INFECTION AND IMMUNITY, Oct. 1981, p. 274-284
0019-9567/81/100274-11$02.00/0

Vol. 34, No. 1

Dissociation of Bactericidal Activity from Other Functions of Activated Macrophages in Exudates Induced by Thioglycolate Medium

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Received 13 April 1981/Accepted 19 June 1981

Macrophages displayed increased spreading, increased Fc-receptor-mediated phagocytosis, and increased secretion of plasminogen activator when collected from the peritoneal cavities of either *Listeria*-immune mice challenged intraperitoneally 3 days earlier with *Listeria* or nonimmune mice injected intraperitoneally 3 days earlier with fluid thioglycolate medium. In contrast, macrophages from the thioglycolate-induced peritoneal exudates were severely impaired in vitro in their ability to destroy *Listeria*. Injection of thioglycolate markedly interfered with the destruction of sublethal intraperitoneal challenge of *Listeria*, which resulted in nonimmune animals dying of an overwhelming systemic infection. In animals immune to *Listeria*, injection of thioglycolate delayed the onset of the expression of immunity to an intraperitoneal challenge of bacteria. The thioglycolate-induced suppression of bactericidal activity was determined to be confined to the site of injection. Results of experiments indicated that the colloidal agar in thioglycolate medium was the cause of the impairment of macrophage bactericidal activity. In addition to the impairment of bactericidal activity induced by agar, additional studies showed that an intraperitoneal injection of colloidal agar (0.075% wt/vol) by itself was a sufficient inflammatory stimulus for the accumulation of a large number of host phagocytic cells.

The acquisition of cell-mediated immunity to bacterial (3, 23, 25), protozoal (27), or neoplastic disease (26, 31) has been shown to result in the systemic activation of macrophages. This has been demonstrated by an enhanced ability of infected mice to nonspecifically destroy a challenge with facultative intracellular bacteria such as *Listeria monocytogenes*. In addition to increased bactericidal functions, activated macrophages have been shown in vitro to display a variety of morphological (12), ultrastructural (3), and physiological (9, 22) changes. Although there is no definitive evidence of a direct correlation between bactericidal functions and other properties of activated macrophages, it has been generally concluded that macrophages which display morphological or physiological characteristics of activation or both are also likely to exhibit enhanced bactericidal activity.

The studies reported in this paper were designed to determine whether the enhancement of certain functions associated with macrophage activation coincides with an increase in bactericidal activity. The results of these studies show that intraperitoneal injection of the phlogogenic agent thioglycolate results in the local accumulation of macrophages that appear activated by morphological and certain functional criteria, but are markedly impaired in their ability to destroy a challenge of *Listeria* in vitro and in vivo. Evidence is also presented that the colloidal agar in thioglycolate medium is the cause of the impairment of macrophage bactericidal activity.

MATERIALS AND METHODS

Animals. Female AB6F$_1$ mice (A/Tru × C57BL/6 Tru) 8 to 12 weeks of age were used throughout. Animals were supplied by the Trudeau Institute Animal Breeding Facility. Mice were free of 11 common murine viruses in tests conducted by Microbiological Associates Viral Testing Service (Cockeysville, Md.). Mice were determined to be free of lactic dehydrogenase virus by the failure of an injection of AB6F$_1$ serum to raise the level of lactic dehydrogenase enzyme in the serum of germfree mice (29). Levels of lactic dehydrogenase were measured in serum with a diagnostic kit (no. 500) from Sigma Chemical Co. (St. Louis, Mo.).

Bacteria. *L. monocytogenes*, strain EGD, was grown to log phase in Trypticase soy broth (TSB; BBL Microbiology Systems), dispensed in 1.0-ml samples, and frozen at −70°C. An inoculum of bacteria was prepared for immunization or challenge by thawing one vial, diluting bacteria in saline, and injecting 0.2 ml of the appropriate dilution. To prepare a log-phase

culture of *Listeria* for use in the in vitro bactericidal assay, several dilutions of bacteria were added to separate 5.0-ml tubes of TSB and grown at 37°C. A standard dose of 2×10^3 *Listeria* was used for intravenous (i.v.) immunization; challenge doses consisted of 5×10^3 i.v. and 5×10^5 or 5×10^6 intraperitoneally (i.p.).

Measurement of bacterial growth in vivo. The ability of immune and nonimmune mice to destroy an i.p. and i.v. challenge of *Listeria* was determined. To follow the fate of an i.p. challenge of *Listeria*, the peritoneal contents were collected in 3 ml of heparinized phosphate-buffered saline (PBS), and the washings were subjected to sonication to release cell-associated bacteria. The number of *Listeria* was quantitated by plating 10-fold dilutions onto Trypticase soy agar (TSA; BBL Microbiology Systems). The fate of a 5×10^3 inoculum of *Listeria* injected i.v. was followed in the livers of experimental and control mice. The number of bacteria in the liver was determined by homogenizing the whole organ in 8 ml of saline and plating 0.1 ml of 10-fold serial dilutions onto TSA.

Preparation of macrophages for in vitro assays. Macrophages were collected from the peritoneal cavities of the following groups, 3 days after i.p. injection of the appropriate material. Mice immunized 6 days earlier with 2×10^3 *Listeria* i.v. (referred to as immune) were challenged i.p. with 5×10^6 bacteria (referred to as immune-boosted). Another group of immune and nonimmune mice were injected i.p. with 1.0 ml of thioglycolate medium. A group of nonimmune controls was injected i.p. with 1.0 ml of PBS. Cells were collected by infusing 1.5 ml of tissue culture medium i.p. The medium consisted of RPMI 1640 (GIBCO Laboratories) supplemented with 5% fetal bovine serum (GIBCO) and 5 U of preservative-free heparin (Sigma Chemical Co.) per ml. Typically 10^7 or greater total cells/animal were collected from the immune-boosted and thioglycolate-injected mice. The PBS-injected controls yielded between 5×10^6 and 7×10^6 total cells, of which 20 to 40% were identified as macrophages by morphology and phagocytosis of heat-killed *Listeria* as previously described (30).

Bactericidal assay. The ability of macrophages to destroy a challenge of *Listeria* in vitro was determined as previously described (30). In brief, cells collected from the peritoneal cavities of immune-boosted or thioglycolate-injected animals were diluted in tissue culture medium to 4×10^6 total cells/ml, and 1.0 ml was added to petri dishes containing three 13-mm circular glass cover slips. In the case of PBS-injected controls, 6×10^6 to 8×10^6 total cells were added to petri dishes in 1.0 to 1.5 ml. Cells were allowed to adhere for 2 h, nonadherent cells were then rinsed off the cover slips, and the remaining cells were challenged 1 h later with 5×10^6 *Listeria* in 1.0 ml of tissue culture medium. After 20 min, extracellular bacteria were washed off the cover slips. When all the cover slips were washed (designated as the T_0 time point), the number of cell-associated viable bacteria was quantitated. Three cover slips were removed from separate dishes at 30-min intervals (designated as the T_1, T_2, T_3, etc. time points) and sonicated to release cell-associated bacteria. Bacteria were enumerated by plating 10-fold dilutions on TSA. Data are presented as the percentage of viable bacteria detected at each time point. This was calculated according to the formula:

$$\frac{\text{number of colony-forming units } T_1, T_2, T_3, \ldots T_n}{\text{number of colony-forming units } T_0} \times 100$$

Macrophage spreading. To measure macrophage spreading, cells were collected from the peritoneal cavities of mice and allowed to adhere to circular glass cover slips for 30 min at 22°C. Nonadherent cells were washed off, the cover slips were placed in warm medium, and spreading was allowed to proceed by placing the dishes at 37°C in a humidified atmosphere of 5% CO_2. Cover slips were removed at timed intervals, rinsed in warm PBS, and fixed in glutaraldehyde diluted to 2% in 37°C PBS. At least 50 cells on each cover slip were photographed with a Zeiss photomicroscope. From the developed negative, the image of the cells was projected onto graph paper, and the outline of each cell was traced. From a knowledge of the magnification of the microscope and enlarger, the area of spreading of each cell was calculated.

Fc-receptor-mediated phagocytic activity. The phagocytic activity of macrophages was assessed by their ability to ingest opsonized sheep erythrocytes (SRBC). Anti-SRBC antibodies (immunoglobulin G [IgG]) were purchased from Cordis Labs (Miami, Fla.), and the agglutination titer was determined to be 1:500. SRBC were washed in PBS, suspended to 5×10^7/ml in tissue culture medium containing a 1:5,000 dilution of anti-SRBC antibody. This suspension was incubated for 20 min at 4°C and then at 37°C for 20 min. The medium overlaying the macrophage monolayers on the glass cover slips was replaced with 1.0 ml of the SRBC suspension. Cover slips were removed at intervals and rinsed in warm saline, and uningested SRBC were lysed in hypotonic PBS. The cells were then fixed in 2% glutaraldehyde, and the number of SRBC ingested per 100 cells was counted using a phase-contrast microscope.

Fibrinolytic assay. The secretion of plasminogen activator from macrophages was quantitated by the release of ^{125}I from labeled fibrinogen as described elsewhere (21, 33). Fibrinogen was labeled with carrier-free ^{125}I (Amersham Corp.) by the chloramine T method (13). To each 16-mm well of a 24-well Linbro plate (Flow Laboratories, Inc.), 10 μg of fibrinogen was added which contained 50×10^3 to 100×10^3 trypsin-releasable cpm. Peritoneal macrophages were harvested in tissue culture medium containing 10 μg of gentamicin per ml (Schering Corp.) and 50 μg of soybean trypsin inhibitor (fraction VI; Miles Laboratories, Inc., Kankakee, Ill.) per ml, the latter to inhibit spontaneous fibrinolysis. Cells (5×10^5 to 2×10^6) were added to the wells and allowed to adhere for 2 h. Nonadherent cells were washed off the plate, and the adherent cells were cultivated for 24 h in medium with soybean trypsin inhibitor. The cells were then washed three times to remove inhibitors, as well as dead cells, and the assay was started by adding 1.0 ml of RPMI 1640 containing 5% acid-treated dog serum. At the indicated times, 0.01 ml of medium was withdrawn from triplicate wells of each group and assayed for the

release of radioactivity in a Searle gamma counter. The actual number of adherent cells in each well at the time of the assay was determined by direct microscopic counts or by removing all cells with cetrimide and pronase (32) and counting in a hemacytometer.

Reagents. Fluid thioglycolate medium (0256-01) was prepared in accordance with the manufacturer's instructions (Difco Laboratories, Detroit, Mich.). Thioglycolate medium was also prepared without agar. All ingredients were purchased from Difco Laboratories and prepared according to the proportions on the fluid thioglycolate medium bottle without the addition of agar (0.75 g/liter) as found in thioglycolate medium. Granulated agar was purchased from BBL Microbiology Systems, (Cockeysville, Md.).

RESULTS

Thioglycolate and immunologically induced enhancement of macrophage function. Experiments were designed to study macrophage spreading, Fc-receptor-mediated phagocytosis, and plasminogen activator activities. Cells were collected from the peritoneal cavities of either *Listeria*-immune mice 3 days after an i.p. challenge of bacteria (immune-boosted) or from normal mice injected i.p. 3 days earlier with thioglycolate or PBS. Injection of thioglycolate into normal mice or bacteria into immune mice induced a cellular exudate which amounted to a three- to fivefold increase in total cell number and consisted mainly of macrophages (60 to 85%) at the time of collection. Examination of cell spreading (Fig. 1A), Fc-receptor-mediated phagocytosis (Fig. 1B), and release of plasminogen activator (Fig. 1C) revealed that macrophages from the immune-boosted and thioglycolate-injected groups showed enhancement in each of these functions which greatly exceeded that of macrophages from PBS-injected controls. In fact, macrophages from the thioglycolate-induced exudate exhibited activities above that of macrophages from immune-boosted mice. Macrophages from both of these groups also displayed the morphological features of activation (Fig. 2).

Thioglycolate-induced impairment of macrophage bactericidal activity. In con-

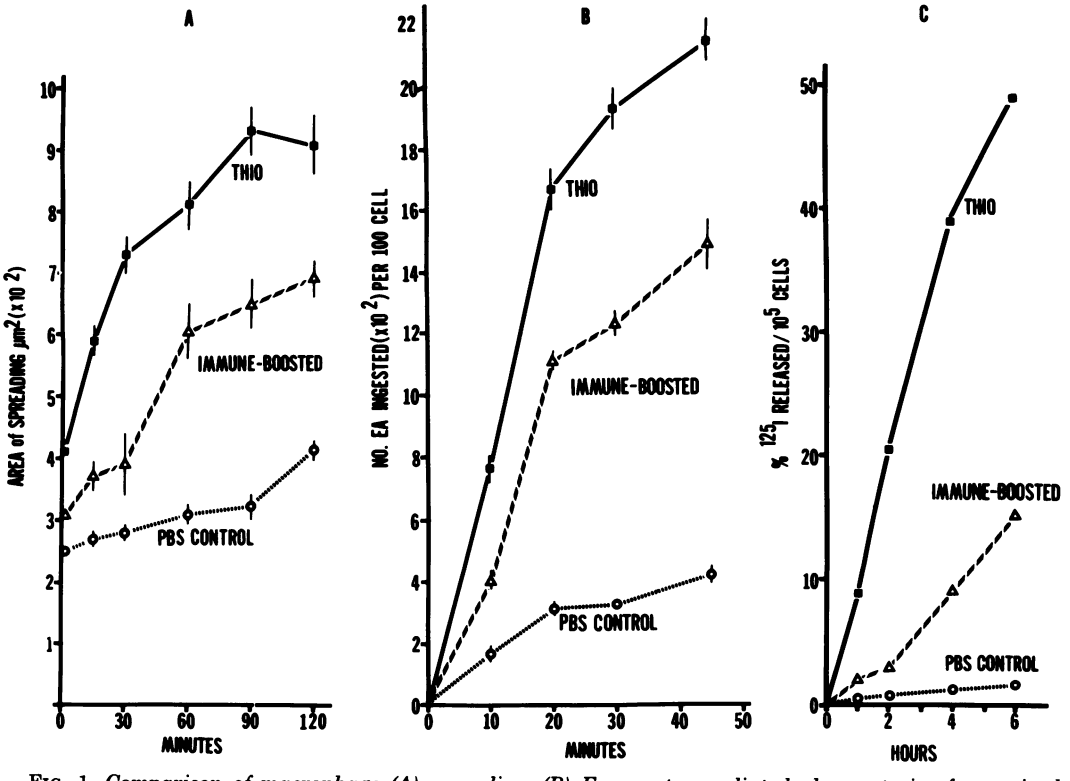

FIG. 1. *Comparison of macrophage (A) spreading, (B) Fc-receptor-mediated phagocytosis of opsonized sheep erythrocytes (EA), and (C) release of plasminogen activator. Macrophages were harvested from the peritoneal cavities of Listeria-immune mice challenged i.p. 3 days earlier with bacteria (IMMUNE-BOOSTED) or from the peritoneal cavities of nonimmune mice 3 days after injection of thioglycolate (THIO) or PBS (PBS CONTROL). (A) Each time point represents the geometric mean of at least 50 determinations ± SEM. (B [± SEM] and C) Each time point represents the mean of triplicate samples assayed.*

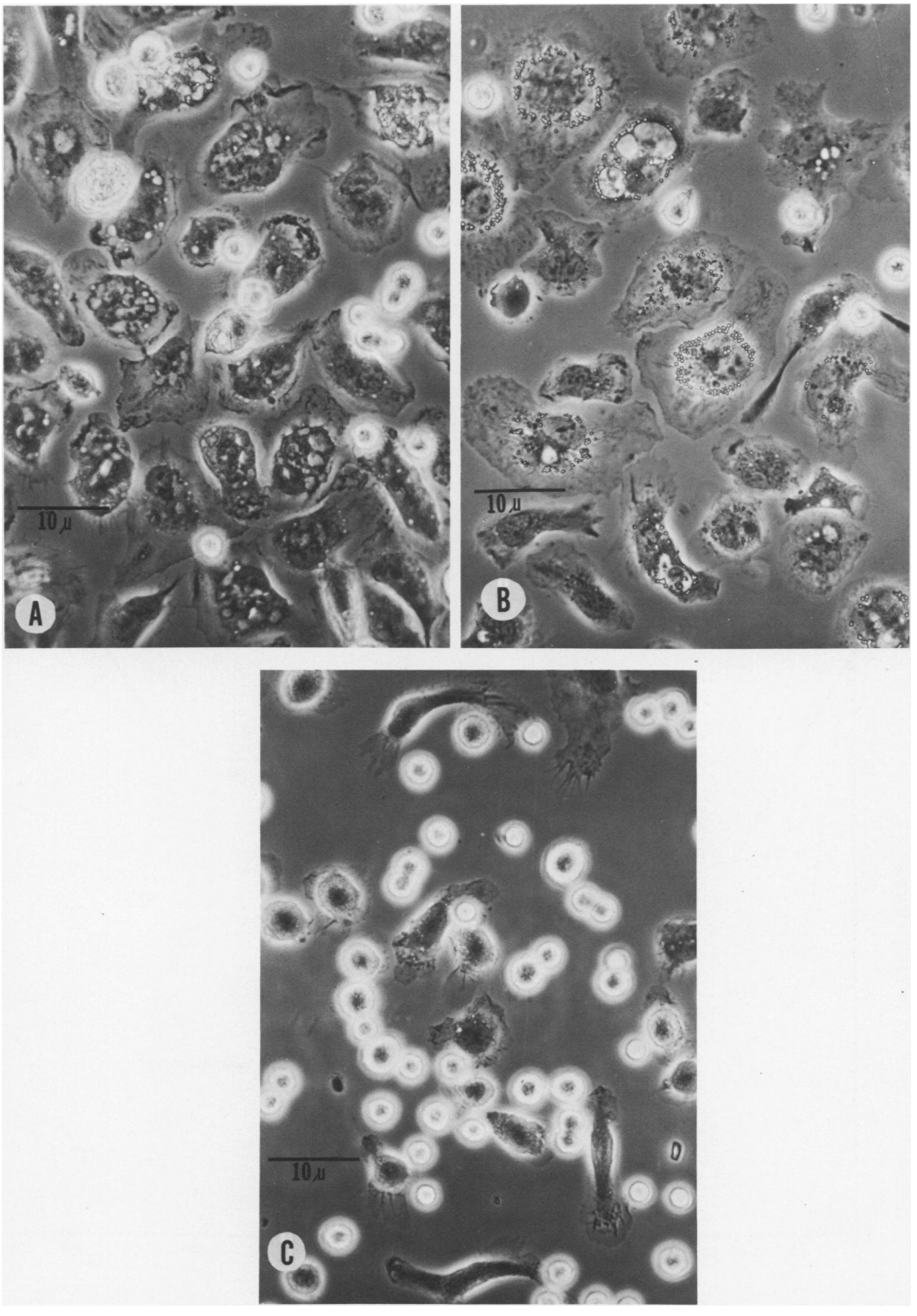

FIG. 2. *Phase-contrast photomicrograph of glass-adherent macrophages. Macrophages were fixed in 2% glutaraldehyde after 1 h in culture. Macrophages were harvested from the peritoneal cavities 3 days after injection of (A) thioglycolate into nonimmune mice, (B) 10^6 Listeria in immune mice, and (C) PBS into nonimmune mice. Macrophages in (A) and (B) show conspicuous features of activation, including highly ruffled plasma membrane, greater tendency to spread, large numbers of lysosomes and pinocytic vesicles.*

trast to the enhancement of macrophage functions shown in the preceding section, results of in vitro studies revealed that macrophages from the thioglycolate-injected mice were impaired in their ability to destroy *Listeria* compared with macrophages from PBS-injected or immune-boosted mice (Fig. 3). Further studies revealed that an injection of thioglycolate interfered with the destruction of an i.p. challenge of *Listeria* (Fig. 4). The impairment of bactericidal activity was so severe in nonimmune mice injected with thioglycolate that *Listeria* growth progressed rapidly and ultimately led to an overwhelming infection. In immune mice injected with thioglycolate, the onset of expression of anti-*Listeria* immunity was delayed for at least 2 h and was then expressed more slowly than in immune-boosted controls. Thus, by 24 h after challenge, the immune-boosted group had completely destroyed the challenge, whereas the thioglycolate-injected mice still contained large numbers of *Listeria* in their peritoneal cavities. The rate and extent of destruction of an i.p. *Listeria* challenge were similar in *Listeria*-immune and immune-boosted animals (data not shown). It should be noted that although immune-boosted mice rapidly destroyed an in vivo challenge infection (Fig. 4), macrophages from immune-boosted mice were certainly no better than those from PBS-injected mice in their ability to destroy a bacterial challenge in vitro (Fig. 3).

Evidence that thioglycolate-induced suppression is confined to the site of injection. To determine whether the impairment of bactericidal activity induced by thioglycolate was confined locally to the site of injection, growth of an i.v. challenge infection was followed on successive days, in the livers of mice injected i.p. 3 days earlier with PBS or thioglycolate. The results show (Fig. 5A) that animals injected previously with thioglycolate or PBS displayed typical expression of nonspecific resistance during the first 12 h of infection (23). Thereafter, bacterial growth was similar until day 4 when thioglycolate animals showed an increase in the number of *Listeria* in their livers. These studies

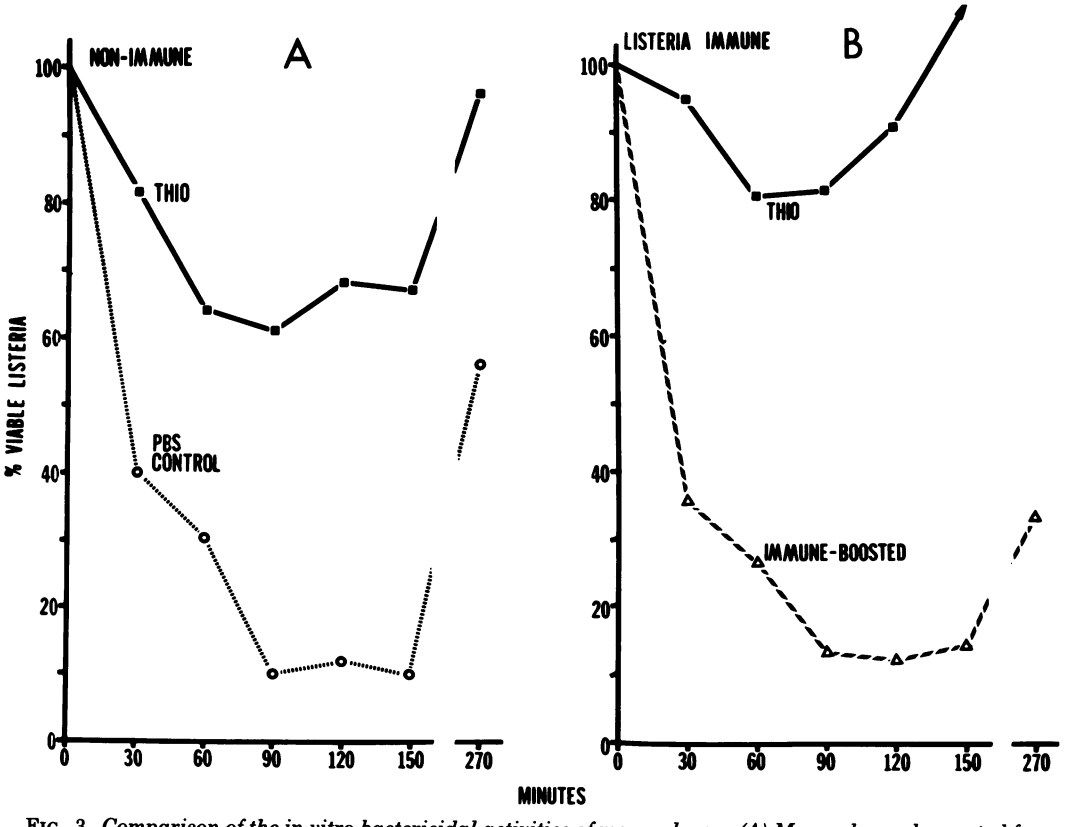

FIG. 3. *Comparison of the in vitro bactericidal activities of macrophages. (A) Macrophages harvested from the peritoneal cavities of nonimmune mice injected 3 days earlier with thioglycolate or PBS. (B) Macrophages were harvested from the peritoneal cavities of Listeria-immune mice injected 3 days earlier with thioglycolate or bacteria. Each time point is the geometric mean of triplicate samples.*

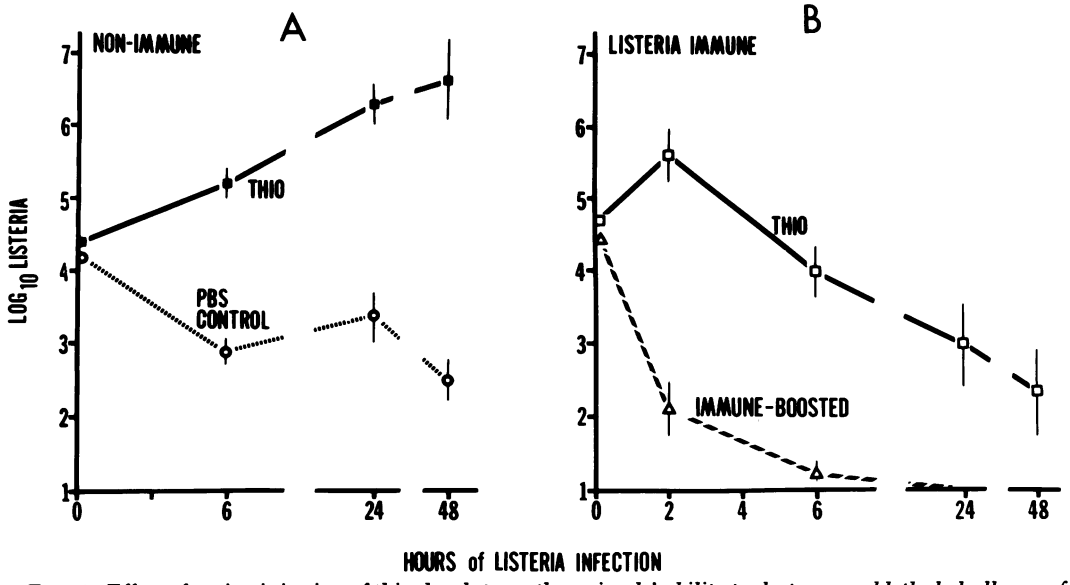

FIG. 4. *Effect of an i.p. injection of thioglycolate on the animals' ability to destroy a sublethal challenge of Listeria injected into the same site. Appropriate groups were injected with either thioglycolate, PBS, or bacteria 3 days before Listeria challenge. (A) Growth of a 10^5 inoculum of Listeria in nonimmune mice. (B) Growth of a similar inoculum in Listeria-immune animals. Geometric mean of five mice per time point ± SEM.*

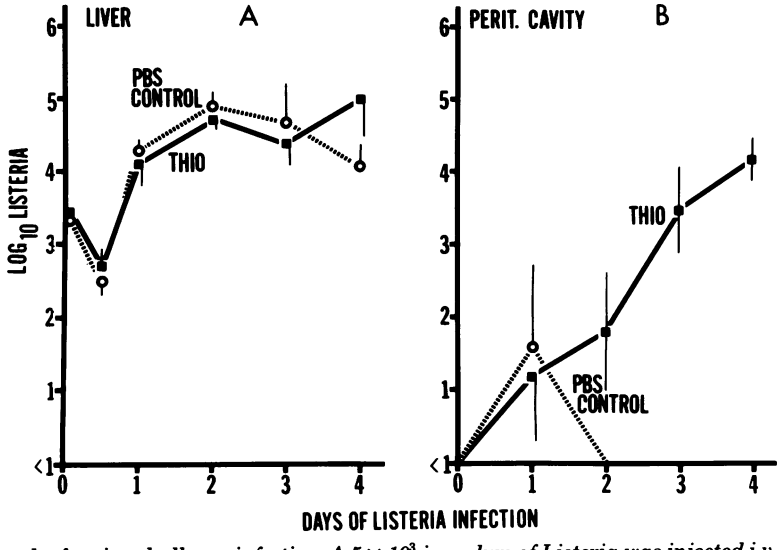

FIG. 5. *Growth of an i.v. challenge infection. A 5×10^3 inoculum of Listeria was injected i.v., and its growth was simultaneously followed in the livers and peritoneal cavities of mice injected i.p. 3 days earlier with either thioglycolate or PBS. Geometric mean of five mice per time point ± SEM.*

also revealed that large numbers of bacteria were present in the peritoneal cavities of the i.v. infected, thioglycolate-treated mice (Fig. 5B). This shows that after i.v. challenge, bacteria spread to the peritoneal cavity where they were able to grow virtually unchecked because of the previous injection of thioglycolate. Hence, under these circumstances it is conceivable that *Listeria* in the peritoneal cavity could, in turn, have spread to the liver, again leading to a fatal infection. This latter possibility is supported by the results in Fig. 6A, which show that a sublethal bacterial challenge grew progressively in the peritoneal cavities of mice injected 3 days earlier

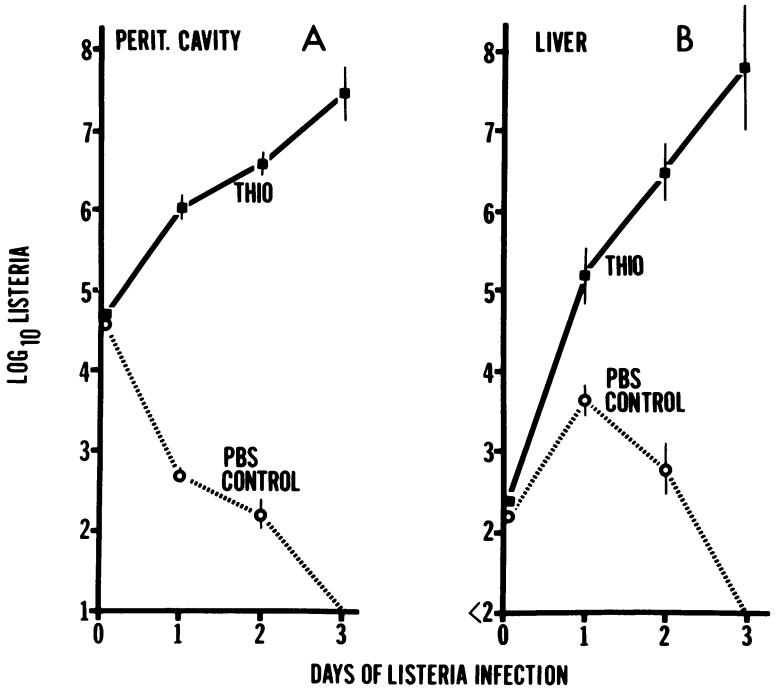

FIG. 6. *Growth of a sublethal bacterial challenge. A 10^5 inoculum of Listeria was injected i.p., and bacterial growth was simultaneously followed in the peritoneal cavities and livers of mice injected i.p. 3 days earlier with either thioglycolate or PBS. Geometric mean of five mice per time point ± SEM.*

with thioglycolate. From the initial site of infection in the peritoneal cavity, bacteria spread to the livers of thioglycolate and control mice (Fig. 6B). However, control mice limited bacterial multiplication, whereas in the livers of thioglycolate mice, *Listeria* growth progressed until the death of the mice.

Evidence that agar in thioglycolate medium is the cause of the impairment of bactericidal activity. Experiments were performed to determine whether the suppression induced by thioglycolate could be attributed to the presence of colloidal agar in the medium. The results in Fig. 7 show that an injection of either complete thioglycolate medium or PBS prepared with a similar concentration of agar (0.075% wt/vol) impaired the expression of antibacterial resistance. Injection of agar-free thioglycolate or PBS failed to interfere with the destruction of the *Listeria* challenge. In vitro studies revealed that macrophages harvested from the peritoneal cavities of the agar-injected mice were severely impaired in their ability to destroy bacteria (Fig. 8). Injection of agar-free PBS or thioglycolate failed to inhibit macrophage bactericidal activity.

Cellular inflammatory property of colloidal agar. In addition to the impairment of bactericidal activity induced by colloidal agar, the results in Table 1 show that i.p. injection of either thioglycolate or PBS prepared with agar induced a cellular exudate that was similar both in the number and types of cells present. In the agar-free preparations, injection of thioglycolate or PBS caused little, if any, change in the composition of peritoneal cells and resulted in a less than twofold increase in total number. These results implicate colloidal agar as being the component of fluid thioglycolate medium able to induce a cellular exudate.

DISCUSSION

The concept that macrophages become activated as part of the host immune response to infection was developed by Mackaness (11, 12, 24) to explain the enhancement in nonspecific bactericidal activity of macrophages from infected mice (3, 24). Since these early studies, enhancement of a variety of physiological or biochemical functions or both has been attributed to activated macrophages (4, 8). The studies presented in this paper show, however, that increases in certain functions associated with activation are not always accompanied by an enhancement in bactericidal activity. Similarly, a previous study (30) demonstrated that macrophages which accumulated in a progressive

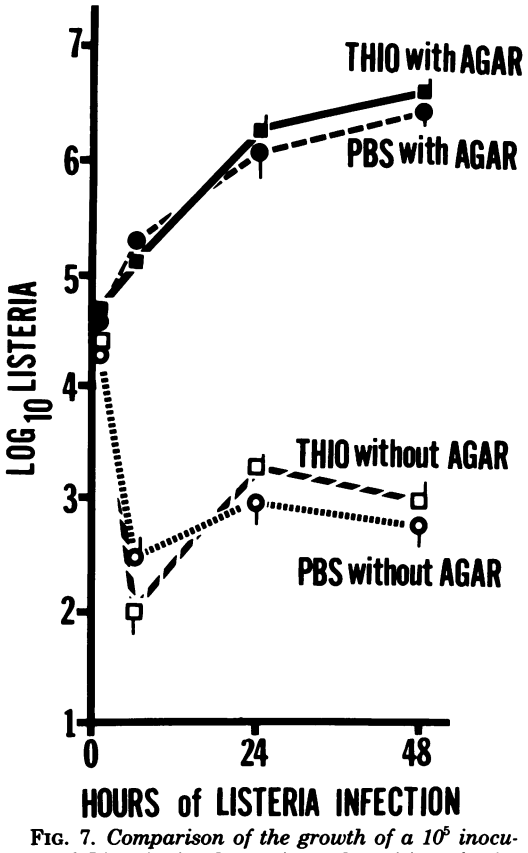

FIG. 7. *Comparison of the growth of a 10^5 inoculum of Listeria in the peritoneal cavities of mice injected i.p. 3 days earlier with 1.0 ml of either complete thioglycolate medium, PBS containing the same concentration (0.075% wt/vol) of agar as in thioglycolate, or agar-free thioglycolate or PBS. Geometric mean of five mice per time point ± SEM.*

syngeneic peritoneal ascites tumor were far better than normal in adherence, spreading, and phagocytosis, but were severely impaired in their ability to destroy a challenge of *Listeria* in vivo. Several other reports have also shown that "activated" macrophages sometimes exhibit decreases in their ability to phagocytose or degrade certain particles (2, 16, 28, 35). Such studies are valuable because they challenge the stereotype of an activated macrophage and are prompting a reevaluation of the concept of macrophage activation.

After local injection of either *Listeria* into immune mice or thioglycolate into nonimmune mice, macrophages collected from the peritoneal cavities of these mice consistently displayed enhancement of spreading, Fc-receptor-mediated phagocytosis, and release of plasminogen activator. Despite these increases, i.p. injection of thioglycolate interfered with antibacterial resistance in vivo, and macrophages from the peritoneal cavities of these mice were severely impaired in their ability to destroy *Listeria* in vitro. Thioglycolate-induced exudate macrophages have also been shown to be no better than normal macrophages in their ability to destroy *Trypanosoma cruzi* in vitro (18). The destruction of *T. cruzi* has been shown to require macrophages activated in vivo in conjunction with the host's response to infection (20) or in vitro by lymphokines (19). In the case of *Listeria*, there is ample evidence that normal macrophages possess some capacity to destroy bacteria in vivo (17, 23) and in vitro (30). The injection of thioglycolate into nonimmune mice virtually abolishes this measure of nonspecific resistance, and the animals ultimately die of an otherwise sublethal infection. On the other hand, the injection of thioglycolate into *Listeria*-immune mice delayed the ultimate onset and expression of antibacterial immunity against an i.p. challenge. The reason immunity was capable of being expressed is not known, but it could perhaps be attributed to either the influx of a new population of cells or the activation of the existing local population via the release of lymphokines. An observation that deserves comment pertains to the finding that immune-boosted mice rapidly destroyed an i.p. challenge of *Listeria* and yet peritoneal macrophages from these mice were no better than normal in their bactericidal activity in vitro. No explanation of this apparent discrepancy is immediately available.

The finding that an i.p. injection of thioglycolate impairs bactericidal activity in vivo and in vitro is in agreement with published studies from other laboratories (1, 14). In our studies, the suppression was shown to be confined to the site of injection, similar to that previously demonstrated in mice bearing peritoneal ascites tumors (30). Hence, i.p. injection of tumor cells or thioglycolate did not interfere with the expression of antibacterial resistance in the liver and spleen. Published studies have shown that, irrespective of the route of infection, bacteria can spread to virtually all tissues and cavities of the body (5). In view of this fact, it was not surprising to find that after i.v. injection of *Listeria*, bacteria were detected in the peritoneal cavities of all mice and were growing rapidly in those animals previously injected with thioglycolate. Consistent with the finding of widespread dissemination of a bacterial infection, our results also showed that the large numbers of bacteria in the peritoneal cavities of the thioglycolate-injected mice rapidly spread to the liver and must have contributed to the overwhelming systemic infection. If this was so, then the reported

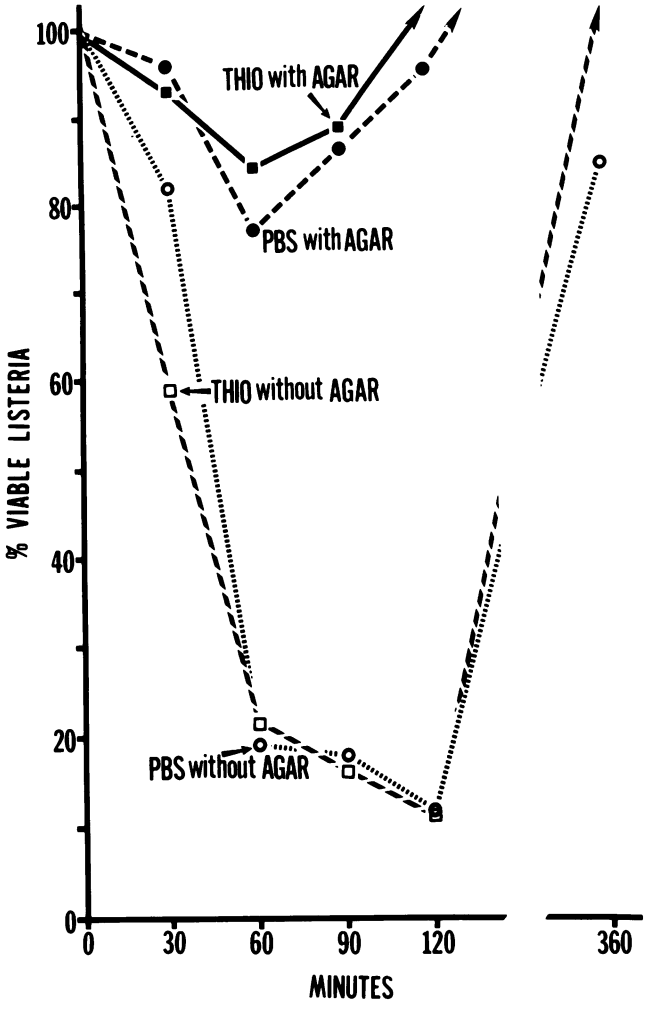

FIG. 8. *Comparison of the in vitro bactericidal activity of macrophages collected from the peritoneal cavities of mice injected i.p. 3 days earlier with 1.0 ml of either complete thioglycolate medium, PBS containing the same concentration (0.075% wt/vol) of agar as in thioglycolate, or agar-free thioglycolate or PBS. Each time point is the geometric mean of triplicate samples.*

reduction in the i.v. 50% lethal dose and the impaired ability of thioglycolate-treated mice to control the growth of *Listeria* in their livers (1) were probably not due to the thioglycolate causing a generalized systemic impairment of bactericidal activity.

Agar or some contaminant in the agar is considered to be the most likely single component of thioglycolate medium which caused the induced suppression of bactericidal activity. Macrophages isolated from the peritoneal cavities of animals injected with either thioglycolate or PBS containing agar exhibited characteristics of cells which had engaged in extensive phagocytosis. This was shown by the large number of vacuoles and lysosomes in the cytoplasm. This finding could be interpreted to suggest that macrophages may have endocytosed the suspension of colloidal agar. If macrophages engulfed the agar, this could have provided sufficient conditions for impairing bactericidal activity. This possibility is not without precedent since other studies have shown that macrophage ingestion of heterologous erythrocytes inhibited the destruction of bacteria (6, 10). Indeed, more recent studies have shown that the ingestion of erythrocytes inhibits the tumoricidal activity of activated macrophages (34). The exposure of activated macrophages to thioglycolate is known to inhibit antibody-dependent cell-mediated cyto-

TABLE 1. *Cellular composition of peritoneal exudates after i.p. injection of thioglycolate medium or PBS, prepared with or without agar*

Group[b]	Cellular composition[a] on day:								
	1			2			3		
	Mononuclear[c] phagocytes	Lymphocytes	PMN[d]	Mononuclear phagocytes	Lymphocytes	PMN	Mononuclear phagocytes	Lymphocytes	PMN
Thioglycolate medium	2.3 ± 0.5	2.7 ± 0.5	6.9 ± 0.6	11.0 ± 1.1	4.0 ± 0.6	7.9 ± 1.5	11.0 ± 0.3	3.0 ± 0.8	2.4 ± 0.8
PBS[e] with agar	7.0 ± 1.0	4.9 ± 0.5	18.0 ± 2.9	10.0 ± 2.3	3.6 ± 0.3	7.7 ± 1.0	9.9 ± 0.9	2.0 ± 0.7	2.7 ± 0.93
Thioglycolate without agar	2.6 ± 0.7	3.2 ± 0.2	1.9 ± 0.5	2.8 ± 0.1	6.7 ± 1.0	0.6 ± 0.02	2.0 ± 0.5	7.3 ± 0.7	0.5 ± 0.03
PBS without agar	1.3 ± 0.06	3.4 ± 0.5	0.48 ± 0.07	3.1 ± 0.06	4.5 ± 0.3	0.47 ± 0.3	3.0 ± 0.4	6.2 ± 1.4	0.7 ± 0.3
Control uninjected	1.7 ± 0.2	3.8 ± 0.9	0.1 ± 0.08	1.8 ± 0.5	5.0 ± 1.3	0.4 ± 0.09	1.9 ± 0.7	3.9 ± 0.3	0.5 ± 0.09

[a] Results are expressed as the number of cells × 10^{-6} ± standard error of the mean (SEM).
[b] Three mice/group per time point.
[c] Mononuclear phagocytes were identified by morphology, as well as by their ability to ingest heat-killed *Listeria*.
[d] PMN, Polymorphonuclear leukocyte.
[e] PBS was prepared with the same concentration of agar (0.075% wt/vol) as in thioglycolate medium.

toxicity and the release of H_2O_2 (15). However, thioglycolate-induced exudate macrophages have been shown to produce greater than normal amounts of superoxide anion (7). From these findings, it is tempting to postulate that the ingestion of agar acted somehow to divert the biochemical pathways or that the agar acted as a scavenger of H_2O_2, which would have resulted, in either case, in a reduction of the bactericidal potential of macrophages.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service grants CA-21360 and RR05705 from the National Cancer Institute and the Division of Research Resources, respectively.

The excellent technical assistance of Patricia Riemensnider and Susan Hutcelseider is gratefully acknowledged.

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