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In Vitro and In Vivo Studies of Macrophage Functions in Amebiasis

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Experimental intrahepatic inoculation of the gerbil with *Entamoeba histolytica* trophozoites was used as a model of liver amebiasis to study the cellular immune response elicited by the parasite. It was shown that abscess-derived macrophages (5 to 20 days old) were deficient in their capacity to develop a respiratory burst, to secrete and express membrane-bound interleukin-1-like activity, and to kill *E. histolytica* trophozoites as well as to respond to lymphokines in vitro. However, macrophages isolated from the spleen and peritoneal cavities from the same infected animals were not significantly down regulated in these functions. Splenocytes from infected gerbils were shown to develop a strong responsiveness to amebic antigen, whereas their response to concanavalin A was suppressed. Crude *E. histolytica* extracts or conditioned medium down regulated murine BALB/c macrophage accessory and effector cell functions in vitro in a manner similar to abscess-derived macrophages, whereas crude extracts of the nonvirulent *E. histolytica*-like Laredo strain did not. Our results indicate that intrinsic or secreted products or both from *E. histolytica* are actively regulating macrophage functions at the abscess site and can possibly mediate other immunoregulatory mechanisms at distant targets.

Entamoeba histolytica is a protozoan parasite that infects up to 10% of the world's population and results in about 100,000 deaths per year (36). Cell-mediated immune mechanisms appear to be important in the pathogenesis and control of invasive amebiasis (6, 12, 13, 19, 27, 28, 30). Recently, an animal model of hepatic amebiasis, the gerbil, has been described in this laboratory (4, 5). This model has been shown to mimic the invasive amebiasis which may occur in humans (4, 5).

Using the gerbil as an experimental host for amebic liver abscess formation, we studied the cellular immune response in this animal during the course of an infection. Functions of macrophages that had infiltrated the amebic granulomas or macrophages from the peritoneal cavity and spleens from infected animals were studied. Our results demonstrate that, in experimental hepatic amebiasis in the gerbil, amebic liver abscess-derived macrophages are profoundly inhibited in both effector functions and accessory cell potential. Furthermore, spleen and peritoneal macrophages are not significantly down regulated. Implication of the involvement of secreted or intrinsic amebic proteins or both in inducing these immune dysfunctions is provided by the demonstration that these proteins could significantly down regulate naive macrophage functions in vitro, using elicited murine BALB/c peritoneal cells. Both the accessory and effector functions of these cells were significantly down regulated following treatment with *E. histolytica* extracts.

MATERIALS AND METHODS

Animals. Male gerbils (*Meriones unguiculatus*) 50 to 60 days old and weighing between 55 and 60 g (Tumblebrook Farms, West Brookfield, Mass.) were used in all experiments.

Parasites. The pathogenic *E. histolytica* HMI-IMSS amebae and the *E. histolytica*-like Laredo strain grown axenically in TY1-S-33 medium (4) were used. Amebic trophozoites were cultured as described previously (4).

Infections. Gerbils were infected intrahepatically with 2×10^5 amebic trophozoites in mid-log phase (72 h) as described previously (4). The evolution of infection and its pathological manifestations have been described elsewhere (4, 5).

Amebic antigens. Amebic trophozoites in mid-log phase were harvested as described, washed twice in Hanks balanced salt solution (HBSS; GIBCO Laboratories, Grand Island, N.Y.), and suspended in phosphate-buffered saline (pH 7.2). The amebae were lysed by three freeze-thaw cycles. The amebic suspensions were centrifuged at 15,000 $\times$ *g* at 4°C for 15 min to remove cellular debris, and the supernatant was used as the crude amebic extract. Conditioned medium was prepared by incubating 5×10^6 amebae in 10 ml of Neumann-Tydell serumless medium (GIBCO) supplemented with 5.7 mM cysteine, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), and 0.5% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) for 4 h at 37°C. Supernatants were collected and stored briefly at 4°C or used fresh for the experiments.

Isolation of abscess-derived macrophages. Liver abscesses of gerbils sacrificed at various times after infection were aseptically harvested and transferred to culture dishes containing complete medium (Neumann-Tydell medium with 10% heat-inactivated fetal calf serum [GIBCO], 25 mM HEPES [Sigma], and 100 U of penicillin and 100 μg of streptomycin sulfate per ml). A suspension was prepared by teasing the tissues apart with forceps. This suspension, after being washed, was transferred to plastic tubes containing 1% collagenase (type 1 from *Clostridium histolyticum*; Sigma) and 100 μg of DNase (type 1; Sigma) per ml in HBSS and agitated in a water bath at 37°C for 1 h. Cell suspensions were then washed three to four times with medium and plated at 10^7 cells per ml in 24-well plates for the interleukin-1 (IL-1) and cytotoxicity assays. Cells were adhered for 2 h, after which they were washed 10 to 15 times with warm HBSS, incubated for 18 h, washed 10 times with warm HBSS, and processed for the various assays. This procedure allowed the recovery of cells which were >97% macrophages as determined by Giemsa staining. Since macrophage yield varied from experiment to experiment, cells were eluted from the plastic surface with cold Mg^{2+}-Ca^{2+}-free HBSS (GIBCO), washed three times in medium, counted, and adjusted to 5×10^5 macrophages per well in 1 ml of

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complete medium in 24-well plates. Cells were incubated for a further 4 to 6 h before the various assays.

Spleen and peritoneal macrophages. The peritoneal cavities of infected and normal gerbils were washed with 10 ml of cold medium, and the harvested cells were washed twice and adjusted at 5×10^6 cells per well in 1 ml of complete medium in 24-well plates. Cells were incubated overnight at 37°C in 5% CO_2, after which they were washed 10 to 15 times with warm HBSS. Spleen macrophages were prepared as described previously (7). In brief, the spleens of infected and normal gerbils were removed aseptically and a cell suspension was made. Cells were washed thrice in medium, freed of erythrocytes with 0.17 M NH_4Cl (Sigma), and plated at 5×10^7 cells in 1 ml of complete medium in 24-well plates. After 2 h of incubation at 37°C in 5% CO_2, cells were washed 10 to 15 times with warm HBSS, after which they were incubated overnight. Cells were then washed in warm HBSS and incubated for 4 to 6 h before the various assays. At 20 days postinoculation, a significant increase in the proportion of macrophages was found in infected gerbils compared with controls; therefore, the cell numbers were adjusted accordingly.

Lymphokines (LKs). Normal gerbil spleen cells were adjusted to 5×10^6 cells per ml in culture flasks (10 ml of complete medium supplemented with 5 μg of concanavalin A [ConA; Sigma] per ml). Cells were incubated for 48 h, and the supernatants were harvested, neutralized with 15 μg of α-methylmannoside (Sigma) per ml, put in aliquots, and stored frozen at −20°C. Recombinant murine gamma interferon (IFN-γ) was obtained from Amgen, Thousand Oaks, Calif.

Secretion of hydrogen peroxide. H_2O_2 production was measured as described by Pick and Keisari (22). Briefly, to each well 125 ng of phorbol myristate acetate (Sigma) and 0.2 mg of horseradish peroxidase (type 2, 190 U/mg; Sigma) per ml were added to a final total volume of 1 ml in phenol-red-free HBSS. Plates were incubated for 1 h at 37°C in 5% CO_2, NaOH was added to stop the reactions, the supernatants were collected and filtered, and the A_{610} was read. Results are expressed in nanomoles of H_2O_2 per milligram of protein per hour, using standard curves generated with H_2O_2 solutions of known concentrations. Protein determinations were made on homogenates prepared by incubating adherent cells with 1 ml of NaOH (1 M) for 24 h by the method of Bradford (2), using bovine serum albumin as a standard.

Generation of conditioned medium. After preparation of macrophage monolayers, lipopolysaccharide (LPS) (L-3129 [Sigma]; *E. coli* O127:B8) was added at 10 μg/ml. After incubation for 24 h, the supernatants were collected and filtered before assay of IL-1 activity.

Membrane-bound IL-1 (mIL-1). Macrophage monolayers were added with 10 μg of LPS per ml and cultured for 24 h. After the removal of supernatants, the wells were washed 10 times with HBSS and the cells were fixed with 0.03% glutaraldehyde (Sigma) for 30 min. After fixation, the wells were washed extensively and cultured in medium overnight. Supernatants were removed, monolayers were washed five times, and 3×10^6 BALB/c thymocytes per well were added in the presence or absence of phytohemagglutinin (1 μg/ml) (24). Proliferation was measured as described below.

Assay of IL-1-like activity. Supernatants were assayed for IL-1 activity by costimulation of BALB/c thymocytes; thymuses from BALB/C mice were aseptically removed, and a cell suspension was prepared. The cells were suspended at 10^7/ml in medium. Supernatants (0.1 ml per well) were added to wells of flat-bottomed 96-well microtiter plates (Corning Glass Works, Corning, N.Y.), and phytohemagglutinin (1 μg/ml) and 0.1 ml of cells were added (24). Plates were incubated for 72 h at 37°C in 5% CO_2. One microcurie of [^{3}H]thymidine (specific activity, 30 Ci/mmol, ICN Radiochemicals, Irvine, Calif.) was added to each well for the final 18 h of culture, and the cells were harvested. Incorporation of radioactivity was determined by liquid scintillation counting.

Cytotoxic potential against *E. histolytica* trophozoites. Macrophages were eluted from plastic surfaces and washed thrice in medium. Amebae (10^4/ml) and macrophages at 2×10^6/ml were suspended in complete medium supplemented with 5.7 mM cysteine in plastic test tubes. Cells were centrifuged at $150 \times g$ for 5 min and incubated at 37°C in 5% CO_2 for 6 h. Tubes were placed on ice, and viability of the cells was assessed by trypan blue exclusion. The data are expressed as the percent viability of controls of amebae incubated alone for 6 h.

In vitro blastogenesis. ConA-induced in vitro lymphocyte blastogenesis was performed in flat-bottomed microtiter plates. Each well received 10^6 cells in medium containing 2 μg of ConA per ml. The plates were incubated for 72 h in a 5% CO_2 atmosphere at 37°C. Antigen-induced in vitro blastogenesis was performed in flat-bottomed plates; each well received 10^6 cells in medium with an optimal concentration of antigen (10 μg/ml) of *E. histolytica*. For assays of antigen-specific lymphocyte blastogenesis, plates were incubated for 120 h at 37°C in 5% CO_2. All cultures received a terminal 8-h pulse of [^{3}H]thymidine. Cultures were harvested on glass-fiber filters and processed for liquid scintillation counting. Results of triplicate cultures are expressed as mean counts per minute ± standard deviation or as a stimulation ratio, i.e., counts per minute of stimulated cultures/counts of unstimulated cultures.

Treatments with amebic extracts. Proteose-peptone-elicited peritoneal macrophages from BALB/c mice (Charles River, St. Constant, Quebec) were isolated as described for gerbils. Secreted mIL-1 was measured as described previously except that, in experimental cultures, various concentrations of amebic extracts or conditioned medium were added with LPS during the stimulation period. The respiratory burst was also measured as described with additions of amebic extracts as indicated in Results. Responsiveness to recombinant IFN-γ (rIFN-γ) was measured by incubating macrophages for 48 h in the presence of 100 U of rIFN-γ per ml with various concentrations of amebic extracts or conditioned medium.

Ia expression. Proteose-peptone-elicited peritoneal macrophages from BALB/c mice were stimulated for Ia expression by exposure to 100 U of rIFN-γ per ml for 3 days. Following this, cells were treated with various concentrations of amebic extracts in medium for indicated periods of time. The macrophages were eluted from the surface, and Ia expression was determined by cytotoxicity as described previously (7).

RESULTS

Characterization of macrophage functions from gerbils with amebic liver abscesses. Macrophages from infected gerbils were analyzed for a variety of functions. Macrophages isolated from the peritoneal cavities and spleens of day-10-infected gerbils were found to secrete higher levels of H_2O_2 and to be more responsive to LKs than their counterparts from normal gerbils (Table 1). In addition, these cells secreted comparable amounts of IL-1-like activity; macro-

TABLE 1. Characterization of macrophage from gerbils inoculated intrahepatically with *E. histolytica* trophozoites 10 days postinoculation[a]

Macrophage source[b]	IL-1 release (10^3 cpm)	Respiratory burst (nmol of H_2O_2/mg per h)	Amebicidal activity (% specific killing)	Responsiveness to LKs (nmol of H_2O_2/mg per h)[c]
Peritoneal (NI)	4.2	115	5	208
Peritoneal (I)	4.9*	153*	45***	291**
Spleen (NI)	2.1	56	3	99
Spleen (I)	2.4	69*	28**	146**
Liver (Kupffer) (NI)	1.3	30	0	58
Amebic abscess (I)	0.23***	13**	0	17***

[a] * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.005$ compared with uninfected homologous gerbil cells by Student's t test.
[b] Functions of macrophages from infected (I) gerbils were compared with those of macrophages from uninfected (NI) gerbils. Shown are representative results of one experiment repeated five times with similar results.
[c] Measured as enhancement of phorbol myristate acetate-triggered respiratory burst by 48-h incubation in 50% (vol/vol) crude LKs.

phages from spleen and peritoneal cavities were found to possess a significant capacity to kill *E. histolytica* trophozoites (28 and 45%, respectively) in vitro (Table 1). However, abscess-derived macrophages from gerbils were, when compared with phosphate-buffered saline sham liver-injected controls, (i) deficient in reactive oxygen intermediate release, (ii) unresponsive to LKs, and (iii) inefficient at secreting IL-1-like activity or expressing mIL-1 (results not shown). When abscess-derived macrophages from day-20-infected gerbils were studied, similar results were obtained; peritoneal and splenic macrophages were activated by infection, whereas abscess-derived macrophages were significantly down regulated (results not shown).

In vitro blastogenesis. Spleen cells from infected gerbils were studied for mitogen- and antigen-induced mitogenesis during the course of infection. Cells from spleens of infected gerbils showed a rapid decrease in responsiveness to ConA (5 days) which persisted until the duration of the experiment at day 20 postinoculation (Fig. 1). However, splenocytes from these animals were readily stimulated to undergo blastogenesis by amebic antigens (Fig. 2). The mitogen reactivity of splenocytes from infected gerbils was examined to determine whether spleen cells from these animals were capable of negatively influencing the responsiveness of normal cells. Spleen cells from day-20-infected gerbils were poorly responsive to ConA (5×10^5 splenocytes from infected gerbils responded to 2 μg of ConA per ml with a proliferation of 62.8×10^{-3} cpm), but failed to suppress the responsiveness of normal spleen cells in cocultivation experiments (5×10^5 normal cells mixed with 5×10^5 cells from infected gerbils responded to ConA with a proliferation of 131.6×10^{-3} versus 126.1×10^{-3} cpm for 10^6 normal splenocytes). Similar results were obtained with day-10-infected gerbils.

Effects of *E. histolytica* extracts on murine BALB/c macrophage functions in vitro. Having demonstrated that abscess-derived macrophages from infected gerbils were significantly down regulated in their functions, we decided to investigate in vitro the effects of amebic extracts on macrophage functions. This set of experiments was performed with elicited murine peritoneal macrophages, which allowed the use of recombinant cytokines and also allowed the study of Ia expression and its inhibition by amebic extracts.

In a first set of experiments, the effects of amebic extracts on IL-1-like activity release and membrane expression were investigated. Addition of 15 μg of extracts of *E. histolytica* led to a significant reduction in the release and expression of IL-1-like activity (Table 2). Furthermore, incubation of macrophage monolayers with 25% (vol/vol) conditioned amebic medium (25 μg of secreted proteins per ml) led to a substantial decrease in secretion of IL-1-like activity and the expression of mIL-1-like activity also (results not shown).

The effect of amebic extracts on the release of reactive

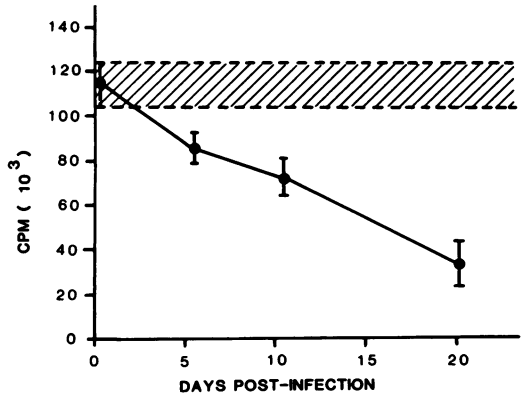

FIG. 1. Response of splenocytes from gerbils with amebic liver abscesses at various times postinfection to ConA (data reported as mean ± standard error of counts per minute). Shaded area represents the response of normal animals. Shown are representative data from one experiment repeated three times with similar results.

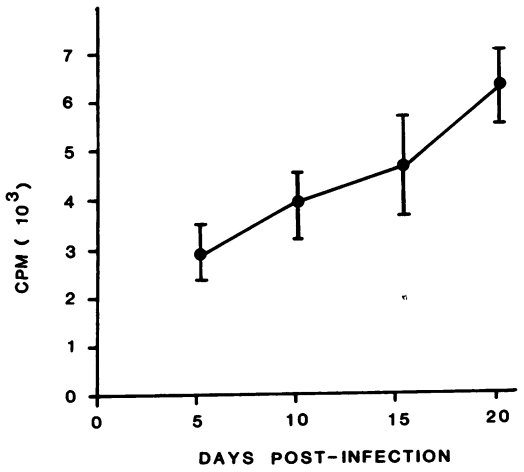

FIG. 2. Response of splenocytes from gerbils with amebic liver abscesses at various times postinfection to 10 μg of *E. histolytica* extracts per ml. Results are means ± standard errors of three separate experiments. Splenocytes from normal gerbils were not significantly stimulated, never >500 cpm.

TABLE 2. Effect of *E. histolytica* extracts on secretion of IL-1-like activity and expression of mIL-1 by elicited murine peritoneal macrophages

Treatment	Activity (10^4 cpm ± SE)[a]	
	IL-1-like	mIL-1
Nil	5.3 ± 0.5*	1.1 ± 0.08
E. histolytica extract, 15 μg/ml	1.6 ± 0.2	0.3 ± 0.03
E. histolytica-like Laredo extract, 15 μg/ml	4.9 ± 1.0	1.3 ± 0.2

[a] Secreted and membrane-bound IL-1-like activity was measured as described in Materials and Methods. Results are representative data from a minimum of four experiments for each experimental group. Addition of *E. histolytica* extracts to proliferating thymocytes did not modify IL-1 measurement, indicating that carryover of extracts was not affecting the assay. *$P < 0.001$ for secreted and membrane-bound IL-1-like activity by Student's *t* test. *E. histolytica*-like Laredo extract-treated macrophages were no different from controls.

oxygen intermediates was also studied (Table 3). Addition of *E. histolytica* extracts (10 μg/ml) led to a significant decrease in the release of H_2O_2, with or without priming with rIFN-γ. Extracts of the *E. histolytica*-like Laredo strain were ineffective at mediating this inhibition. In the next set of experiments, a dose response of the effect of amebic extracts on reactive oxygen intermediate release after IFN-γ priming was performed (Fig. 3). With 10 to 30 μg of the *E. histolytica* extract per ml, 60 to 80% inhibition was observed. Amebic extracts as low as 8 μg/ml caused partial inhibition (Fig. 3). This inhibition of H_2O_2 release induced by the amebic extract was not due to prostaglandins, as the addition of indomethacin (5 μM) failed to restore the reactive oxygen intermediate release (results not shown). The effect was not due to a depletion of activation, as shown by demonstrating that amebic extracts incubated with macrophages did not stimulate a respiratory burst higher than untreated cells at any time during incubation.

In the last experiment, the effect of *E. histolytica* extract on Ia expression induced by LKs was studied. Elicited BALB/c mouse peritoneal macrophages were cultured for 3 days with rIFN-γ and treated with various extracts. After culture, Ia expression was evaluated relative to that of controls. Treatment with 10 μg of the *E. histolytica* extract per ml caused a 55% suppression of Ia antigens when compared with untreated controls or to cells exposed to the

TABLE 3. Macrophage deactivation by *E. histolytica* extracts; effect on respiratory burst of elicited murine peritoneal macrophages

Treatment (15 μg/ml)	Priming	H_2O_2 release (nmol/mg per h)[a]
None		75 ± 17*
E. histolytica extracts		12 ± 5
None	rIFN-γ[b]	258 ± 19**
E. histolytica extracts	rIFN-γ	71 ± 12
E. histolytica-like Laredo extracts		98 ± 10
E. histolytica-like Laredo extracts	rIFN-γ	299 ± 26

[a] Mean ± standard error for triplicates; representative data from three experiments. *$P < 0.002$ and **$P < 0.001$ compared with *E. histolytica* extract-treated cells by Student's *t* test. H_2O_2 release from untreated macrophages or macrophages primed with rIFN-γ were not significantly different when treated with *E. histolytica*-like Laredo extract.

[b] Priming by 48-h exposure to 100 U of rIFN-γ per ml with or without amebic extracts before phorbol myristate acetate triggering (see Materials and Methods).

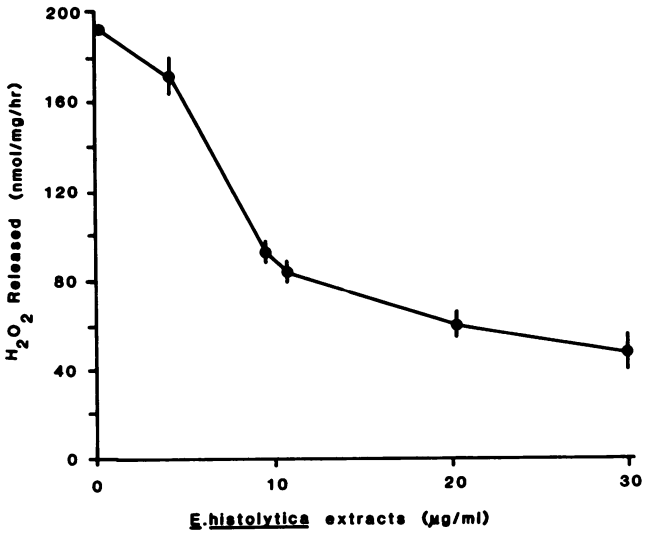

FIG. 3. Effect of *E. histolytica* extract on hydrogen peroxide release by macrophages. Macrophages from BALB/c mice were incubated for 48 h with 100 U of rIFN-γ per ml and various concentrations of *E. histolytica* extracts. H_2O_2 release was determined after phorbol myristate acetate stimulation. Results are means ± standard errors of three separate experiments.

E. histolytica-like Laredo amebic extract (Table 4). Indomethacin (5 μM) was ineffective at restoring Ia expression.

DISCUSSION

Numerous studies have indicated the importance of cell-mediated mechanisms in invasive amebiasis (6, 11–13, 19, 27, 28). Humoral immunity is evident in invasive disease (14, 17), but is not protective (1, 23, 35). However, peripheral lymphocytes, spleen cells, and, particularly, macrophages are competent effector cells against *E. histolytica* (6, 28, 30, 32). The phagocytic cell, thus, plays an important role in the pathogenesis of amebiasis. Parasites possess a complex repertoire of mechanisms that help them invade the host and evade its defenses. A recent study has shown that supernatant fluid of axenically grown *E. histolytica* inhibits chemotaxis, chemokinesis, and mobility of human mononuclear phagocytes (15).

Perversion of macrophage functions by microbes, parasites, tumor cells, and/or their products is a recurrent theme in recent research on macrophage study. A product of murine tumor cell lines was found to remove the capacity of mouse peritoneal macrophages to secrete H_2O_2 (34). In addition, this factor was found to suppress the killing of

TABLE 4. Effects of *E. histolytica* extracts on Ia expression in elicited murine peritoneal macrophages[a]

Amebic extracts (10 μg/ml)	Ia expression (%)[b]	
	−LK	+LK
None	2.9 ± 1.3	76 ± 2.9*
E. histolytica	2.6 ± 1.3	21 ± 7
E. histolytica-like Laredo	1.6 ± 1.0	80 ± 3.6

[a] BALB/c macrophages were incubated for 3 days with 100 U of rIFN-γ per ml with various amebic extracts present or not present during incubation.

[b] *$P < 0.002$ compared with *E. histolytica*-treated cells. *E. histolytica*-like Laredo-treated cells were not significantly different from untreated macrophages by Student's *t* test.

intracellular protozoan pathogens (33). The adenylate cyclase toxin of *Bordetella pertussis* was shown to inhibit chemiluminescence and O_2^- of monocytes (21) by increasing cyclic AMP levels in monocytes. Recently, a polysaccharide from *Haemophilus actinomycetemcomitans* was found to suppress IL-1 release from LPS-stimulated macrophages (18). Extracts of oral treponemes contain a potent inhibitor of O_2^- production by human polymorphonuclear leukocytes (31). *Leishmania* species were found to inhibit the respiratory burst (3), IL-1 secretion (24), and the expression of class I and II major histocompatibility complex products of mouse macrophages (25). In addition, a recent report showed that sulfatides, a component of mycobacterial cell walls, block priming of cultured human monocytes (20).

Overall, these results show that microbes and parasites have evolved exquisitely subtle mechanisms to escape macrophage effector functions. Our study was aimed at determining the potential in terms of accessory and effector cells of macrophages from gerbils with amebic liver abscesses, a model which mimics the human disease (4, 5). Our results show that abscess-derived macrophages were deficient in reactive oxygen intermediate release, IL-1 release, and LK responsiveness. This supports the contention that abscess-derived macrophages are defective in their response to macrophage-activating signals. In addition, it was shown that extracts of *E. histolytica* trophozoites could down regulate significantly the ability of naive murine macrophages to secrete IL-1-like activity and express Ia antigen as well as to secrete H_2O_2, with or without priming with IFN-γ. The ability of extracts of *E. histolytica* to down regulate IL-1 expression may explain, in part, the remarkable absence of scarification around amebic liver abscesses in humans or in experimental models (4, 16), since IL-1 is a very important molecule, involved in stimulating collagen deposition, a crucial part of the wound-healing process (10). However, we do not know whether bone marrow- or monocyte-derived macrophages from infected animals are similarly impaired. As spleen and peritoneal macrophages from uninfected animals were unaffected, this suggests that amebic products in close proximity to macrophages (as in amebic granulomas) are required to cause inhibition of their functions. Furthermore, the inhibition of murine macrophage functions in vitro by amebic extracts was not seen with the Laredo strain, which indicates that the inhibition is specific to *E. histolytica* trophozoites.

Our study showed that splenocytes from infected gerbils developed a depressed responsiveness to ConA, whereas a strong reactivity to amebic antigens was seen. A similar pattern of reactivity was observed in peripheral blood leukocytes of patients with liver abscesses (27). It should be noted that soluble proteins from virulent amebae are mitogenic for human and murine lymphocytes (8, 9, 29). This mitogenicity correlated with amebic lectin activity, which in turn correlated with virulence (29). Studies on the mitogenic principle of virulent *E. histolytica* trophozoites have shown that different subsets of T cells proliferate in response to *E. histolytica* extracts as compared with ConA (8, 9). In any case, as pointed out by other investigators (8), that *E. histolytica* can act as a polyclonal activator of T lymphocytes suggests that this increased response to *E. histolytica*-derived mitogen in humans or other animals (or both) with hepatic amebiasis might be associated with disturbances of the immune system.

In summary, our studies clearly demonstrate that intrinsic or secreted amebic products (conditioned medium) or both can actively regulate macrophage functions which are important in immunoregulatory mechanisms in invasive amebiasis. Studies are under way to define these products more specifically and to define their mode of action.

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