

REGULATION OF GRANULOMATOUS INFLAMMATION IN MURINE SCHISTOSOMIASIS

II. T Suppressor Cell-derived, I-C Subregion-encoded Soluble Suppressor Factor Mediates Regulation of Lymphokine Production*

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The spontaneous modulation of granulomatous inflammation in chronic murine schistosomiasis represents a naturally occurring immune process that leads to diminished T cell-mediated inflammatory responses around parasite ova in the tissues (1-4). A recent series of studies has shown that this spontaneous modulation is associated with the appearance of regulatory T cells (5-11) that can adoptively suppress granuloma formation *in vivo* and lymphokine production *in vitro* (5, 6). One population was active in the long-term adoptive suppression of acutely infected recipients and expressed the $\text{Lyt-1}^+, 2^-, 3^-, \text{Ia}^+$ phenotype (6, 9), and another caused short-term suppression of responses in uninfected recipients and was defined as $\text{Lyt-1}^-, 2^+, 3^+$ (11). Because lymphocytes of mice undergoing modulation had an impaired capacity to produce migration inhibition factor (MIF)¹ and eosinophil stimulation promoter (ESP)-active lymphokines (3, 4) that are presumed to function in the formation of the granuloma (12-14), we postulated that modulation of the granulomatous response is carried out by regulatory suppressor T (T_S) cells that suppress lymphokine production by lymphokine secretor T (T_DH) cells. This postulate was confirmed by demonstrating that spleens of chronically infected mice contained $\text{Lyt-1}^-, 2^+, 3^+, \text{I-J}^+/\text{I-C}^+$ T_S cells that suppress *in vitro* MIF production by $\text{Lyt-1}^+, 2^-, 3^-, \text{Ia}^-$ T_DH cells of acutely infected animals (8).

The present study was undertaken to further examine mechanisms by which inflammatory and suppressor T cell subpopulations interact in the modulation of granuloma formation. We specifically wished to examine the mechanism(s) by which $\text{Lyt-1}^-, 2^+, 3^+$ T_S cells suppress the production of MIF. Our results suggest that

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¹ *Abbreviations used in this paper:* BSA, bovine serum albumin; ESP, eosinophil stimulation promoter; FCA, Freund's complete adjuvant; MHC, major histocompatibility complex; MIF, migration inhibition factor; MIF-SF, migration inhibition factor suppressor factor; MLR, mixed leukocyte reaction; NHS, normal human serum; NMS, normal mouse serum; NPEC, normal peritoneal exudate cells; NSP, spleen cells of normal mice; PBS, phosphate-buffered saline; PPD, purified protein derivative of mycobacteria; SEA, soluble schistosome egg antigens; SPEC, peritoneal exudate cells of mice sensitized with complete Freund's adjuvant and antigen, T_DH , lymphokine secretor T cell; T_S , suppressor T cell; 8-wk SP, spleen cells of 8-wk infected mice; 20-wk SP, spleen cells of 20-wk infected mice.

suppression of lymphokine secretion is mediated by a soluble suppressor factor (MIF-SF). MIF-SF appears to belong to a new family of I-C antigen bearing suppressor factors first described to function in the MLR system (15). Initial characterization of MIF-SF showed that its production involves $\text{Lyt } 1^{-}, 2^{+}, 3^{+}$ T cells, it is antigen specific, acting only on soluble schistosome egg antigen (SEA) -primed cells, and minimally requires I-AB or I-C compatibility with target cells to exert its effect. Thus, at least under in vitro conditions, I-C-encoded suppressor factors may act as signals in the regulation of lymphokine secretion by $\text{Lyt } 1^{+} 2^{-} 3^{-}$ effector T_{DH} .

Materials and Methods

Animals and Infection. Female, CBA/J (H-2^k) mice obtained from The Jackson Laboratory, Bar Harbor, ME were used in all experiments. In those experiments comparing strain responses, BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were kindly provided by Dr. Y. M. Kong and Dr. H. C. Rauch, respectively. All other strains were acquired from colonies maintained at the Mayo Medical School, Rochester, MN. Mice were maintained under standard laboratory care and provided with food and water ad lib. 7–8-wk-old CBA mice were infected subcutaneously with 25 cercariae of the Puerto Rican strain of *Schistosoma mansoni*.

Preparation of SEA. SEA were prepared from homogenized eggs isolated from the livers of infected mice (16).

Preparation of MIF-SF. Spleen cells of 20-wk infected CBA mice were harvested aseptically and suspended to 1×10^7 nucleated cells/ml of RPMI 1640 containing 2 mM glutamine, 100 U/ml penicillin and 100 $\mu\text{g/ml}$ streptomycin (Grand Island Biological Co., Grand Island, NY), and 1 $\mu\text{g/ml}$ SEA. 2-ml aliquots of the suspensions were distributed into $16 \times 125\text{-mm}$ tissue culture tubes (BioQuest; Falcon Labware, Becton, Dickinson & Co., Oxnard, CA) and incubated at 37°C for 90 min in a humidified 5% CO_2 atmosphere. After this pulse with SEA, the cells were centrifuged and washed twice in prewarmed unsupplemented medium. Finally, the cells were resuspended in 2 ml of RPMI 1640 supplemented with antibiotics and 10% heat-inactivated normal human serum (RPMI-NHS) (Grand Island Biological Co.) that was absorbed with 1:5 volume of a mixture of mouse spleen and peripheral blood cells (30 min at room temperature) before use. The cells were cultured for 24 h, then centrifuged at 500 g for 10 min. Supernatants were then collected from above the cell pellet and stored at -70°C . Control supernatants were obtained from SEA-pulsed normal spleen cells.

Assays for Suppression of MIF Activity. Mineral oil-induced peritoneal cells were obtained from SEA-Freund's complete adjuvant (FCA)-sensitized mice (SPEC) as described previously (8). SPEC gave consistent migration inhibition in the presence of 0.5–10 $\mu\text{g/ml}$ SEA or 5–10 $\mu\text{g/ml}$ purified protein derivative (PPD) (Parke, Davis and Co., Detroit, MI). To test antigen specificity, PEC were also obtained from mice sensitized with 30 μg crystallized bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO) in FCA. Such SPEC were inhibited in the presence of 10–20 $\mu\text{g/ml}$ BSA or 5–10 $\mu\text{g/ml}$ PPD, but were unaffected by SEA. Spleen cell supernatants of 20-wk infected mice (20-wk SP) to be tested for suppressor activity were diluted in fresh RPMI-NHS then supplemented with 1 $\mu\text{g/ml}$ SEA. The standard capillary migration inhibition assay was performed using SPEC with dilutions of 20-wk SP supernatants as incubation medium. Conditions of the MIF assay and methods of measuring migration areas have been described in detail (8). If the expected migration inhibition was significantly abrogated, then it was concluded that suppressor factor was present in the test supernatant.

In some experiments, 20-wk SP were tested for the capacity to suppress SEA-elicited MIF production by cultured spleen cells of 8-wk infected mice (8-wk SP). The latter cells were cultured for 24 h in the presence of serially diluted putative suppressor factor supplemented with 1 $\mu\text{g/ml}$ SEA. After incubation, the culture supernates were tested for MIF activity using normal peritoneal exudate cells (NPEC) as previously described (8).

Antisera and Complement Treatments. Anti-Thy-1.2 serum was prepared by multiple immunizations of AKR mice with CBA thymocytes; this preparation was highly specific for CBA T lymphocytes. Anti-Lyt-1.1 and anti-Lyt-2.1 were generously provided by Dr. F.-W. Shen (Memorial Sloan-Kettering Cancer Center, New York). Anti-I(ABJ)^k [A.BY \times B10.HTT] anti-

A.TL], anti-I (EC)^k [B10.S(7R) anti-B10.HTT], anti-I(JE)^k [(B10.B2 × A.BY) anti-B10.A (5R)], and anti-I-C^k [A × B10) anti-B10.AM] sera were prepared at the Mayo Medical School. Rabbit serum absorbed with mouse tissues (Accurate Chemicals Inc., Westbury, NY) served as a source of complement. Antisera and complement were used for cytolysis as follows: Spleen cells (1×10^7 /ml) were incubated for 30 min at 4°C in antiserum diluted in RPMI 1640 (anti-Thy-1.2, 1:10; anti-Lyt-1.1, 1:100; anti-Lyt-2.1, 1:50). Control serum consisted of normal (BALB/c × 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, then incubated for 30 min at 37°C. After incubation, cells were washed three times, their viability was determined by dye exclusion, and cell concentrations were adjusted according to numbers of live cells.

Preparation and Use of Insoluble Immunoabsorbents. Immunoabsorbents were prepared as described by Rich et al. (15). Heat inactivated sera were dialyzed for 18 h against coupling buffer (0.1 M NaHCO₃, 0.5 M NaCl, pH 8.0). A volume of 1.0 ml of serum was mixed with a 2.5-ml bed volume of cyanogen bromide-activated Sepharose 4B beads (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) in a sealed siliconized glass tube and gently rotated overnight at 4°C. Unbound protein was removed by washing with coupling buffer, then the beads were suspended in 1 M ethanolamine (pH 8.0) and rotated for an additional 1.5 h to block any remaining active sites. Using a sintered glass filter, the coupled beads were washed with three alternating cycles of 0.1 M acetate buffer (pH 4.0) and 0.1 M borate buffer (pH 8.0) both containing 1 M NaCl. The material was next washed with 100 ml of phosphate-buffered saline (PBS) pH 7.2 and then mixed with 4.0 ml of a 3% BSA solution in 0.05 M Tris-HCl (pH 7.6) and incubated for 1 h at 4°C to block nonspecific binding sites. After incubation, the bed was washed extensively with PBS and excess fluid was removed. 4 ml of culture supernatant was then added to the bed. This mixture was returned to a sealed glass tube and gently rocked for 2 h at 4°C. After rocking, the slurry was transferred to a 0.7 × 12-cm plastic column with a sintered glass base and the adsorbed supernatant was drawn off using negative pressure. Virtually all the applied supernatant was recovered by this method with minimal dilution.

After rinsing the immunoabsorbent with PBS, bound suppressor material was recovered by adding 4 ml of cold glycine-HCl buffer (pH 2.8) and incubating the mixture for 15 min at 4°C. The glycine eluate was drawn off then dialyzed against RPMI for 24 h. Before testing, the dialyzed eluate was mixed with an equal volume of RPMI-NHS having twice the concentration of supplementary ingredients.

Statistical Methods. 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: [(migration area of control - migration area of experimental)/(migration area of control)] × 100. Inhibitions of ≥20 percent represented significant differences in migration areas. The degree of suppression was calculated as follows: [(percent inhibition of control - percent inhibition of experimental)/(percent inhibition of control)] × 100.

Results

Demonstration of MIF-SF in the Culture Supernates of 20-wk SP. We previously showed that 20-wk SP can directly suppress SEA-induced MIF production by both 8-wk SP and SPEC of syngeneic mice (8). To determine whether this suppression was mediated by soluble suppressor factor(s), we examined supernatant from 20-wk SP in both indirect and direct MIF assays. Using the indirect assay, 8-wk SP were cultured for 24 h in the presence of SEA and serially diluted supernatants from 20-wk SP or normal spleen cells (NSP). The culture supernatant were subsequently assayed for MIF activity. As shown in Fig. 1, supernatants of 20-wk SP strongly suppressed MIF production by 8-wk SP as compared with controls. Significant suppressive activity was present even at 1:8 dilution of supernatant. At high concentration, normal supernatant also showed moderate suppressive activity. This may be explained by the presence of small amounts of suppressor factor produced by SEA-pulsed normal

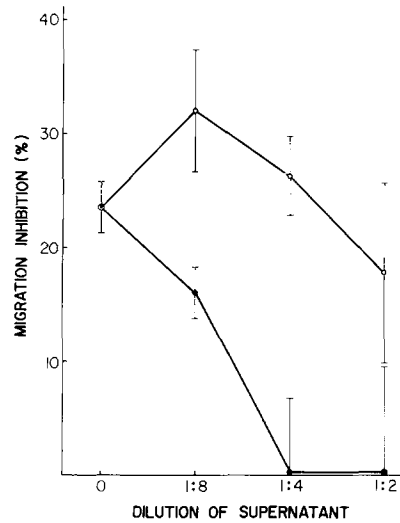


FIG. 1. The suppressive effect of 20-wk SP supernatants on MIF production of SEA-stimulated 8-wk SP cultures. 8-wk SP were cultured in the presence of serial dilutions of suppressor (●) or control (○) supernatants supplemented with 1 μg/ml SEA. At 24 h, the culture supernatants were collected and assayed for MIF activity using NPEC as indicator cells. The 0-point baseline consisted of a 1:2 dilution of supernatant of NSP not pulsed with SEA. Points indicate mean ± SE of seven individual 8-wk supernatant preparations.

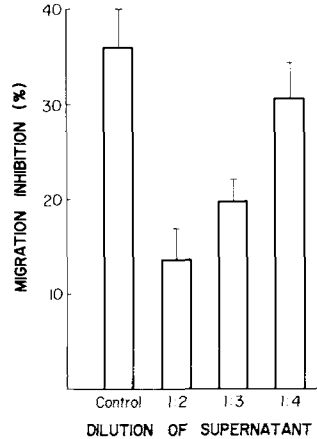


FIG. 2. The suppressive effect of 20-wk SP supernatants on the SEA-elicited MIF-response of SPEC. Suppressor supernatants were serially diluted with fresh medium, then supplemented to 1 μg/ml SEA before injection into assay chambers. Control is a 1:2 dilution of supernatant of NSP pulsed with SEA. Bars represent the mean ± SE of two to three separate experiments.

spleen cells. Spleen cells unpulsed by SEA showed no activity (Fig. 1, 0 point). We next tested the suppressive activity of supernates in the direct MIF assay using SPEC as indicator cells (Fig. 2). At a 1:2 or 1:3 dilution, 20-wk SP supernatant clearly suppressed the MIF activity of SEA-stimulated SPEC. However, suppression was usually lost at a 1:4 dilution of supernatant. Note that in this system normal control supernatants showed no suppression even at a 1:2 dilution. For the sake of its ease and rapidity, the direct assay was used for all of our subsequent analyses.

Examination of the dose-response kinetics of MIF-SF production showed that greatest suppressive activity was obtained in cultures where 20-wk SP were briefly pulsed with 0.1 and 1.0 $\mu\text{g/ml}$ of SEA. It is of note that detectable activity was produced even in the absence of exogenous antigenic stimulation (Table I). By direct assay, it was also demonstrated that the suppressive activity (MIF-SF) was nondialyzable, indicating that it has a molecular weight of $>12,000$ (data not shown).

Characterization of the Cells Involved in the Production of MIF-SF. Having previously shown that $\text{Lyt-1}^- \text{,2}^+ \text{,3}^+$, T cells of 20-wk SP suppressed MIF production (8), it was of interest to determine whether the same subset of cells was involved in the production of MIF-SF. To answer this question, 20-wk SP were pretreated with Thy-1 and Lyt antigen-specific alloantisera and complement, then cultured and assayed for MIF-SF production. Fig. 3 shows that MIF-SF activity was eliminated by anti-Thy-1.2 treatment, indicating a role for T cells. Furthermore, the subset of T cells involved appeared to be $\text{Lyt-1}^- \text{,2}^+ \text{,3}^+$, because anti-Lyt-2.1, but not anti-Lyt-1.1, could elimi-

TABLE I
Antigen Dose-Response of MIF-SF Factor Production

Dose of SEA to elicit MIF-SF*	Migration inhibition‡	Suppression
$\mu\text{g/ml}$	%	%
Control	28.5 ± 3.7	—
0	17.3 ± 3.3	39
0.1	3.6 ± 25.7	87
1.0	2.3 ± 12.8	92
10.0	17.7 ± 5.7	38

* Spleen cells of 20-wk SP mice were pulsed for 90 min with graded doses of SEA then washed and cultured for 24 h. Supernatants were then collected and assayed against SPEC.

‡ Value represent mean migration inhibition \pm SD derived from two separate experiments. Each experiment was performed in duplicate.

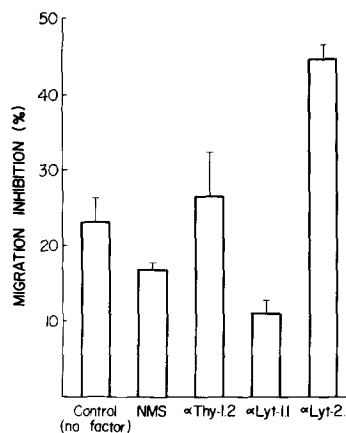


FIG. 3. Characterization of the surface phenotype of cells involved in MIF-SF production. 20-wk SP were pretreated with the indicated alloantisera and complement, washed, then cultured for the production of MIF-SF as described in Materials and Methods. Bars represent mean \pm SE of three separate experiments.

nate activity. In fact, supernatants of anti-Lyt-2.1 pretreated 20-wk SP augmented rather than suppressed the migration inhibition of SPEC.

Antigen and H-2 Specificity of MIF-SF. Antigen specificity of MIF-SF was examined directly using SPEC from SEA-FCA or BSA-FCA-sensitized mice. As Fig. 4 illustrates, MIF-SF suppressed MIF activity elicited by either SEA or PPD, using SPEC from SEA-FCA immunized mice. However, the factor was inactive when tested in a system that lacked schistosome specificity. Thus MIF-SF did not suppress either the BSA- or PPD-elicited migration inhibition using SPEC of BSA-FCA-sensitized mice. These results indicate the antigen-specific nature of MIF-SF.

When CBA (H-2^k) MIF-SF was tested on SPEC from a battery of histoincompatible and H-2-recombinant strains, a clear H-2 specificity was revealed (Table II). MIF-SF had no significant effect upon the lymphocytes of incompatible mice such as BALB/

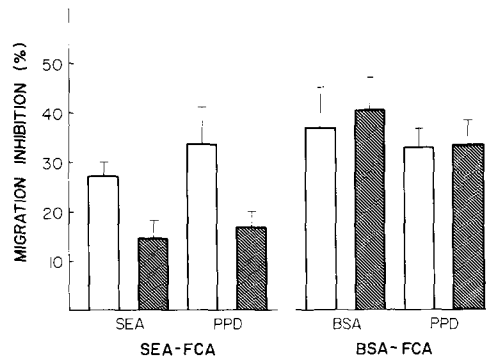


FIG. 4. Demonstration of antigen specificity of MIF-SF. Suppressor (▨) and control (□) supernatants were diluted with an equal volume of fresh medium, then supplemented with 1 µg/ml SEA, 5 µg/ml PPD, or 10 µg/ml BSA before transfer into MIF assay chambers. Bars represent mean ± SE of three separate experiments.

TABLE II
Genetic Requirement for Suppressor Function of MIF-SF

Strain*	H-2								Percent migration inhibition				Mean per- cent sup- pression
									1 µg		10 µg		
	K	A	B	J	E	C	S	G	CON	MIF-SF	CON	MIF-SF	
CBA/J	k	k	k	k	k	k	k	k	43.9	16.7	46.9	29.3	49.8
BALB/c	d	d	d	d	d	d	d	d	33.6	44.3	53.3	48.7	4.3
C57BL/6	b	b	b	b	b	b	b	b	27.6	22.9	35.7	29.7	17.0
B10.BR	k	k	k	k	k	k	k	k	26.5	16.6	51.1	17.5	51.6
B10.A	k	k	k	k	d	d	d	d	54.2	13.1	35.8	16.6	64.7
A/Sn	k	k	k	k	d	d	d	d	—‡	—	46.2	15.4	66.0
C3H.OL	d	d	d	d	d	k	k	k	37.5	30.3	38.5	42.9	9.6
C3H.OH	d	d	d	d	d	d	d	k	—‡	—	24.5	21.2	13.5
B10.AQR	q	k	k	k	k	d	d	d	38.0	16.9	34.3	2.5	74.1
B10.S (9R)	s	s	s	k	k	d	d	d	—‡	—	31.2	28.1	9.9
B10.HTT	s	s	s	s	k	k	k	k	31.5	9.2	50.3	33.4	52.2

* SPEC from five to six mice of each strain were pooled and tested in duplicate or triplicate with supernatants of normal (CON) or 20-wk SP (MIF-SF) cultures. MIF chambers contained 1:3 dilutions of supernatant supplemented to 1 µg/ml or 10 µg/ml SEA.

‡ Less than 20% migration inhibition achieved with 1 µg SEA.

c (H-2^d) and C57BL/6 (H-2^b). However, strong activity was seen with B10.BR (H-2^k), which shares only H-2 genes with CBA, arguing against the involvement of non-H-2 gene products. Further analysis revealed a significant suppression with B10.A and A/Sn, but not with C3H.OL and C3H.OH strains. These results suggested that K and I region genes but not S, G, and D genes determined susceptibility to MIF-SF. A more detailed examination showed that suppression could be obtained with I-ABJE (B10.AQR) or I-EC, SG (B10.HTT) but not with I-JE [B10.S(9R)] compatibility. Taken together, our findings suggest that I-AB or I-C subregion compatibility is the minimal requirement for target cell susceptibility to MIF-SF.

Expression of I Region-encoded Antigens by MIF-SF. Because the activity of MIF-SF appeared to be H-2 restricted, we wished to determine if this factor carried major histocompatibility complex (MHC)-encoded antigens. Specifically, we tested for the presence of I region-associated antigens, which have been found in other soluble mediators of suppression (15, 17-22). To this end, insoluble immunoabsorbent columns were prepared with various bound alloantisera directed against I subregion determinants. Samples of MIF-SF active supernatants were absorbed as described in Materials and Methods and tested for suppressor activity. Table III shows the result of these experiments. Suppressor activity was not removed by normal mouse serum (NMS), anti-(ABJ)^k, or anti-(JE)^k, but was completely eliminated with anti-(EC)^k and anti-C^k alloantisera. Because only the columns with anti-I-C antibodies were able to adsorb the suppressor activity, it appeared that I-C subregion determinants were expressed by MIF-SF.

To further substantiate these findings and eliminate the possibility that MIF-SF was simply inactivated by the column adsorption, we next attempted to recover the column-bound MIF-SF by elution with glycine-buffer at low pH. After elution, the material was dialyzed against fresh medium, restored to its original volume, then tested for suppressor activity. As shown in Fig. 5, MIF-SF was recovered from both the anti-(EC)^k and C^k columns, whereas no activity was found on the NMS column.

Discussion

The spontaneous modulation of granulomatous hypersensitivity in experimental schistosomiasis *mansoni* provides a unique model for the analysis of natural mechanisms that regulate chronic, cell-mediated inflammations. In previous studies (3-11),

TABLE III
Expression of I-C Subregion Determinants by MIF-SF

Supernatant	Column adsorption*	Migration inhibition‡	Suppression
		%	%
Normal	None	23.1 ± 3.3	—
20-wk	NMS	11.3 ± 2.9	51.0
	Anti-I(ABJ) ^k	6.5 ± 7.1	71.9
	Anti-I(EC) ^k	30.6 ± 4.9	0
	Anti-I(JE) ^k	4.8 ± 12.8	79.2
	Anti-IC ^k	26.2 ± 6.0	0

* 20-wk SP supernatants were adsorbed with solid-phase immunoabsorbents as described in Materials and Methods.

‡ Values represent mean ± SE of three separate experiments, using three different 20-wk SP supernatant preparations.

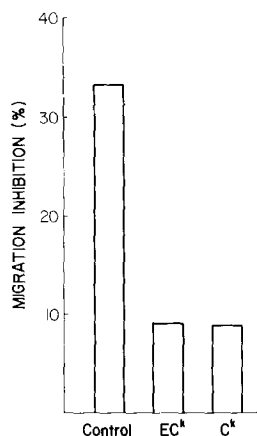


FIG. 5. Suppressive effect of acid eluates from anti-I subregion immunoadsorbents. Immunoadsorbents that had bound MIF-SF were treated with glycine buffer (pH 2.8) to elute bound material. Eluent material was dialyzed against fresh medium, reconstituted with antibiotics and serum, then tested for suppressive activity. Control: migration inhibition in presence of eluate from the NMS column, which did not remove suppressor activity. Dotted line shows baseline inhibition in a 1:2 dilution of NSP supernatant. Bars represent means of two separate experiments.

the involvement of suppressor T cells in the modulation of granuloma formation and lymphokine production was delineated. Numerous observations indicate that the modulated granulomatous response is the result of a dynamic equilibrium maintained between effector (T_{DH}) and suppressor (T_S) cells. (a) The granulomatous response is diminished, but not abrogated, during the process of modulation (3, 4). (b) Mice displaying modulation regain their vigorous granulomatous response and/or the capacity to produce MIF after the elimination of T_S lymphocytes by cyclophosphamide (7, 9) or anti-I-J subregion alloantisera (10). (c) Splenic lymphocytes of mice undergoing modulation are capable of transferring accelerated granulomatous response to naive recipients (11). (d) Cotransfer of equal numbers of splenic T cells from acutely and chronically infected mice suppresses granuloma formation in naive recipients (11). (e) The $Lyt-1^-,2^+,3^+$, $I-C^+/I-J^+$ subset of T_S lymphocytes from spleens of chronically infected mice admixed with $Lyt-1^+$, $Ia^- T_{DH}$ cells suppressed in vitro the production of MIF by the latter (8). Because an association between inflammatory lymphokines and the schistosome egg-induced granulomatous response has been established (12-14), regulation of lymphokine production would be a likely mode for the maintenance of diminished granulomatous inflammatory responses.

The present study was designed to further elucidate the mechanism(s) of interaction between effector and suppressor lymphocytes that occurs during the modulated granulomatous response. Results show that splenic lymphocytes of mice displaying modulation secrete or shed into the culture fluid a soluble factor that can suppress in vitro the production of MIF. The factor, whose production involves the $Lyt-1^-,2^+,3^+$ subset of splenic T cells, can replace in concentrated or dilute form the suppressor activity of live T_S cells. Though MIF-SF is also produced by unstimulated splenocytes, the in vitro production of the factor seems to be antigen dependent. This observation may bear relevance to in vivo conditions. In chronically infected mice, higher antigen concentrations may prevail in the modulated often less cellular granulomas. Thus, strong local antigenic stimuli emanating from the parasite ova could induce and

sustain the production of MIF-SF by Ia^+ T cells that infiltrate the immunomodulated lesions (6).

In repeated experiments, MIF-SF inhibited lymphokine production in the SEA and in the unrelated tuberculin immune systems. However, inhibition occurred only when SEA and tuberculin-sensitive lymphocytes were present together. In the absence of the SEA-specific component, MIF-SF was ineffective in either the BSA- or FCA-primed systems. Based on this we assume, that MIF-SF bears specificity for SEA-sensitive, H-2-compatible target cell(s). Interaction between factor and target cell(s) causes the release of a nonspecific factor(s) that suppresses mediator production in heterologous cell-mediated systems as well. This assumption concurs with recent reports (23, 24) that describe the participation of antigen-specific and nonspecific components in cell-mediated systems that are regulated by various T_S cell-initiated pathways. We previously observed (6) that T_S cells adoptively transferred from immunomodulated donors did not suppress heterologous granulomas in the lungs of recipients. In the light of present observations, this is explicable if we postulate as prerequisites for suppression (a) the specific triggering by MIF-SF of local granuloma lymphocytes, and (b) the short range, intralesional effect of the released nonspecific immunoregulatory products.

Suppression of T_{DH} function by MIF-SF was H-2 restricted. More specifically, effective interaction required I-AB or I-C region compatibility of the target cell with the source of factor. Further analysis by immunoabsorption indicated that MIF-SF expressed I-C subregion-encoded determinants. Thus, MIF-SF seems to belong to the group of genetically restricted, immunoregulatory molecules that derive from I region genes (15, 17-22). Some of these molecules expresses I-J, others I-C specificities. To date, I-C antigen-bearing ($I-C^+$) suppressor factors have been shown to regulate mixed leukocyte reaction (MLR) (15) and contact sensitivity (22). Our present observations extend their involvement to the regulation of inflammatory lymphokine production during a chronic granulomatous disease. It is noteworthy that MIF-SF differed in genetic requirement from the suppressor factor active in contact sensitivity. Restriction of the latter was determined by the K and D regions of the MHC. This difference may reflect the nature of the antigen and its particular mode of presentation. Moorhead (22) has shown that the acceptor molecules for the suppressor factor of contact sensitivity are hapten-modified H-2K and/or D alloantigens. In contrast, soluble protein/glycoprotein antigens such as SEA are likely to be presented with I region-encoded determinants (25). However, further genetic analysis and delineation of molecular targets will be necessary to precisely define the relationship of MIF-SF to the MHC.

Presently, MIF-SF most closely resembles the factor that suppressed cell proliferation in the MLR (MLR- T_S F). This factor was I-C subregion restricted, antigen nonspecific, did not bind foreign antigens, and required $I-J^+/I-C^+$ $Lyt\ 2^+$ T cells for its production (15, 24). Though we have not directly shown that MIF-SF production involves $I-J^+/I-C^+$ T_S cells, this would be expected as such cells were required for the suppression of MIF production in recent in vitro admixture experiments (8).

Based on this previous observation, detection of both $I-J^+$ and $I-C^+$ factors were predicted. Experiments showed, however, the presence only of an $I-C^+$ factor. Various explanations may be offered for this discrepancy. A simple one would be the insensitivity of the fairly crude bioassay (abrogation of migration inhibition), which

could not detect very small amounts of I-J⁺ materials. A more plausible assumption would assert that I-J⁺ factors went undetected because they do not interact directly with T_{DH} target cells. This is an attractive explanation, in the light of recent observations showing that long-term in vivo administration of anti-I-J alloantiserum to schistosome infected mice abrogated the onset of modulation of the granulomatous response (10). Thus, conceivably I-J⁺ factors may participate in the specific recruitment and activation of suppressor precursor lymphocytes (9), whereas I-C⁺ factors would maintain the modulated inflammatory response via the regulation of lymphokine production. The demonstration that I-J⁺ factors participate in the recruitment of suppressor populations that regulate humoral (26) and cell-mediated responses (19, 20) lends further weight to these speculations.

In summary, in the present study we extended previous observations and showed that in vitro lymphokine production is regulated by soluble I subregion gene products derived from Lyt 1⁻,2⁺,3⁺ T_S lymphocytes. Though the final target of this factor is the T_{DH} effector cell, an indirect interaction between MIF-SF and such a cell via an intermediary T cell (24) or macrophage (27) is not excluded. The in vivo relevance of such a suppressor factor in the process of modulation of the granulomatous response is under current investigation.

Summary

In the present study, we extended the analysis of the regulation of inflammatory lymphokine production in mice with schistosomiasis mansoni. Splenic lymphocytes of chronically infected mice were briefly pulsed in vitro by soluble egg antigens, washed, and then cultured overnight. The supernatant culture fluid added to cultures of splenic cells of acutely infected or peritoneal lymphocytes of antigen-sensitized mice inhibited the production of migration inhibition factor (MIF). Elaboration of MIF suppressor factor (MIF-SF) required the Lyt-1⁻,2⁺,3⁺ subset of T lymphocytes. MIF-SF acted only on egg antigen-primed cells and required H-2 compatibility with the target cell for its suppressive effect. Further analysis with recombinant strains revealed that the factor interacted with I-AB or I-C subregion-compatible target cells. Experiments using immunoadsorbent columns with bound anti-I subregion alloantisera indicated that MIF-SF contained I-C subregion-encoded determinants. Extrapolation of this in vitro model to in vivo conditions would indicate that the granulomatous response is modulated by I region-derived suppressor factor(s) that regulate lymphokine production by T_{DH} effector cells.

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References

1. Andrade, Z. A., and K. S. Warren. 1964. Mild prolonged schistosomiasis in mice: alterations in host response with time and development of portal fibrosis. *Trans. R. Soc. Trop. Med. Hyg.* **58**:53.
2. Domingo, E. O., and K. S. Warren. 1968. Endogenous desensitization: changing host granulomatous response to schistosome eggs at different stages of infection with *Schistosoma mansoni*. *Am. J. Pathol.* **52**:369.
3. Boros, D. L., R. P. Pelley, and K. S. Warren. 1975. Spontaneous modulation of granulomatous hypersensitivity in schistosomiasis mansoni. *J. Immunol.* **114**:1437.

4. Colley, D. G. 1975. Immune responses to a soluble schistosomal egg antigen preparation during chronic primary infection with *Schistosoma mansoni*. *J. Immunol.* **115**:150.
5. Colley, D. G. 1976. Adoptive suppression of granuloma formation. *J. Exp. Med.* **143**:696.
6. Chensue, S. W., and D. L. Boros. 1979. Modulation of granulomatous hypersensitivity. I. Characterization of T lymphocytes involved in the adoptive suppression of granuloma formation in *Schistosoma mansoni*-infected mice. *J. Immunol.* **123**:1409.
7. Colley, D. G., F. A. Lewis, and C. W. Todd. 1979. Adoptive suppression of granuloma formation by T lymphocytes and by lymphoid cells sensitive to cyclophosphamide. *Cell. Immunol.* **46**:192.
8. Chensue, S. W., D. L. Boros, and C. S. David. 1980. Regulation of granulomatous inflammation in murine schistosomiasis. In vitro characterization of T lymphocyte subsets involved in the production and suppression of migration inhibition factor. *J. Exp. Med.* **151**:1398.
9. Chensue, S. W., S. R. Wellhausen, and D. L. Boros. 1981. Modulation of granulomatous hypersensitivity. II. Participation of Ly-1⁺ and Ly-2⁺ T lymphocytes in the suppression of granuloma formation and lymphokine production in *Schistosoma mansoni*-infected mice. *J. Immunol.* **127**:363.
10. Green, W. F., and D. G. Colley. 1981. Modulation of *Schistosoma mansoni* egg-induced granuloma formation: I-J restriction of T-cell-mediated suppression in a chronic parasite infection. *Proc. Natl. Acad. Sci. U. S. A.* **78**:1152.
11. Wellhausen, S. R., S. W. Chensue, and D. L. Boros. 1980. Modulation of granulomatous hypersensitivity. Analysis by adoptive transfer of effector and suppressor T lymphocytes involved in granulomatous inflammation in murine schistosomiasis. In *Basic and Clinical Aspects of Granulomatous Diseases*. D. L. Boros and T. Yoshida, editors. Elsevier/North-Holland, Amsterdam. 219-234.
12. Boros, D. L., K. S. Warren, and R. P. Pelley. 1973. The secretion of migration inhibitory factor by intact schistosome egg granulomas maintained *in vitro*. *Nature (Lond.)*. **246**:224.
13. Boros, D. L., H. J. Schwartz, A. Powell, and K. S. Warren. 1973. Delayed hypersensitivity, as manifested by granuloma formation, dermal reactivity, macrophage migration inhibition and lymphocyte transformation, induced and elicited in guinea pigs with soluble antigens of *Schistosoma mansoni* eggs. *J. Immunol.* **110**:1118.
14. James, S. L., and D. G. Colley. 1975. Eosinophils and immune mechanisms. Production of the lymphokine eosinophil stimulation promoter (ESP) *in vitro* by isolated intact granulomas. *J. Reticuloendothel. Soc.* **18**:283.
15. Rich, S. S., C. S. David, and R. R. Rich. 1979. Regulatory mechanisms in cell-mediated immune responses. VII. Presence of I-C subregion determinants on mixed leukocyte reaction suppressor factor. *J. Exp. Med.* **149**:114.
16. Boros, D. L., and K. S. Warren. 1970. Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from *Schistosoma mansoni* eggs. *J. Exp. Med.* **132**:488.
17. Tada, T., M. Taniguchi, and C. S. David. 1976. Properties of the antigen-specific suppressive T-cell factor in the regulation of antibody responses in the mouse. IV. Special subregion assignment of the gene(s) that codes for the suppressive T-cell factor in the H-2 histocompatibility complex. *J. Exp. Med.* **144**:713.
18. Theze, J., J. A. Kapp, and B. Benacerraf. 1977. Immunosuppressive factor(s) extracted from lymphoid cells of nonresponder mice primed with L-glutamic acid⁶⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT). III. Immunochemical properties of the GAT-specific suppressive factor. *J. Exp. Med.* **145**:839.
19. Green, M. I., A. Pierres, M. E. Dorf, and B. Benacerraf. 1977. The I-J subregion codes for determinants on suppressor factor(s) which limit the contact sensitivity response to picryl chloride. *J. Exp. Med.* **146**:293.

20. Perry, L. L., B. Benacerraf, and M. I. Greene. 1978. Regulation of the immune response to tumor antigen. IV. Tumor antigen-specific suppressor factor(s) bear I-J determinants and induce suppressor T cells *in vivo*. *J. Immunol.* **121**:2144.
21. Liew, F. Y., D. Y. Sia, C. R. Parish, and I. F. C. McKenzie. 1980. Major histocompatibility gene complex (MHC)-coded determinants on antigen-specific suppressor factor for delayed-type hypersensitivity and surface phenotypes of cells producing the factor. *Eur. J. Immunol.* **10**:305.
22. Moorhead, J. W. 1979. Soluble factors in tolerance and contact sensitivity to 2,3-dinitrofluorobenzene in mice. III. Histocompatibility antigens associated with the hapten dinitrophenol serve as target molecules on 2,4-dinitrofluorobenzene-immune T cells for soluble suppressor factor. *J. Exp. Med.* **150**:1432.
23. Germain, R. N., and B. Benacerraf. 1981. A single major pathway of T-lymphocyte interactions in antigen-specific immune suppression. *Scand. J. Immunol.* **13**:1.
24. Beckwith, M., and S. S. Rich. 1982. Suppressor-target interaction in alloantigen induced responses: induction of a second cell in the suppressive pathway. *J. Immunol.* **128**:791.
25. Vadas, M. A., J. F. A. P. Miller, A. M. Whitelaw, and J. R. Gamble. 1977. Regulation of the H-2 gene complex of delayed-type hypersensitivity. *Immunogenetics.* **4**:137.
26. Tada, T., M. Taniguchi, and K. Okumura. 1977. Regulation of antibody response by antigen-specific T-cell factors bearing I-region determinants. *In Progress in Immunology III*. T. E. Mandel, C. Cheers, C. Hosking, I. McKenzie, and G. Nossal, editors. Australian Academy of Science, Canberra City. 369-377.
27. Ptak, W., M. Zembala, and R. K. Gershon. 1978. Intermediary role of macrophages in the passage of suppressor signals between T cell subsets. *J. Exp. Med.* **148**:424.