and then adjusted to 4 x 10^7 cell/ml in RPMI-NHS without SEA. Assay of supernates was performed as described by David (20). The NPEC were placed into glass clot tubes (70- x 1-mm) (Arthur H. Thomas Co., Philadelphia, Pa.) with a seal at one end. The tubes were then centrifuged for 3 min at 200 g to pellet the cells. With an aseptic technique, the tubes were cut at the cell-medium interface and placed into Sykes-Moore chambers (Bellco Glass, Inc., Vineland, N. J.). The chambers were filled with control or test supernates diluted 1:1 with fresh medium and then incubated 18-24 h at 37°C. Assays were performed in duplicate with three capillary tubes per chamber. After incubation, the cell migration patterns were projected and traced with an overhead projecting microscope (Bausch & Lomb Inc., Analytical Systems Div., Rochester, N. Y.). The area of migration was measured from the tracings by planimetry (Los Angeles Scientific Instrument Co., Inc., Los Angeles, Calif.).

Direct assay of MIF-producing cells was performed as described by David et al. (21). 1 million NPEC, supplemented with 25-100 x 10^5 spleen cells, were placed into clot tubes and subjected to the migration inhibition assay in the presence of RPMI-NHS as described above. Controls were exposed to RPMI-NHS without antigen or consisted of unsupplemented NPEC exposed to antigen.

Assay for Suppression of MIF Activity. In this technique, peritoneal exudate cells were obtained from sensitized mice (SPEC) as follows: normal 7- to 8-wk-old CBA mice were each injected in the nape of the neck with 0.1 ml s.c. of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) that contained 30 µg of SEA. 5 d later the mice were given an injection of 2.0 ml i.p. of light mineral oil. 4 d after oil injection (day 9 of sensitization), the SPEC were harvested and washed as described above. This protocol of sensitization was predetermined as optimal for obtaining SPEC that gave consistent migration inhibition in the presence of 0.5-5 µg/ml SEA or 0.5-5 µg purified protein derivative (PPD) (Parke, Davis & Co., Detroit, Mich.). To test for suppression of MIF activity, 25-100 x 10^5 spleen cells of normal or 20-wk infected mice were added to 1 x 10^6 SPEC; then the MIF assay was performed in the presence of 1 µg/ml of SEA. If the expected migration inhibition was abrogated, it was concluded that cells that suppress MIF activity were present in the spleen cell population.

Statistical Methods of Calculations. The Student's t test was used to determine significant differences between migration areas of control and experimental groups. Values of P > 0.05 were considered not significant. Percent migration inhibition was calculated as follows:

\[
\frac{(\text{migration area of control} - \text{migration area of experimental})}{\text{migration area of control}} \times 100.
\]
Inhibitions of ≥20% represented significant differences in migration areas. Data from suppressor cell assays also include value for the degree of suppression, calculated as follows:

\[
\left( \frac{\text{percent inhibition of control} - \text{percent inhibition of experimental}}{\text{percent inhibition of control}} \right) \times 100.
\]

Results

Demonstration of Suppressed MIF Activity During Spontaneous Modulation. In a previous study, Boros et al. (7) showed that the onset of modulated granuloma formation is paralleled by a decreased production of MIF by SEA-stimulated spleen cells. Because those results were obtained from outbred mice, with xenogeneic peritoneal macrophages used to detect MIF activity, the experiments were repeated in a totally syngeneic system. Spleen cells of 8- (8-wk SP) and 20-wk (20-wk SP) infected CBA/J mice were cultured for 24 h with graded doses of SEA. Supernates were then collected and tested for MIF activity with NPEC as indicator cells. As shown in Fig. 1, the previous findings were confirmed with inbred CBA mice. Supernates of 8-wk SP cultures showed strong MIF activity over the antigen dose range employed. In contrast, the antigen-stimulated 20-wk SP cultures produced no significant MIF activity.

Essentially identical results were obtained by directly supplementing NPEC with 8- or 20-wk SP. Graded doses of spleen cells were added to the NPEC, and a direct MIF assay was carried out in the presence of 1 μg/ml of SEA. Fig. 2 shows that 8-wk SP caused significant migration inhibition, whereas 20-wk SP or normal spleen cells (NSP) did not. Because direct supplementation of NPEC accurately reflected the activities observed in supernates, this technique provided a rapid method of assaying for MIF and obviated the need for the collection and storage of supernates.

Demonstration of Active Suppression of MIF Production by 20-wk SP. The lack of MIF activity in supernates of 20-wk SP suggested that regulatory mechanism(s) may be

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**Fig. 1.** Comparison of MIF activity produced by (●) 8-wk SP and (○) 20-wk SP in response to graded doses of SEA. Points represent the mean of two experiments.
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Fig. 2. MIF production by spleen cells of 8- and 20-wk infected and normal mice. Graded doses of spleen cells were added to 10^6 NPEC (solid, 2.5 x 10^4; open, 5 x 10^4; and hatched, 1 x 10^5) and then assayed for MIF activity. Bars represent the mean and SE of three to five separate experiments.

Fig. 3. Suppression of MIF activity of 8-wk SP by 20-wk SP. Equal numbers of spleen cells from 8- and 20-wk infected mice were mixed and added to 10^6 NPEC in graded doses (open, 5 x 10^4; hatched, 1 x 10^5) and then assayed for MIF activity. Bars represent mean and SE of three separate experiments.

operating to prevent lymphokine production. Therefore, it was of interest to examine the effect of 20-wk SP on the production of MIF by 8-wk SP. Fig. 3 shows that the MIF activity of 8-wk SP was totally ablated in the presence of 20-wk SP. In contrast, NSP had no effect on MIF activity. To determine if 20-wk SP produced a factor that directly inactivated MIF, supernates of 20-wk SP were mixed with equal volumes of MIF-rich supernates and were tested for MIF activity. Apparently, 20-wk SP did not
produce factors that negated the effect of MIF, as full activity was retained in the supernatant mixtures (Fig. 4).

The suppressive effect of 20-wk SP was also demonstrable with SPEC rather than 8-wk SP. The SPEC consistently showed migration inhibition in the presence of SEA or PPD and provided a reliable, independent system to examine the regulation of MIF production. As shown in Fig. 5, the addition of 20-wk SP to SPEC abrogated MIF activity in a dose-dependent fashion, whereas NSP had no such effect.

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**Fig. 4.** Failure of supernates of cultured 20-wk SP to inactivate MIF-rich supernates of 8-wk SP. Pooled supernates of cultured spleen cells from four to five normal or 20-wk infected mice were added to an equal volume of supernate prepared from 8-wk SP. These mixtures were diluted 1:1 with fresh medium and then assayed for MIF activity. Five individual supernates from 8-wk SP were treated in this manner. Bars show the mean and SE of the five supernates. The MIF activities of the pooled supernates from 20-wk SP and NSP are also shown.

**Fig. 5.** Suppression of the MIF activity of peritoneal exudate cells from SEA sensitized mice by 20-wk SP. 20-wk SP (O) or NSP (■) were added to 10⁶ SPEC in graded doses and then assayed for MIF activity. Points represent the mean ± SE of three separate experiments.
Specificity of the Suppression of MIF Production. If suppression is mediated by SEA-specific cells, then migration inhibition elicited by PPD presumably should not be abrogated by 20-wk SP. This was tested by supplementing SPEC with doses of 20-wk SP and assaying in parallel for SEA- or PPD-elicited MIF production. Table I shows that the response to SEA was indeed suppressed, whereas that of the adjuvant-sensitized lymphocytes was unimpaired.

Characterization of Cells Involved in MIF Production with Thy-1 and Lyt Antisera. To characterize the cell(s) that produced MIF, NPEC were supplemented with 8-wk SP that had been pretreated with anti-Thy-1.2 and anti-Lyt alloantisera and complement. As shown in Fig. 6 both anti-Thy-1 and anti-Lyt-1.1 sera abrogated MIF activity. In contrast, anti-Lyt-2.1 treatment tended to augment or enrich MIF-producing cells. This was manifested by the appearance of significant MIF activity at the lower dose of spleen cells ($5 \times 10^4$) that was suboptimal for MIF detection in the normal mouse serum (NMS)-treated control. These results indicate that Lyt-1$^+$ T cells are responsible for MIF production in the schistosome-infected mice. Furthermore, mixing the anti-Lyt-1.1 and anti-Lyt-2.1 antisera-treated spleen populations resulted in a loss of significant MIF activity, which suggests a possible suppressive role for Lyt-2$^+$ cells.

Characterization of Cells Involved in MIF Production with Anti-I-Subregion Sera. Further characterization of MIF-producing T cells was performed with a battery of anti-I-subregion sera. T cells, enriched from 8-wk SP were treated with the various anti-I-subregion sera and complement before the addition to NPEC and MIF assay was carried out. As shown in Fig. 7, sera directed against A$^k$, J$^k$, E$^k$, EC$^k$, and C$^k$-subregion determinants had no significant effect, which suggests that I-region determinants are not expressed by MIF-producing T cells.

Characterization of Cells That Suppress MIF Production with Thy-1 and Lyt Antisera. To identify the cells involved in the suppression of MIF production, 20-wk SP were treated with anti-Thy-1.2, anti-Lyt-1.1, or anti-Lyt-2.1 alloantisera and complement before addition to SPEC. Table II shows that anti-Thy-1.2 serum totally ablated the suppressive effect of 20-wk SP (compare groups 1 and 2). Furthermore, suppression was eliminated by treatment with anti-Lyt-2.1 but not with anti-Lyt-1.1 serum.

| Table 1 | Antigen Specificity of the Suppression of MIF Production |
|---------|---------------------------------------------------------|
| Antigen | Number of spleen cells added* ($\times 10^4$) | Migration inhibition‡ | Degree of suppression |
| SEA     |                                               |                      |                        |
| 0       | 35.9 ± 4.7                                      |                        |
| 5.0     | 18.4 ± 2.5                                      | 48.7                  |
| 10.0    | 14.6 ± 3.9                                      | 59.3                  |
| PPD     |                                               |                      |                        |
| 0       | 22.6 ± 6.5                                      |                        |
| 5.0     | 29.5 ± 3.6                                      | 0                     |
| 10.0    | 22.0 ± 3.2                                      | 2.7                   |

* The indicated doses of 20-wk SP were added to $10^6$ SPEC and then assayed for MIF activity in the presence of 1 μg/ml SEA or 5 μg/ml PPD.
‡ The values represent the mean ± SE of three separate experiments.
Characterization of Cells That Suppress MIF Production with Anti-I-Subregion Antisera.

Further characterization of cells suppressing MIF production was performed on T cell-enriched fractions from 20-wk SP. As shown in Fig. 8, the splenic T cell (compare groups 3-5). These data indicated a clear involvement of Ly-2+ T cells in the suppression of MIF production.

Characterization of Cells That Suppress MIF Production with Anti-I-Subregion Antisera.

FIG. 6. Characterization of MIF-producing cells with anti-Thy-1 and anti-Lyt antisera. Bars represent the mean and SE of three to four separate experiments. 8-wk SP were treated with serum and complement, added to 10⁶ NPEC in graded doses (open, 5 x 10⁴; hatched, 1 x 10⁵) and then assayed for MIF activity.

FIG. 7. Characterization of MIF-producing cells with anti-I-subregion antisera. Bars represent the mean and SE of three to four separate experiments. T cell-enriched fractions of 8-wk SP were treated with serum and complement, added to 10⁶ NPEC in graded doses (open, 5 x 10⁴; hatched, 1 x 10⁵), and then assayed for MIF activity.
Table II
Characterization of the Cells That Suppress the Production of MIF with Anti-Thy-1 and Anti-Lyt Antisera

| Group | Serum treatment | Number of spleen cells added* (X 10^-4) | Migration inhibition‡ | Degree of suppression |
|-------|-----------------|------------------------------------------|-----------------------|----------------------|
|       |                 |                                          | %                     | %                    |
| 1     | NMS (AKR)       | 0                                        | 21.5 ± 2.7            | 21.5 ± 2.7           |
|       |                 | 5.0                                      | 6.7 ± 3.6             | 67.0                 |
|       |                 | 10.0                                     | -7.9 ± 6.6            | 136.7                |
| 2     | anti-Thy-1.2    | 0                                        | 21.5 ± 2.7            | 21.5 ± 2.7           |
|       |                 | 5.0                                      | 36.8 ± 12.4           | 0                    |
|       |                 | 10.0                                     | 43.4 ± 2.3            | 0                    |
| 3     | NMS (BALB/c × C57BL/6) | 0                                      | 32.4 ± 5.0           | 32.4 ± 5.0           |
|       |                 | 2.5                                      | 9.6 ± 4.3             | 70.4                 |
|       |                 | 5.0                                      | 14.4 ± 8.2            | 55.6                 |
| 4     | anti-Lyt-1.1    | 0                                        | 32.4 ± 5.0            | 32.4 ± 5.0           |
|       |                 | 2.5                                      | 12.4 ± 6.5            | 61.7                 |
|       |                 | 5.0                                      | 15.1 ± 3.9            | 53.4                 |
| 5     | anti-Lyt-2.1    | 0                                        | 32.4 ± 5.0            | 32.4 ± 5.0           |
|       |                 | 2.5                                      | 30.8 ± 4.1            | 50.0                 |
|       |                 | 5.0                                      | 45.7 ± 3.9            | 0                    |

* 20-wk SP were treated with serum and complement and added to SPEC at the indicated doses and then assayed for MIF activity.
‡ The values represent the mean ± SE of three to four separate experiments.

Fig. 8. Suppression of MIF activity by T cell-enriched fractions from 20-wk SP. T cell fractions of (O) NSP and (C) 20-wk SP were added to 10^6 SPEC in graded doses and then assayed for MIF activity. Points represent the mean ± SE of two experiments.

fraction of 20-wk-infected, but not normal, mice was capable of suppressing the MIF activity of SPEC. These T cells were subsequently treated with various anti-I-subregion sera and complement and then were assayed for their capacity to suppress the MIF activity of SPEC. As shown in Table III, suppression was abrogated with
TABLE III

Characterization of the Cells That Suppress the Production of MIF with Anti-I-Subregion Antisera

| Group | Serum treatment | Number of Spleen T cells added* (× 10^4) | Migration inhibition‡ | Degree of suppression |
|-------|-----------------|------------------------------------------|----------------------|-----------------------|
| 1     | NMS (BALB/c × C57BL/6) | 0 ± 2.6 | 26.6 ± 2.6 | 73.7 |
|       | (A.BY × B10.MM) | 2.5 ± 6.0 | 70.4 ± 10.2 | 0 |
| 2     | anti-ABJ\k | 0 | 26.6 ± 2.6 | 73.7 |
|       | (A.BY × B10.HTT) | 2.5 | 30.4 ± 10.2 | 0 |
|       | anti-A.TL | 5.0 | 31.5 ± 4.2 | 0 |
| 3     | anti-A\k [ATFR.5 × B10.S(9R)] | 2.5 | 10.8 ± 5.7 | 59.4 |
|       | anti-A.TL | 5.0 | 0.6 ± 10.6 | 97.7 |
| 4     | anti-J\k | 0 | 26.6 ± 2.6 | 73.7 |
|       | B10.HTT anti-B10.S(9R) | 2.5 | 32.0 ± 3.1 | 0 |
|       | 5.0 | 26.0 ± 9.8 | 0 |
| 5     | anti-E\k | 0 | 26.6 ± 2.6 | 73.7 |
|       | (B10.D2 × A.BY) | 2.5 | −4.8 ± 20.0 | 118.0 |
|       | anti-B10.A(3R) | 5.0 | −9.7 ± 12.3 | 136.5 |
| 6     | anti-C\k | 0 | 26.6 ± 2.6 | 73.7 |
|       | (A × B10) anti-B10.AM | 2.5 | 21.4 ± 3.2 | 19.5 |
|       | 5.0 | 27.4 ± 6.0 | 0 |

* T cell-enriched fractions of 20-wk SP were treated with serum and complement and added to SPEC at the indicated doses and then assayed for MIF activity.
‡ The values represent the mean ± SE of three separate experiments.

Evidence accumulated in recent years indicates that lymphokines may play an important role in the initiation and maintenance of the granulomatous host response to schistosome eggs. In sensitized guinea pigs, augmented granulomatous reaction coincided with the appearance of MIF activity in the peritoneal lymphocytes (22). Conversely, the diminished (spontaneously modulated) granulomatous response of chronically infected mice was correlated with the cessation of lymphokine production (7, 8). Recently, adoptive transfer studies have shown that modulation of the granulomatous response may be mediated by T suppressor lymphocytes (13–15). Because cyclophosphamide treatment of chronically infected mice reversed the modulation of the granulomatous response, it is possible that lymphokine-producing lymphocytes are still present in the infected mice but are under the regulation of a suppressor cell(s) (14). Thus, modulation of the host granulomatous response may be based on T-T cell interaction, with the resultant curtailment of mediator production and cellular mobilization.

In the present in vitro investigations we show that such a mechanism may possibly
function in murine schistosomiasis during the stage of the modulated response. Splenic lymphocytes of mice with modulated granulomatous responses, mixed with mediator-producing splenic cells of animals that displayed peak granulomatous response indeed abrogated mediator production by the latter. A similar effect was seen when instead of splenocytes, antigen-sensitized peritoneal lymphocytes were used as target cells for the suppression. The suppression was elicited by specific antigen and, apparently, acted at the cellular level rather than on the released mediator. The lymphocytes involved in mediator production and suppression were then characterized. The MIF-producer lymphocytes present in the spleens of 8-wk infected mice were shown to be T cells that belong to a Lyt-1+ subpopulation that lacked I region-encoded determinants. This profile is consistent with recent reports that characterized mediator-secreting lymphocytes (23–25). In contrast to these findings, Newman et al. (26) have shown that Lyt-2+ cells also produce MIF. However, their results were obtained with mixed lymphocyte cultures (MLC) and not soluble protein antigens. The lack of detectable Ia antigens on the surface of the Lyt-1+ MIF-producing lymphocytes makes these cells similar to, or identical with, the TDL cells active in the adoptive transfer of delayed-type hypersensitivity (27).

Lymphocytes from the spleens of 20-wk infected mice that abrogated the in vitro production of MIF-active mediator were identified as T cells that belong to the Lyt-2+ subpopulation classified as suppressor/killer cells by Cantor and Boyse (28). Although clearly demonstrable at 20 wk of infection, suppressor cells seemed to be present, albeit in smaller numbers, in 8-wk SP. When an enriched Lyt-2+ population from such spleens was added to Lyt-1+ cells, abrogation of MIF activity was seen (Fig. 6). Further characterization of the suppressor cells revealed that they also expressed the I-J- and I-C-subregion gene products. As yet it is not known if such markers are expressed on the same or separate different cells. These findings are consistent with reports from other laboratories that have shown the presence of I-J antigen on the membranes of suppressor T cells (29), auxiliary suppressor T cells (30), and soluble suppressor factors active in the regulation of antibody production (31) and the suppression of contact sensitivity (32). So far, the I-C subregion-encoded determinants have been associated with soluble suppressive factors active in cell proliferation of MLC (33). We furnish evidence that I-J+ and I-C+ T suppressor (Ts) lymphocytes are involved in yet another cell-mediated system: the regulation of lymphokine production. It is still to be seen whether such cells would exert a similar effect locally, within the granulomas. Our previous finding that shows the virtual absence of Ia+ T lymphocytes in the granulomas of 8-wk infected mice, but the pronounced presence of such cells in the modulated lesions of 20-wk infected animals, seems to support this notion (15). A phenotypic and functional analysis of the various subpopulations of the granuloma lymphocytes is presently under way in our laboratory to answer this question. Such an analysis should shed light on local interaction of Lyt-2+, Ia+ T cells with precursor suppressor cells (34) and/or the Lyt-1+, Ia− mediator producer lymphocytes.

As yet there are only a few reports on Ts lymphocyte-mediated regulation of lymphokine production. Fox and Rajaraman (35) have shown that T lymphocytes of sensitized guinea pigs and humans suppress in vitro MIF production. In another report by Suko et al. (36), exposure of sensitized lymphocytes to large doses of antigen elicited the production of a soluble factor, which nonspecifically suppressed MIF.
production in heterologously sensitized lymphocytes. Such factor(s) may be the products of the $T_s$ cells we have described. Furthermore, in view of previous descriptions of soluble suppressor factors (31–33) these might be predicted to express I-region gene products. We are currently investigating these possibilities.

$T_s$ cell regulation of cell-mediated inflammation may be relevant to human schistosomiasis as well as other granulomatous diseases. Ottesen et al. (37) have shown that anti-SEA blastogenic responses of peripheral blood lymphocytes from patients with schistosomiasis mansoni decreases with the duration of infection. Moreover, Colley et al. (38) have identified factor(s) in the sera of chronically infected patients that suppress blastogenic responses to parasite antigens. These changes were attributed to $T_s$ cell activity; however, their relationship to granuloma formation has yet to be established. Suppressor cells that cause depressed delayed-dermal responses have been described in experimental leprosy (39) and histoplasmosis (40). Interestingly, comparison of groups of patients with tuberculoid or lepromatous leprosy revealed that the peripheral blood lymphocytes of the latter have a reduced capacity to produce MIF (41) or macrophage aggregation factor (42) in response to lepromin. One may postulate from such studies that $T_s$ cells are blocking lymphokine-mediated granuloma formation, thereby causing the dissemination of organisms seen in lepromatous leprosy. A better understanding of the mechanism(s) active in the modulation of granulomatous inflammation should help to assess the risks and benefits of this phenomenon and will improve the clinical management of various granulomatous diseases.

Summary

Host granulomatous inflammation in murine schistosomiasis mansoni is a $T$ cell-mediated immune response, which, at the chronic stage of the disease, undergoes $T$ suppressor lymphocyte-dependent modulation. In the present study this phenomenon was further analyzed in vitro. Spleen cells of mice undergoing modulation (20 wk of infection) when mixed with spleen cells of animals exhibiting vigorous granulomatous responses (8 wk of infection) abrogated in vitro migration inhibition factor (MIF) production by the latter. Characterization of the delayed-type hypersensitivity $T$ lymphocytes involved in lymphokine production showed that they belonged to the Lyt-1+ subset and did not express I region-encoded antigens. In contrast, $T$ lymphocytes involved in the suppression of MIF activity belonged to the Lyt-2+ subpopulation of cells, which expressed I-J- and I-C-subregion determinants. These results suggest that the modulation of the granulomatous hypersensitivity response in mice is the result of $T$-$T$ cell interaction with subsequent regulation of inflammatory lymphokine production.

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