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Schistosomal Egg Granuloma-Derived Fibroblast-Stimulating Factor Is Apparently Distinct from Interleukin-1

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We have previously reported that egg granulomas isolated from livers of *Schistosoma mansoni*-infected euthymic mice in vitro elaborate a factor(s) that stimulates a variety of fibroblast responses including fibroblast proliferation and enhanced synthesis of extracellular matrix proteins. We have postulated that these factors play a role in the pathogenesis of hepatic fibrosis in schistosomiasis mansoni. Serum-free supernatants from egg granuloma cultures also stimulate thymocyte proliferation in an assay that detects interleukin-1 (IL-1). Thymocytes and fibroblasts are stimulated to proliferate by the same fractions of egg granuloma culture supernatant separated by gel filtration, isoelectric focusing, and ion-exchange chromatography. This suggested that granuloma-derived IL-1 is responsible for the observed fibroblast stimulation. Here we report that the ability of granuloma culture supernatants to stimulate the IL-1-sensitive D10.G4.1 cells but not fibroblasts is removed by treatment with immobilized anti-IL-1 antibody. We also observed that dialyzed culture supernatants from egg granulomas obtained from infected congenitally athymic (nude) mice also stimulate fibroblast proliferation. Treatment with anti-IL-1 antibody did not abrogate this response. In contrast to our experience with egg granulomas isolated from euthymic mice, IL-1- and fibroblast-stimulating activity could be separated by gel filtration and isoelectric focusing. We conclude that the fibroblast growth-stimulating activities elaborated by egg granulomas from *S. mansoni*-infected euthymic and athymic mice may be different but both appear to be distinct from IL-1.

Tissue fibrosis that causes organ dysfunction can occur as a serious complication of a variety of granulomatous diseases (e.g., tuberculosis, sarcoidosis, and schistosomiasis) (3). Recent studies suggest that there is a molecular link between chronic granulomatous inflammation and subsequent fibrogenesis (26). Investigations from our laboratory have shown that soluble products secreted in vitro by isolated schistosomal egg granulomas can stimulate a number of fibroblast responses including proliferation (30) and enhanced extracellular matrix synthesis (28), responses that contribute to scar formation. Studies by several other investigators have shown that soluble products secreted by certain monodispersed inflammatory cells appropriately stimulated in vitro can also activate such fibroblast responses (6, 12, 15, 18, 22, 25). Among the fibrogenic cytokines that have been specifically identified is interleukin-1 (IL-1) (19, 20). IL-1 can promote fibroblast proliferation, collagen and collagenase synthesis, and prostaglandin E_2 secretion (7).

During our earlier characterization studies, we observed that the same fractions prepared by gel filtration, isoelectric focusing, and ion-exchange chromatography from crude egg granuloma supernatants stimulated both fibroblast proliferation and thymocyte proliferation (in an assay that detects IL-1) (29). This suggested the possibility that the granuloma-derived fibroblast-stimulating factor is IL-1. We now report evidence that these factors are in fact distinct. Furthermore, in related studies, we determined that egg granulomas obtained from congenitally athymic mice also elaborate a fibroblast-stimulating factor that is apparently distinct from IL-1.

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MATERIALS AND METHODS

Animals and infection. Female C57BL/6 mice 5 to 7 weeks old (Taconic Farms, Germantown, N.Y.) were infected at the University of Lowell Research Foundation (Lowell, Mass.) by intraperitoneal injection of approximately 50 cercariae of *Schistosoma mansoni* (Puerto Rico strain) suspended in sterile 0.15 M sodium chloride solution. Congenitally athymic outbred (NIH Swiss background) female mice (Taconic Farms) were infected at New England Medical Center Hospital in a similar manner.

Isolation of granulomas and preparation of granuloma supernatant. At 7 to 8 weeks after infection, mice were killed by inducing CO_2 narcosis. Livers were removed aseptically into chilled Hanks balanced salt solution (HBSS). Livers from individual outbred nude mice were processed separately; livers from inbred euthymic mice were pooled and processed. They were washed three times with cold HBSS and homogenized in the presence of HBSS with a Waring blender. Intact viable granulomas were isolated from the liver homogenate by serial sedimentation at $1 \times g$ as previously described (30). To adjust for the reduced cellularity of athymic granulomas (4), both athymic and euthymic granulomas were washed, pelleted ($200 \times g$), and then suspended to a 10% (vol/vol) density in RPMI 1640 medium supplemented with 4 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml, and incubated for 24 h at 37°C in an atmosphere of 5% CO_2 and 95% air. Cell-free supernatants were obtained after centrifugation ($200 \times g$ for 10 min at 20°C). These were dialyzed (6- to 8-kilodalton nominal exclusion; American Scientific Products, McGaw Park, Ill.) at 4°C against two changes of large volumes of HBSS and finally against RPMI 1640 medium. Prior to immunoaffinity adsorption and isoelectric focusing, culture supernatants from athymic granulomas were concentrated

(three to five times) by vacuum dialysis (nominal exclusion, 12 kilodaltons). In our initial experiments, we found that biological activity was lost from unconcentrated but not concentrated athymic granuloma supernatants when these were incubated in polypropylene tubes or subjected to isoelectric focusing.

Treatment of granuloma culture supernatant with anti-IL-1 antiserum. Antibody to recombinant IL-1 was elicited in New Zealand White rabbits. Recombinant human IL-1α and IL-1β were produced and used separately to immunize rabbits (8). The antisera prepared in this manner neutralize only the specific IL-1 form; they are not cross-reactive. The antisera were conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) by standard methods (5). The Sepharose beads were washed in 0.1 M glycine to bind all remaining reactive sites following antibody conjugation. Just prior to their use, the beads were washed several times with phosphate-buffered saline (PBS) (0.15 M, pH 7.4) and equilibrated with serum-free RPMI 1640 medium. Concentrated granuloma supernatant from athymic mice or unconcentrated granuloma supernatant from euthymic mice (2 ml) was mixed with an equal volume of conjugated beads and incubated at 37°C for 2 to 3 h in polypropylene culture tubes (Falcon; Becton Dickinson Labware, Lincoln Park, N.J.). The tubes had been pretreated with bovine serum albumin (0.5 mg/ml) in PBS to diminish nonspecific adsorption of bioactive material. The supernatant was retrieved following centrifugation (1,000 × *g* for 10 min at 20°C) and was filter sterilized. Conjugated beads were reused after being washed three to four times with 0.2 M citric acid (pH 2.2) and then PBS (pH 7.4). The regenerated conjugated beads were stored at 4°C in the presence of PBS containing 0.05% sodium azide (Sigma Chemical Co., St. Louis, Mo.) and thoroughly washed (see above) just prior to reuse.

Gel filtration chromatography. Gel filtration chromatography was performed at 4°C with a column (1 by 40 cm) of Bio-Gel P-30 (Bio-Rad Laboratories, Rockville Center, N.Y.) equilibrated with PBS (pH 7.4). The column was calibrated with blue dextran, ovalbumin, chymotrypsin A, RNase A (gel filtration calibration kit; Pharmacia, Inc., Piscataway, N.J.), carbonic anhydrase, and myoglobin (gel filtration markers; Sigma). Approximately 1 ml of unconcentrated dialyzed granuloma culture supernatant from either athymic or euthymic mice was loaded onto the column, which was then run with PBS (pH 7.4) at a rate of 5 to 6 ml/h. Fractions of 1 ml were collected and dialyzed either separately or in pools of 2 to 3 ml at 4°C (nominal exclusion, 6 to 8 kilodaltons) against two changes of HBSS followed by RPMI 1640 medium. Retentates were filter sterilized.

Isoelectric focusing. Preparative isoelectric focusing of granuloma supernatant was done as described previously (29). Briefly, 4 to 5 ml of fivefold-concentrated granuloma culture supernatant from athymic mice was mixed with a solution containing 4.5 ml of Ampholine (LKB-Produkter AB, Bromma, Sweden) pH 3.5 to 10; 0.5 ml of Ampholine pH 5 to 8; 4 g of Ultrodex (LKB); and enough distilled water to bring the final volume to 100 ml. Equine cytochrome *c* (Sigma) (2 to 3 mg) was added to the final mixture as a marker. This mixture was poured onto a glass tray (22 by 10 cm) and was dried with a low-speed fan until the surface developed a pasty appearance. Electrical contact with the flat bed was established with filter paper saturated with NaOH (0.1 M) and acetic acid (0.5 M). The flat bed was placed on a cooling plate (~4°C) and was subjected to a constant power of 8 W (~900 V) for 18 h with an LKB power supply (model 2197). Slices 1 cm wide were removed from the flat bed and mixed with 2 ml of distilled water; the pH of the fractions was determined. PBS (pH 7.4) (2 ml) was then added to the fractions, and supernatants were retrieved by centrifugation (1,000 × *g* for 10 min) and dialyzed for 24 h. Fractions were filter sterilized.

Fibroblasts. Human diploid fibroblast cultures were established from newborn foreskin (29). Cells were suspended in supplemented RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) and grown to confluency in polystyrene tissue culture flasks (Costar, Cambridge, Mass.). Confluent fibroblasts were passaged twice weekly. For the fibroblast proliferation assay, 5×10^4 to 6×10^4 cells in supplemented medium were seeded in a 1-ml volume in each well of a 24-well polystyrene tissue culture plate and incubated overnight. Cells were then washed and replenished with serum-free medium. Twenty-four hours later, test samples were added (total volume, ~100 μl) and incubated from 24 h. Tritiated thymidine (1 μCi; specific activity, 6.7 Ci/mM; Dupont, NEN Research Products, Boston, Mass.) was added per well for the final 4 h of incubation. Cells were trypsinized and harvested onto glass fiber filters with an automatic cell harvester (Titertek; Flow Laboratories, McLean, Va.). The magnitude of [^{3}H]thymidine uptake was determined by scintillation spectrometry (model LS7000 spectrophotometer; Beckman Instruments, Inc., Fullerton, Calif.). We have previously demonstrated that stimulated net uptake of [^{3}H]thymidine by fibroblasts in this assay is an appropriate measure of fibroblast proliferation (30).

IL-1 assay. IL-1 activity in granuloma supernatants was detected in a comitogen assay that employed either thymocytes (29) or the D10.G4.1 cloned T-cell line (13) as the indicator cells. In the former assay, 10^6 thymocytes per ml from 6- to 12-week-old female A/J mice (Jackson Laboratory, Bar Harbor, Maine) were cultured in round-bottom 96-well plates (Nunc, Roskilde, Denmark) in minimal essential medium containing 5% FBS, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer (supplemented minimal essential medium), and 2 μg of phytohemagglutinin (Difco Laboratories, Detroit, Mich.) per ml. Cells were cultured for a total of 72 h at 37°C in a 5% CO_2–95% humid air atmosphere. [^{3}H]thymidine (0.5 μCi) was added for the final 24 h of incubation. Cells were harvested with a cell harvester (model M12V; Brandel, Rockville, Md.) onto glass fiber filters that were dried, placed in scintillation vials, and counted in the presence of 5 ml of Econofluor (Dupont, NEN Research Products) in a liquid scintillation spectrometer. For most assays, D10.G4.1 cloned T-cells were used. A total of 2×10^4 cells per well were cultured and otherwise treated in a manner identical to that described for thymocytes. The arithmetic mean counts per minute (cpm) determined in triplicate cultures were calculated. In all cases, the standard error of the mean (SEM) was ≤10% of the mean.

IL-2 and IL-4 assays. Detection of interleukin-2 (IL-2) and interleukin-4 (IL-4) was performed with the CTLL-2 cell line (1). The assay was performed by a slight modification of methods described previously (10). Assay cells were washed free of growth medium and suspended in RPMI 1640 supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM glutamine. A total of 4,000 cells (50 μl) were placed in each well of a 96-well microplate (Costar 3596; Costar Data Packaging, Cambridge, Mass.) followed by 50 μl of serially diluted samples. Microplates were incubated at 37°C in a humidified atmosphere of 5% CO_2–95% air. At 20 h, the cells were pulsed for 4 to 6 h with 0.5

μCi of [^{3}H]thymidine (specific activity, 6.7 Ci/mM). Cultures were harvested onto glass fiber filters with a manifold harvester (Richter Scientific, Vancouver, Canada) and washed extensively with water. Dried filters were placed in a solution of toluene and Omnifluor (Dupont, NEN Research Products), and their radioactivity was counted in a scintillation spectrometer.

To determine the proliferative response of the cells to IL-2 or IL-4 specifically, we used monoclonal antibodies specific for either IL-2 or IL-4. The rat monoclonal antibody specific for murine IL-4, 11B11, was produced and provided by J. Ohara and W. Paul (17). Anti-IL-2 antibody was a gift of T. Mosmann (DNAX Corp., Palo Alto, Calif.).

Production of IL-4- and IL-2-containing supernatants. IL-4-containing supernatants were prepared from a helper T-cell line (CDC35) by a previously described procedure (24). T cells were suspended at 10^6 cells per ml in fresh medium containing 10% FBS and 5 μg of concanavalin A (ConA) per ml and cultured for 24 h. ConA was removed by passing the supernatants over *p*-aminophenyl-α-mannoside (Sigma) coupled to cyanogen bromide-activated Sepharose 4B. IL-2-containing supernatant was prepared by incubating rat spleen cells at 10^7 cells per ml for 24 h in medium containing 10% FBS and 10 μg of ConA per ml (21). Harvested supernatant was precipitated with $(NH_4)_2SO_4$, dialyzed, and used at the indicated concentrations.

RESULTS

Granulomas from euthymic and athymic mice secrete D10.G4.1-stimulating and fibroblast-stimulating activities. Dialyzed culture supernatants from granulomas of euthymic mice induced [^{3}H]thymidine incorporation in human diploid fibroblasts and in IL-1-responsive D10.G4.1 cells. The magnitude of [^{3}H]thymidine uptake by both cell types was dose dependent in a similar manner (Fig. 1A). These observations with the D10.G4.1 cell assay confirm our previous finding with the thymocyte assay that egg granulomas of euthymic mice elaborate a factor(s) that is similar to IL-1 in its ability to stimulate growth of T cells and fibroblasts. We also established for the first time that supernatant of egg granulomas obtained from *S. mansoni*-infected athymic (nude) mice also contains fibroblast-stimulating activity (Fig. 1B) with similar dose dependence.

Egg granuloma culture supernatants from euthymic and athymic mice do not contain IL-2 or IL-4. Although growth stimulation of D10.G4.1 cells has been widely used as an assay for IL-1, it recently has been determined that this cell line produces an autocrine growth factor, IL-4 (16), in response to IL-1 and a costimulant. These cells also grow in response to IL-2 (14). Therefore, to consider the growth responses by D10.G4.1 cells to be appropriate assessments of the presence of IL-1 in test material, it was important for us to also assay for the presence of IL-2 or IL-4 in our culture supernatants.

CTLL-2, a cytotoxic T-cell line, is stimulated to grow by both IL-2 and IL-4. CTLL-2 cells were incubated in the presence of culture supernatants of egg granulomas obtained from infected euthymic mice or athymic mice (euthymic supernatant or athymic supernatant), IL-2-containing medium, or IL-4-containing medium. Neither euthymic nor athymic culture supernatants stimulated the growth of these cells (Table 1). The inability of supernatants to stimulate the cells at lower dilutions was not due to the presence of an inhibitor since addition of egg granuloma culture supernatants (50%, vol/vol) did not alter the response of cells to IL-2- or IL-4-containing medium (data not shown).

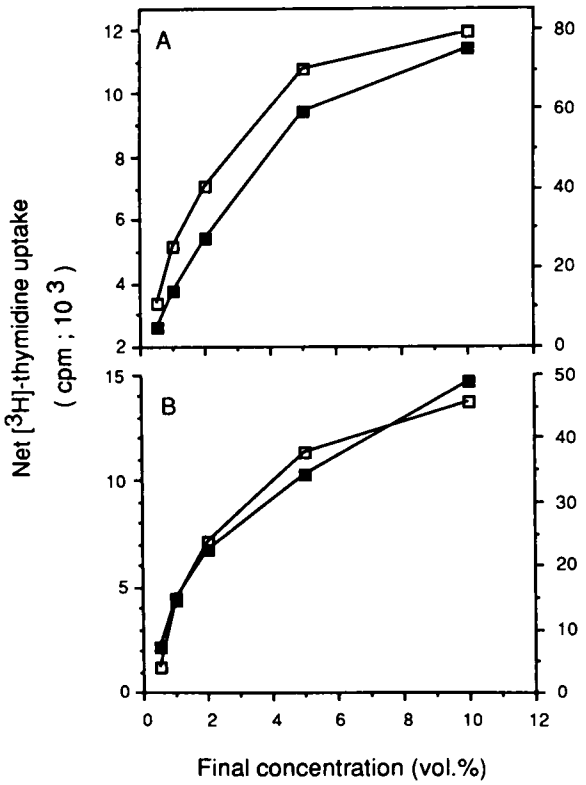

FIG. 1. Net tritiated thymidine uptake by fibroblasts (□; ordinate on left) and D10.G4.1 cells (■; ordinate on right) in response to culture supernatants of granulomas obtained from *S. mansoni*-infected euthymic mice (A) and athymic mice (B). Final concentration of unconcentrated dialyzed supernatant is shown. Each point represents triplicate determinations in a representative experiment (of five performed) (SEM ≤10% of the mean in all cases). The magnitude of tritiated thymidine uptake by fibroblasts or D10.G4.1 cells incubated in the absence of granuloma supernatant was reflected by cpm of approximately 3,000 and 15,000 to 20,000, respectively.

Anti-IL-1 antibody removes D10.G4.1-stimulating activity but not fibroblast-stimulating activity from granuloma culture supernatants. Since egg granuloma culture supernatants from euthymic or athymic mice do not contain either IL-2 or IL-4, it seems likely that D10.G4.1-stimulating activity is due to IL-1. To confirm this, we attempted to remove the activity that stimulates D10.G4.1 cells by adsorption with rabbit anti-human IL-1 (α and β) antibody conjugated to Sepharose 4B. When egg granuloma culture supernatant from euthymic mice was incubated with this immunoadsorbant, greater than 70% of the D10.G4.1-stimulating activity was removed. This treatment, however, did not reduce the fibroblast-stimulating activity (Table 2). Incubation of granuloma culture supernatants from athymic mice with the immunoadsorbant removed approximately 80% of the D10.G4.1-stimulating activity but did not significantly reduce the fibroblast-stimulating activity. Since treatment of samples of the same granuloma supernatant preparations with normal serum coupled to Sepharose 4B reduced neither the D10.G4.1-stimulating activity nor the fibroblast-stimulating activity (Table 2) (27), we conclude that the effects of treatment with immobilized anti-IL-1 antiserum resulted from specific adsorption of IL-1.

IL-1 activity and fibroblast-stimulating activity in granuloma culture supernatants from athymic mice are physically

TABLE 1. [^{3}H]thymidine uptake by CTLL-2 cells

Final concn of supernatant (% vol)	Net [^{3}H]thymidine uptake (cpm) by CTLL-2 cells in response to:			
	IL-2[a]	IL-4[b]	Granuloma supernatant[c]	
			Nude[d]	Euthymic
50			18	29
10	14,564	2,248	42	41
5	15,846	1,726		
2.50	12,808	1,154		
2			19	31
1.250	6,599	680		
0.625	8,226	499		
0.400			23	18
0.320	7,368	250		
0.160	3,828	62		
0.080	1,335	37	35	26
0	42			

[a] IL-2-containing supernatant was prepared by incubating rat splenic cells at 10^7 cells per ml with ConA (10 μg/ml) for 24 h at 37°C.

[b] Cells from the helper T-cell line CDC35 were suspended at a density of 10^6/ml in medium containing 10% FBS and stimulated with ConA (5 μg/ml) to yield IL-4 containing supernatant.

[c] Granulomas from *S. mansoni*-infected congenitally athymic (nude) or euthymic mice were cultured in serum-free medium at 37°C for 24 h.

[d] [^{3}H]thymidine uptake by CTLL-2 cells cultured in the presence of the relevant supernatant. Each value is a mean of three determinations (SEM of ≤10% of the mean).

separable. We were able to separate IL-1 and fibroblast-stimulating activity by gel filtration and isoelectric focusing. When granuloma culture supernatant from athymic mice was subjected to gel filtration chromatography, IL-1 activity and the fibroblast-stimulating activity eluted from Bio-Gel P-30 with distinct profiles (Fig. 2). Peak fibroblast-stimulating activity was present in fractions with an estimated M_r of ~25,000; peak IL-1 activity was present in fractions with an estimated M_r of ~17,000 to 18,000. These activities were also separable by isoelectric focusing (Fig. 3). Maximum fibroblast-stimulating activity was present in fractions with a pH of approximately 6.5; maximum IL-1 activity, on the other hand, was present in fractions with pHs of 7.3 to 7.5. Our ability to separate IL-1- and fibroblast-stimulating activity present in athymic supernatants by these physicochemical methods contrasts with our prior studies in which we observed that the two activities cofractionated (29).

TABLE 2. Adsorption of granuloma culture supernatant with anti-IL-1 antibody conjugated to Sepharose 4B

Source of granuloma supernatant	Treatment of granuloma supernatant[a]	Net [^{3}H]thymidine uptake (cpm) by:	
		D10.G4.1 cells	Fibroblasts
Euthymic mice	None[b]		
	1:20	101,120[c]	16,670[d]
	1:50	85,750	8,640
	Immobilized normal serum		
	1:20	97,150	12,360
	1:50	74,600	6,370
	Immobilized anti-IL-1		
	1:20	59,470	12,276
	1:50	27,340	6,414
Athymic mice	None (1:10[e])	17,286	16,602
	Immobilized normal serum (1:10[e])	15,157	22,800
	Immobilized anti-IL-1 (1:10[e])	2,050	19,214

[a] Granuloma culture supernatants were incubated for 2 to 3 h with either normal serum or rabbit anti-human IL-1 antibodies conjugated to Sepharose 4B. Unbound material was assayed in the fibroblast proliferation and IL-1 assays. Values represent a mean of triplicate determinations (SEM of ≤10% of the mean).

[b] Control, untreated granuloma supernatant maintained for 2 to 3 h at 37°C.

[c] Baseline cpm (untreated D10.G4.1 cells) = 35,000 ± 2,200.

[d] Baseline cpm (untreated fibroblasts) = 3,500 ± 550.

[e] Concentrations at which maximum response of D10.G4.1 cells were observed in this experiment.

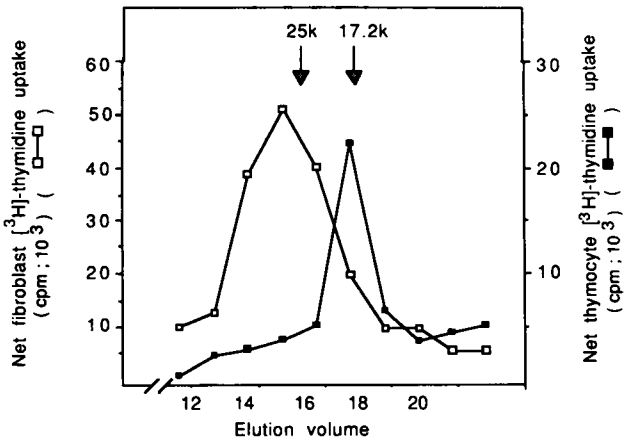

FIG. 2. Gel filtration chromatography of unconcentrated granuloma culture supernatant from *S. mansoni*-infected athymic mice (Bio-Gel P-30). Samples of each fraction were assayed for the ability to stimulate tritiated thymidine uptake by fibroblasts and thymocytes at optimal concentrations. Each point represents a mean of three determinations (SEM of ≤10% of the mean). Elution positions of relevant molecular weight standards are shown. k, 10^3.

DISCUSSION

Serum-free culture supernatants of egg granulomas obtained from the livers of *S. mansoni*-infected euthymic and athymic mice can stimulate proliferation of fibroblasts, thymocytes, and D10.G4.1 cells (29) (Fig. 1). We previously reported that the fibroblast- and thymocyte-stimulating activities present in culture supernatants of egg granulomas from euthymic mice were present in the same fractions prepared by gel filtration, isoelectric focusing, and ion-exchange chromatography. Furthermore, macrophages isolated from egg granulomas of athymic mice spontaneously elaborate thymocyte-stimulating activity as detected in an IL-1 assay (23). These observations prompted the present study to determine more precisely whether the thymocyte and fibroblast growth-stimulating activities both derive from the presence of IL-1 in the granuloma supernatants.

The proliferative response of D10.G4.1 cells is a highly sensitive bioassay for IL-1. Concentrations as low as 1 pg/ml stimulate these cells to proliferate (8). Since IL-2 alone can also promote the proliferation and IL-4 can augment the ability of IL-1-dependent growth of D10.G4.1 cells (14, 16), we first assessed whether the granuloma culture supernatants contained IL-2 or IL-4. Since we found that these supernatants failed to stimulate IL-2- and IL-4-responsive CTLL-2 cell lines (Table 1), we conclude that the granulomas we studied do not elaborate detectable amounts of these interleukins. We cannot of course exclude that IL-2 or IL-4 is produced by cells of granuloma and is rapidly sequestered by other cells within the granuloma. In any case, the absence of detectable IL-2 and IL-4 activities in the supernatants

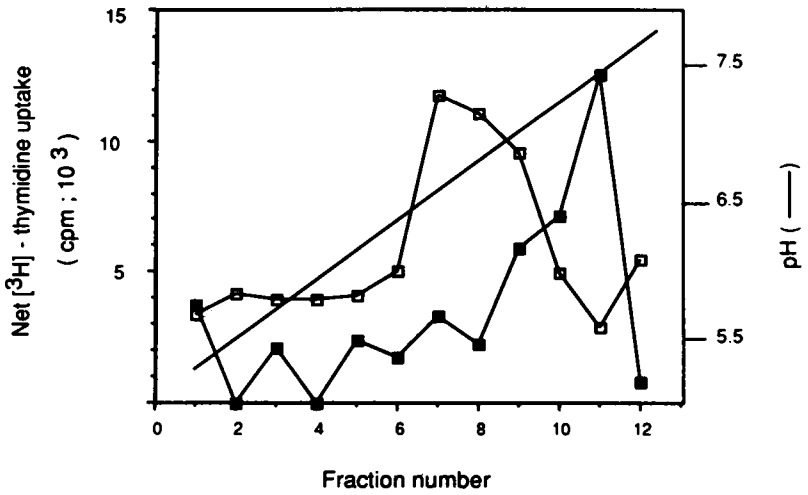

FIG. 3. Isoelectric focusing of crude athymic granuloma supernatant from *S. mansoni*-infected athymic mice. Each fraction was assayed for its ability to stimulate tritiated thymidine uptake by fibroblasts (□) and thymocytes (■). Fractions at the extreme cathodic and anodic ends were not tested in the assay.

excludes the participation of these lymphokines in the IL-1 assay. Moreover, since D10.G4.1-stimulating activity was substantially reduced following treatment with Sepharose-conjugated IL-1 antibody, it appears that the response of these indicator cells is primarily due to the presence of IL-1 in granuloma culture supernatants.

Although we used immobilized anti-human IL-1 antibody for adsorption of mouse-derived culture supernatants, the sequence homology of each form of IL-1 (α and β) between human and mouse is greater that 78% in the mature protein (11). Therefore, it is not surprising that most of the IL-1 activity in the egg granuloma supernatants is removed by these antibodies. The ability of treatment with anti-IL-1 antibody to reduce D10.G4.1-stimulating activity, however, contrasts with the inability of this treatment to remove fibroblast-stimulating activity from granuloma culture supernatants (Table 2). Our observation that the two cell types exhibited similar patterns of dose dependence to the untreated granuloma supernatants (Fig. 1) suggests similar sensitivities of these cells to the respective granuloma-derived growth factor(s). Accordingly, we consider it unlikely that residual IL-1 which might have been present after adsorption with anti-IL-1 accounted for the unmodified fibroblast responses. The findings suggest that IL-1 is not the primary fibroblast mitogen elaborated by egg granulomas. On the other hand, we do not exclude the possibility that granuloma-derived IL-1 serves as an accessory factor in promoting fibroblast stimulation. In related studies, we also have determined that the granuloma-derived growth factor is not transforming growth factor β or tumor necrosis factor (S. Prakash, J. Keske-Oja, B. Sherry, and D. Wyler, unpublished data).

Our conclusion that the major fibroblast growth-stimulating activity in granuloma supernatants is not IL-1 is supported by the results of our fractionation experiments with athymic granuloma supernatant. Fractions prepared by gel filtration (Fig. 2) or preparative isoelectric focusing (Fig. 3) that maximally stimulated fibroblasts were distinct from those that were maximally stimulatory in an IL-1 assay. This contrasts with our prior observations with euthymic granuloma supernatants in which both activities cofractionated. The basis for this distinction between euthymic and athymic granuloma supernatants is uncertain but suggests that the fibroblast-stimulating factors from the two sources are different. In our present studies, immunoadsorption proved useful in discriminating fibroblast-stimulating factors that apparently exhibit similar physicochemical properties. IL-1 activity and fibroblast-stimulating activity in macrophage supernatants have been separated by others using standard chromatographic techniques (2, 9). On the other hand, there are also reports of identification of a macrophage-derived fibroblast factor as IL-1 based solely on cofractionation of biological activities (19, 20). More rigorous criteria are obviously now needed to substantiate these conclusions in light of the recent recognition that these assays also detect cytokines other than IL-1 (14, 31).

Conditions may exist in vivo under which IL-1 might serve as an important fibroblast-stimulating factor. Most studies of this property of IL-1 have utilized culture supernatants from monocytes or macrophages stimulated in vitro (19), establishing a potential fibrogenic role for this cytokine. Our studies employ explants of chronic inflammatory cells stimulated in vivo. We believe that the cytokines spontaneously elaborated by these cells in explant cultures are also likely to be secreted in vivo. If this premise is correct, the results of our present investigation implicate a cytokine(s) other than IL-1 in the pathogenesis of hepatic fibrosis in schistosomiasis (26).

In related studies, we determined that culture supernatants from athymic granulomas contain a dialyzable substance that inhibits the fibroblast growth-stimulating activity also present in these supernatants (S. Prakash, A. E. Postlethwaite, G. P. Stricklin, and D. J. Wyler, submitted for publication). Furthermore, we found that athymic granulomas, in contrast to euthymic granulomas, do not elaborate factors that stimulate collagen or glycosaminoglycan synthesis by fibroblasts. These findings presumably explain why athymic granulomas fail to become fibrotic. Inasmuch as athymic granulomas lack mature T cells and eosinophils (4), our observations implicate a role for one or both of these cells in production of fibrogenic factors by euthymic granulomas.

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