TRYPANOSOMA CRUZI: THE IMMUNOLOGICAL INDUCTION OF MACROPHAGE PLASMINOGEN ACTIVATOR REQUIRES THYMUS-DERIVED LYMPHOCYTES*

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Macrophages have been implicated for many years as an important effector cell in host defense against a variety of facultative and obligate intracellular parasites (1–9). Their ability to spread, to phagocytize, and to display microbicidal and tumoricidal effects are enhanced in the course of these infections and the macrophages are said to be activated. Some of the activated macrophage properties have been achieved in vitro by lymphocyte mediators (10). Prior studies of others have demonstrated the requirement of thymus-derived lymphocytes in the adoptive transfer of anti-bacterial immunity (11, 12). We have shown in the preceding paper that, in the course of BCG and *Trypanosoma cruzi* infection in mice, macrophages become activated as indicated by their enhanced spreading and phagocytic capacities, their ability to secrete plasminogen activator, and their ability to kill trypomastigotes of *T. cruzi* in vitro (13).

In this paper, we report that normal, unstimulated macrophages can be activated in vitro by a lymphocyte product(s) derived from the interaction of sensitized peritoneal or spleen cells with *T. cruzi* antigen. This activation is expressed as the secretion of high levels of plasminogen activator and requires the participation of T lymphocytes.

Materials and Methods

Parasites. The Y strain of *T. cruzi* was obtained from Dr. S. C. Correa, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, and the Tulahuén strain from Dr. B. Bloom, Albert Einstein School of Medicine, Bronx, N. Y. The parasites were grown in Tobie's medium and passed weekly (14). Parasites were harvested from 8-day-old cultures, washed five times with ice-cold phosphate-buffered saline (PBS)[1] (Dulbecco's, Grand Island Biological Co., Grand Island, N. Y.) at 750 *g* for 15 min, resuspended in ice-cold PBS or Dulbecco's modified Eagle's medium (GIBCO), and counted in a hemocytometer with a 40 × objective. The cultures of Y strain contained 85% epimastigotes and 15% trypomastigotes. The Tulahuén strain contained 95% epimastigotes. Purified trypomastigotes were obtained as previously described (15).

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* Supported in part by grants AI 07012 and AI 12975 from the U. S. Public Health Service.

‡ Scholar of the Leukemia Society of America, Inc.

[1] *Abbreviations used in this paper:* AT-DS, acid-treated dog serum; D2FBS, Dulbecco's medium containing 2% fresh fetal bovine serum plus 5 × 10^{-5} M mercaptoethanol; D10FBS, Dulbecco's medium containing 10% heat-inactivated fetal bovine serum plus 5 × 10^{-5} M mercaptoethanol; FBS, fetal bovine serum; HBSS, Hanks' balanced salt solution; HKT, heat-killed trypanosomes; ME, mercaptoethanol; MIF, migration inhibitory factor; PBS, phosphate-buffered saline; PPD, purified protein derivative of tuberculin.

Cell Cultures

NORMAL MACROPHAGES. Mouse peritoneal macrophages were obtained from female Swiss mice maintained at The Rockefeller University, and C57BL/6J, A/J, and C3H/HeJ mice obtained from The Jackson Laboratory, Bar Harbor, Maine. Cells were harvested according to the methods of Cohn and Benson (16). They were cultivated on 13-mm round glass cover slips or in ^{125}I-fibrin-coated 16-mm Linbro plates (Linbro Chemical Co., New Haven, Conn.), prepared as previously described (17) in Dulbecco's medium and 2% fresh fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. 2×10^6 total peritoneal cells were plated per well, 45–50% of which were macrophages.

T. CRUZI-"PRIMED" PERITONEAL CELLS. NCS, C57BL/6J, A/J, and C3H/HeJ mice were infected intraperitoneally with 5×10^6 live culture forms of *T. cruzi*, Y strain, in PBS. At several times after the infection, peritoneal cells were harvested, usually pooled from three to four mice. Average yields were 10×10^6 cells per mouse, from which 45–50% were lymphocytes, 45–50% macrophages, and about 5% polymorphonuclear leukocytes. 4×10^6 total peritoneal cells were plated on nonradiaoctive 16-mm Linbro plates in Dulbecco's medium plus 2% FBS, 5×10^{-5} M mercaptoethanol (ME) (D2FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, and glutamine, in the presence of the desired antigen.

T. CRUZI-PRIMED SPLEEN CELLS. Spleens were removed from three to four mice infected as described above, pooled, teased with forceps, and passed through a sterile stainless steel wire mesh. The screen was washed with cold medium, and the cells dispersed by pipetting the suspension up and down several times. The cells were then washed once in cold D2FBS, resuspended at the desired concentration in the same medium, and plated in nonradiaoctive Linbro plates in the presence of the desired antigen.

NYLON-WOOL SEPARATION OF LYMPHOCYTES. Suspension of spleen or peritoneal cells were obtained in Dulbecco's 10% heat-inactivated FBS plus 5×10^{-5} M ME (D10FBS) and overlayed on a nylon-wool column (Fenwal Laboratories, Inc., Morton Grove, Ill.), preincubated with D10FBS at 37°C for 1 h (18). The column containing the cells was then incubated at 37°C for 45 min, and the cells eluted with 50 ml of D10FBS. The cells were washed once, counted, suspended to the desired concentration in D2FBS, and plated on nonradioactive Linbro plates containing a monolayer of normal macrophages (2×10^5 macrophages per well), in the presence of the desired antigen.

ANTI-Thy 1.2 AND ANTI-κ TREATMENT OF SPLEEN CELLS. 10^8 spleen cells were incubated for 20 min at 4°C in 1 ml of anti-Thy 1.2 serum (AKR anti-SBLE thymocytes) diluted 1:10 in Dulbecco's medium or in 1 ml of anti-κ serum (rabbit anti-κ γ-myeloma) diluted 1:100 in D2FBS. Both antisera were kindly supplied by Dr. U. Hämmerling of The Sloan-Kettering Cancer Institute, New York. The cells were centrifuged and incubated for 45 min at 37°C in 3 ml of a selected rabbit complement (C) (for anti-Thy 1.2 treatment) or guinea-pig C (for anti-κ treatment) diluted 1:22 in D2FBS in the presence of 30 μg/ml DNase (from beef pancreas, noncrystalline, 1,180 Kunitz units/mg protein; lot no. 73C-0320; Sigma Chemical Co., St. Louis, Mo.). The cells were then washed and resuspended to the desired concentration in D2FBS. Controls were immune cells incubated with the antisera or C alone and untreated spleen cells from noninfected mice.

Antigens. Heat-killed trypanosomes (HKT) were obtained by mild heat-treatment (80°C/10 min) of a suspension of 5×10^6 culture forms/ml PBS (five times washed) of either Y or Tulahuén strains; purified protein derivative of tuberculin (PPD), 2 mg/ml was obtained from Connaught Medical Research Laboratory, Willowdale, Ontario, Canada.

Reagents. Acid-treated dog serum (AT-DS) was prepared by adjusting the pH of dog serum (GIBCO) to 2 with 2 N HCl in isotonic saline; after 30 min at room temperature the pH was adjusted to 7.4 with 2 N NaOH in isotonic saline and filter sterilized. Polystyrene latex particles 1.101 μm were obtained from Dow Diagnostics, Indianapolis, Ind.

Fibrinolytic Assay. The assay was performed in a two-step process. First, supernates were prepared by incubation of cells with antigen. These supernates were then added to normal, unstimulated mouse macrophages plated on ^{125}I-fibrin-coated Linbro plates, incubated for 1–3 days, at the end of which fibrinolytic activity was assayed.

PREPARATION OF SUPERNATES. Control and *T. cruzi*-primed total or fractionated peritoneal or spleen cells were incubated with the antigen at 37°C in a CO_2 atmosphere for the desired period of time. The supernates were then collected, centrifuged at 750 g for 15 min to remove cells and debris, and millipored in a 0.45 μm Millex filter (Millipore Corp., Bedford, Mass.). These

supernates are very stable upon storage at −20°C and −70°C and activity was found to be preserved over 4 mo.

INDUCTION OF FIBRINOLYTIC ACTIVITY IN UNSTIMULATED MACROPHAGES. The supernates were added at the desired concentration to monolayers of normal macrophages (kept for 2 h at 37°C after explanting, washed twice in Hank's balanced salt solution (HBSS) to remove nonadherent cells), on ^{125}I-fibrin-coated Linbro wells, prepared as described (17). The plates contained 10 μg fibrinogen/cm^2 and 5 × 10^5 trypsin-releasable cpm. Supernatant concentration was 25%, unless stated otherwise. The cells were incubated for 1–3 days in D2FBS. The fibrinolytic activity was then assayed by washing the cells twice in HBSS, followed by incubation in 0.5 ml Dulbecco's medium containing 5% AT-DS. 100-μl samples of medium were withdrawn and assayed for release of radioactivity in a Packard gamma counter (Packard Instruments, Inc., Downers Grove, Ill.). Secretion of plasminogen activator was measured in samples of conditioned medium from normal macrohages exposed to the supernatant fluids, washed, and cultivated in serum-free medium for 24 h and assayed as previouly described (13).

Results

To obtain a better understanding of the mechanisms involved in macrophage activation, attempts were made to carry out this process under in vitro conditions. Two parameters of macrophage activation were of particular interest: the trypanocidal activity against trypomastigotes of *T. cruzi* and secretion of plasminogen activator. For this purpose, a two-step system was employed. First, peritoneal or spleen cells from mice infected with *T. cruzi* were incubated in vitro with HKT and cell-free supernatant fluids prepared. Next, normal unstimulated macrophages were exposed to these supernatant fluids for varying periods of time. The cells were then either infected with *T. cruzi* or assayed for enzymatic activity. No trypanocidal or trypanostatic activity has as yet been induced under these conditions. Because of these difficulties we chose to focus on the induction of enzyme secretion.

Enhanced Secretion of Plasminogen Activator by Normal Macrophages Induced by Antigen-Stimulated Sensitized Cells. Fig. 1 shows a representative experiment in which mixed peritoneal cells from 4-wk-infected mice were exposed in vitro to viable trypomastigotes of *T. cruzi*. 72 h later supernatant fluids were collected, millipored, and incubated for 3 days with normal macrophages. These cells exhibited a 10-fold increase in fibrinolytic activity. Similar results were obtained when HKT were used, indicating that the supernatant activity was not dependent on the living parasite. HKT were therefore used as antigen in all the subsequent experiments. Control supernates were obtained by incubating peritoneal cells from noninfected mice with the same antigens. Additional controls included supernatant fluids derived from the incubation of immune or control cells without antigen (S No Ag) and finally macrophages incubated without any added supernate (No S). No fibrinolysis was observed when supernates were added in the absence of macrophages.

The characteristics of this induced fibrinolytic activity in normal macrophages was in all respects similar to that observed with in vivo activated cells and described in the accompanying paper (13). The activity was plasminogen dependent, linear with time over the 5- to 6-h assay period, and predominantly associated with the extracellular medium. This suggests that the induced fibrinolytic activity in these in vitro activated macrophages is due to an increased secretion of plasminogen activator.

The Time-Course of Plasminogen Activation Induction. Next, optimal con-

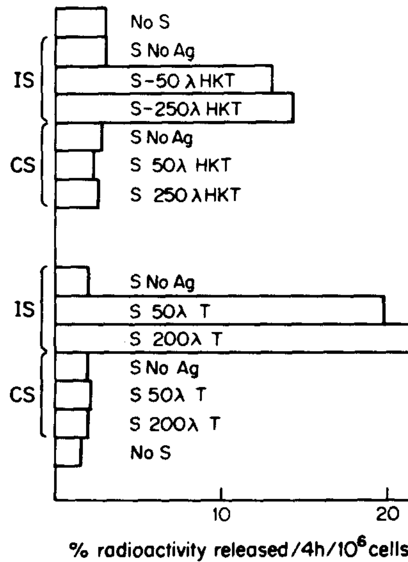

FIG. 1. Enhanced fibrinolytic activity by normal macrophages induced by supernatant fluids from antigen-exposed sensitized peritoneal cells from 4-wk *T. cruzi*-infected mice. Antigens were live trypomastigotes or heat-killed mixed culture forms (HKT). HKT and T concentration was 5×10^6/ml. IS, supernatant fluids from incubated sensitized cells with or without antigen exposure. CS, supernatant fluids from incubated control cells with or without antigen exposure.

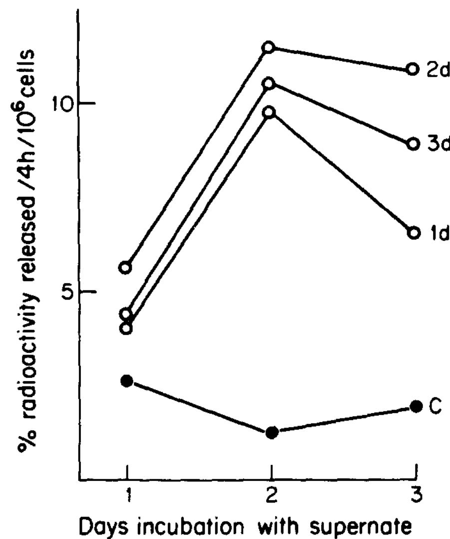

FIG. 2. In vitro time-course of generation of active supernatant fluids and the induction of plasminogen activator secretion.

ditions were sought for both the production of the active supernatant fluids and the induction of plasminogen activator secretion by normal macrophages. Fig. 2 shows the time required for optimal activity in each of these parameters. Total peritoneal cells from 3-wk-infected mice were incubated for 24, 48, and 72 h with HKT. The resulting supernatant fluids were then incubated for 24, 48, and

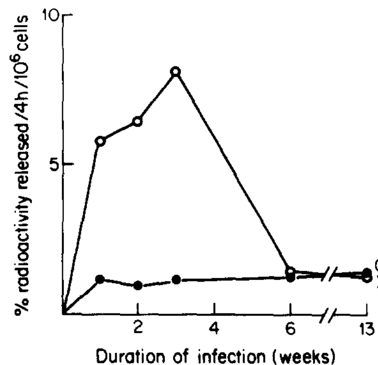

FIG. 3. Temporal requirement for the sensitization of peritoneal cells. Peritoneal cells were harvested from *T. cruzi*-infected mice 1-13 wk after infection and exposed to HKT in vitro. Supernatant fluids were then incubated with normal macrophages for 48 h, and fibrinolysis assayed.

72 h with normal macrophages and secretion of plasminogen activator assayed. Although some enhancement of plasminogen activator secretion was noted as early as 24 h, optimal supernatant fluids were obtained when antigen and sensitized peritoneal cells were incubated for 48 h. Similarly the exposure of macrophages to these supernates for 48 h yielded maximal values.

Fig. 3 shows that total peritoneal cells were capable of producing the active supernatant fluids, when exposed to HKT, as early as 1 wk after *T. cruzi* infection. The activity peaked at 3 wk, and was absent from the 6th to 13th wk. This is in contrast with our findings in vivo in which peritoneal macrophages obtained after an intraperitoneal challenge with HKT were active in secreting plasminogen activator for up to 14 wk after infection (13). Spleen cells were also capable of producing the supernatant factor and the activity was detectable from 1 to 12 wk postinfection (Table I). A sensitized cell population is therefore present in the spleen for up to 3 mo after infection, whereas their peritoneal counterpart disappears after 6 wk. The decline in the supernatant activity at high cell densities is presumably the result of poor viability under these conditions. 10^7 spleen cells therefore appeared to be optimal under the culture conditions used. It should be pointed out that peritoneal cells are more efficient in producing the supernatant fluids on a per cell basis, since 4×10^6 unfractionated peritoneal cells yield comparable activity to 10^7 spleen cells.

Specificity of the Production of Active Supernatant Fluids. Initial experiments were designed to demonstrate that the active supernatant fluids were derived from a specific interaction of sensitized cells and antigen. Spleen and peritoneal cells from *T. cruzi*- or BCG-infected mice were incubated in vitro with HKT, PPD, or latex particles for 48 h and the supernates assayed on unstimulated macrophages. Table II shows that the active supernatant fluids were only elicited by incubation of immune spleen cells with the sensitizing antigen. Incubation of *T. cruzi*-immune cells with PPD or of BCG-immune cells with HKT did not elicit this activity. Neither did the addition of nonspecific particles in the form of polystyrene latex. These results indicated that nonspecific factors or phagocytosis of a particulate were not responsible for the production of the activating factor(s).

TABLE I
*The Ability of Spleen Cells during a T. cruzi Infection to Produce Active Supernatant Fluids**

Duration of *T. cruzi* infection	No. of spleen cells exposed to antigen	Plasminogen activator, % radioactivity released/4 h/10^6 cells
Control	5×10^5	0.63
	5×10^6	0.57
	5×10^7	0.69
T. cruzi 3 wk	5×10^5	0.63
	5×10^6	5.80
	5×10^7	3.30
T. cruzi 12 wk	5×10^5	1.75
	5×10^6	5.21
	1×10^7	9.70
	5×10^7	3.34

* Mice were injected intraperitoneally with 5×10^6 culture forms of *T. cruzi* and 3 and 12 wk later their spleens removed, and serial dilutions of spleen cells incubated with 5×10^5 HKT for 48 h. Supernates were collected and incubated with normal, unstimulated macrophages for 48 h, in ^{125}I-fibrin-coated plates. The cells were then washed three times with HBSS and the assay started by adding Dulbecco's medium containing 5% AT-DS.

TABLE II
*The Role of Specific Antigen in the Production of Active Supernatant Fluid**

Infection	Antigen added	Plasminogen activator, % radioactivity released/4 h/10^6 cells
Control	HKT	1.4
	PPD	1.7
	Latex	1.7
BCG	HKT	1.8
	PPD	8.2
	Latex	1.8
T. cruzi	HKT	10.0
	PPD	2.5
	Latex	1.0

* Mice were infected 3 wk previously with either 5×10^6 culture forms of *T. cruzi* intraperitoneally or $2\text{-}4 \times 10^7$ viable BCG intravenously. 10^7 spleen cells were incubated for 48 h with 5×10^5 HKT, 5 μg PPD, or 5×10^6 latex particles. Supernates were collected and incubated for 48 h with normal unstimulated macrophages on a ^{125}I-fibrin-coated plate and assayed for fibrinolytic activity.

Dose-Response Titration of the Supernatant Fluids. Fig. 4 shows a dose response titration obtained when different concentrations of spleen cell supernates were added to unstimulated macrophages. Immune supernates at a concentration of 2.5% was enough to induce a significant enhancement of secretion.

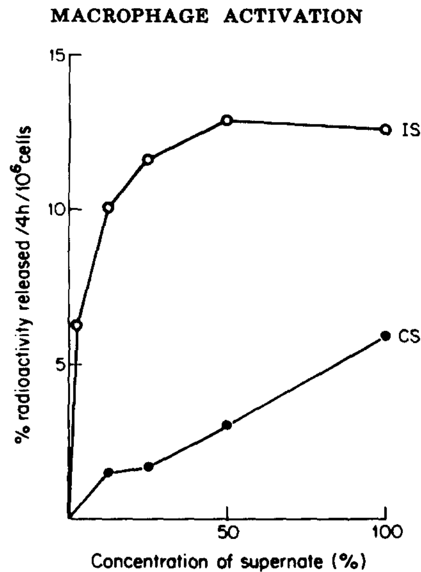

FIG. 4. Dose-response titration of supernatant fluid activity. 10^7 spleen cells fom control mice or 3-wk *T. cruzi*-infected mice were exposed to HKT for 48 h. Supernatant fluids were added at different concentrations to 10^6 unstimulated mouse peritoneal macrophages on ^{125}I-fibrin-coated plates and fibrinolytic activity assayed 48 h later.

TABLE III
*The Ability of Supernatant Fluids to Stimulate Macrophages of Other H-2 Specificities**

Origin of active supernatant fluids	Plasminogen activator, % radioactivity released/4 h/10^6 cells		
	Unstimulated macrophages from:		
	NCS	C57/BL	A/J
NCS	19.0 (1.3)	15.7 (2.7)	14.2 (3.3)
C57BL/6J	15.3 (1.9)	16.2 (2.1)	14.4 (3.1)
A/J	16.1 (2.3)	15.8 (1.5)	11.5 (2.0)

* Mice were infected intraperitoneally with 5×10^6 culture forms of *T. cruzi* and 3 wk later spleens were removed, and 10^7 spleen cells incubated with 5×10^5 HKT for 48 h. Supernates were then incubated for 48 h with normal, unstimulated macrophages from NCS, C57BL/6J, and A/J mice. Numbers in parentheses represent values obtained by incubating macrophages with supernates derived from antigen-exposed control spleen cells.

The response plateaued at about 50% and higher concentrations did not elicit any further increases in secretion. Supernates derived from the interaction of nonimmune spleen cells with antigen (CS) had a lower, but significant effect at concentrations greater than 50%. All subsequent experiments were performed using a concentration of 25%.

Effect of Supernatant Fluids on Allogeneic Macrophages. The induction of plasminogen activator on unstimulated macrophages by the active supernatant fluids was independent of histocompatibility differences in either spleen cells or macrophages. These results are illustrated in Table III. Similar results were

TABLE IV
*The Ability of Anti-Thy 1.2 Serum to Suppress the Production of Active Supernatant Fluids**

Infection	Treatment of spleen cells	Plasminogen activator, % radioactivity released/ 4 h/10^6 cells
Control	–	1.4
T. cruzi	–	8.6
	Anti-Thy 1.2	9.5
	Complement	9.8
	Anti-Thy 1.2 + complement	2.6

* 10^7 spleen cells from either control or infected C3H/HeJ mice (5×10^6 live culture forms of Y strain of *T. cruzi* injected intraperitoneally 4 wk previously) were incubated with anti-Thy 1.2 serum and rabbit C as described in the Materials and Methods. After treatment, cells were washed and incubated in vitro for 48 h with 5×10^5 HKT. Supernates were collected and incubated for 48 h with normal macrophages on a ^{125}I-fibrin-coated plate and assayed for fibrinolytic activity.

obtained with supernates obtained from C3H/HeJ mice tested on NCS macrophages (not shown).

The Requirement for T Lymphocytes in the Production of Active Supernatant Fluids. Lymphocytes were the obvious candidates to produce such activating factor(s). To confirm this, initial experiments were performed to test the ability of nylon-wool separated peritoneal or spleen cells to generate the active supernatant fluids. This procedure has been shown to result in depletion of macrophages and B lymphocytes and an enrichment of T lymphocytes in the column effluent (18). Nylon-wool-separated peritoneal or spleen cells were indeed able to generate the active supernatant fluids.

More definitive experiments were then performed by using a specific antithymocyte serum, kindly supplied by Dr. U. Hämmerling of the Sloan-Kettering Cancer Institute. A 1:10 dilution of this antiserum, in the presence of rabbit C, killed 25–30% of spleen cells, as determined by trypan-blue exclusion. As seen in Table IV, pretreatment of spleen cells with anti-Thy 1.2 serum and C suppressed the production of the active supernates. This indicates that T cells are required for the generation of this factor(s). Pretreatment of spleen cells with either anti-Thy 1.2 serum or C alone had no effect on the generation of the active supernatant fluids.

Treatment of spleen cells with anti-κ serum and C did not affect significantly the production of the supernatant factors. Such anti-κ and C-treated spleen cells were, however, able to reconstitute the activity of a spleen cell population previously treated with anti-Thy 1.2 serum and C. This suggests that the suppression obtained with killing of T lymphocytes is indeed due to a requirement for these cells and not due to inhibition by factors released by the lysed cells.

Discussion

Macrophages activated by the intraperitoneal injection of thioglycollate medium (17) or after a secondary intraperitoneal challenge in the course of a BCG or *T. cruzi* infection (13) secrete large amounts of plasminogen activator. The immunological specificity exhibited in the induction of plasminogen activator secretion and trypanocidal activities in these macrophages suggested that this could be the result of the influx of sensitized lymphocytes and the release of mediators upon contact with antigen. If that was the case then we would expect that sensitized lymphocytes should be able to generate these mediators in vitro, upon exposure to the appropriate antigen. In addition, the incubation of such lymphocyte products with unstimulated macrophages in vitro should elicit similar activated properties. Indeed, our results show that the exposure of sensitized peritoneal or spleen cells to the specific antigen leads to the generation of factor(s) that induce the secretion of large amounts of plasminogen activator by unstimulated macrophages. In addition, this activating factor(s) is generated by macrophage-depleted cell populations and requires T lymphocytes for its production. Recent work has provided evidence that other lymphokines such as migration inhibitory factor (MIF) (19–22), mitogenic factor (23, 24), lymphotoxin (25), and chemotactic factor (26) can be produced by B as well as T lymphocytes. Bloom and Shevach (27) have recently reported that B lymphocytes can only be stimulated by antigen in the presence of T cells and that MIF production is a thymus-dependent response. We have described a T-cell requirement for the production of the macrophage induction factor(s), but have not yet excluded the possibility that other cell types may be involved in its production.

A variety of other macrophage secretory products has been described (28) and it would be of interest to know if these are also released by the addition of the lymphocyte factor(s). Enhanced secretion of collagenase by guinea pig peritoneal exudate cells exposed to antigen-stimulated spleen cell supernates has been reported previously (29). Induction of macrophage plasminogin activator by lectin-stimulated spleen cell supernates has also been recently described (30).

There are a number of advantages in using plasminogen-activator induction to study the influence of lymphokines on the activation of normal macrophage populations. These include: First, the sensitivity of the fibrinolytic system allows accurate quantitation of the activity of this factor(s), which has not been possible in other systems. None of the systems previously described to evaluate lymphokine(s) activity such as MIF, macrophage chemotactic factor, and lymphotoxin provide a precise dose-response relationship. Under the conditions employed, a 2.5% concentration of the active supernatant fluid produced an easily detectable response and maximum secretion occurred at a 50% concentration. This dose-response relationship may be explained either by quantitative alterations in the level of secretion and/or the recruitment of new secretory cells in response to increasing levels of the factor. A recent report (30) favors the latter possibility: using a lectin-stimulated spleen cell supernate and a casein-agar procedure that detects fibrinolysis in single cells, a majority of macrophages was found to be secreting plasminogen activator after induction. A second advantage is that it allows the study of the cell types involved in the generation of the factor. In particular it can employ homogeneous cell populations and normal, unstimulated macrophage targets.

This is an advantage not only of the assay system but of the mouse model in general. Finally, it would be of considerable interest to examine these cell populations with more highly defined antigens.

The fact that neither trypanostatic nor trypanocidal activity could be induced by this factor(s) in unstimulated resident macrophages suggests that either other mediators are required for these properties to be expressed and/or that macrophages must undergo metabolic and functional changes to become responsive to the lymphocyte supernatant factor(s). This could occur for example when exposed to a nonspecific inflammatory environment. In this regard, it is of interest that in every instance in which an in vitro microbistatic or microbicidal activity has been generated against protozoa, macrophages were obtained from peritoneal exudates, sensitized animals, or had been manipulated in vitro by exposure to heart-infusion broth, thioglycollate medium, or Ficoll-Hypaque (4, 6–8, 31). Efforts are in progress to define the conditions under which a trypanocidal activity can be evoked under in vitro conditions.

Summary

In this article we describe methods in which unstimulated mouse peritoneal macrophages were induced to secrete high levels of plasminogen activator under in vitro conditions. The exposure of sensitized peritoneal or spleen cell populations from *Trypanosoma cruzi*-infected animals to either viable or heat-killed trypanosomes lead to the release of an inducing factor(s). Maximal levels of plasminogen activator secretion are achieved by the incubation of such factor(s) with unstimulated macrophages for 48 h. A significant increase in enzyme secretion was already observed after a 24 h incubation. The production of the inducing factor(s) by sensitized cells was immunologically specific and unrelated antigens did not stimulate the production of the factor(s) by sensitized peritoneal or spleen cell populations. The inducing factor(s) was produced by nylon-wool-fractionated spleen and peritoneal cells which had been depleted of macrophages. Pretreatment of sensitized spleen cells with anti-θ serum and C abolished the production of the activating factor(s). The active supernatant fluids were able to induce secretion of macrophage plasminogen activator across *H-2* barriers. Attempts to induce trypanocidal activity in unstimulated macrophages have not been successful.

We want to thank Ms. Marianne Polimeni for excellent technical assistance and Ms. Grace Silvestri for typing the manuscript.

Received for publication 29 March 1977.

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