INFECTION AND IMMUNITY, Jan. 1982, p. 124–132
0019-9567/82/010124-09$02.00/0

Vol. 35, No. 1

Fate of *Listeria monocytogenes* in Murine Peritoneal Macrophage Subpopulations

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Received 20 July 1981/Accepted 15 September 1981

Peritoneal macrophages derived from CD-1 and C57BL/6 mice were separated into distinct groups based on their buoyant densities on discontinuous gradients of Percoll and assayed for antibacterial activity against *Listeria monocytogenes*. Subpopulations of peritoneal macrophages derived from *Listeria*-immune mice present a wide variation in their ability to control intracellular infection. Distinct subsets were found which exhibited bacteriostatic and listericidal activity. The fractionation procedure yielded a population of peroxidase-positive macrophages which were devoid of antilisterial action. Subpopulations of resident and elicited macrophages were also functionally heterogeneous in their ability to restrict intracellular growth of bacteria. In some experiments, subclasses were examined for secretion of plasminogen activator and phagocytosis of latex particles. These activities varied considerably with the status of activation of the macrophages, but failed to correlate with antimicrobial activity within given subpopulations.

In recent years, investigation has proved that mononuclear phagocytes, like lymphocytes, represent a heterogeneous population of cells. By using density gradients to effect separation, it has been shown that populations of macrophages from a single tissue source are heterogeneous in a variety of characteristics and activities, including the expression of phagocytosis (39, 45, 54), Fc receptors (45, 51), Ia molecules (4, 11, 12), immunogenic RNA production (39, 52), enzyme content (14, 38), chemotaxis in vitro (13), and response to lymphokines (13, 26). Moreover, several workers have isolated subpopulations of macrophages differing in tumor-inhibitory capacity (8, 9, 24, 25, 30, 32, 53). An early indication of the functional heterogeneity of macrophages with respect to antibacterial activity was derived from in vitro studies showing that although some bacteria are killed after phagocytosis, others survive and multiply (20, 21, 27). Observations from reinfection studies (27) and single-cell interactions (28) demonstrated more directly that heterogeneity resided with the macrophages and not the microorganisms. The present report deals with experiments which show that buoyant density subpopulations of resident, elicited, and immunologically activated macrophages differ in their expression of antibacterial activity. This manuscript also describes relationships among phagocytosis, peroxidase cytochemistry, secretion of plasminogen activator, and listericidal activity within murine peritoneal macrophage subpopulations.

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MATERIALS AND METHODS

Bacterium. Smooth *Listeria monocytogenes* strain A4413 (serotype 4b), a virulent, facultative, intracellular parasite, was utilized as the target microorganism in all experiments.

Collection and cultivation of macrophages. Peritoneal macrophages from CD-1 and inbred C57BL/6 mice (Charles River Breeding Laboratories Inc., Wilmington, Mass.) were harvested, processed, and cultivated by the method of Harrington-Fowler et al. (19). Activated macrophages were obtained from mice after intraperitoneal immunization and after elicitation with viable and heat-killed *Listeria*, respectively, as described previously (19). Elicited macrophages were obtained 5 days after intraperitoneal injection of 1.0 ml of 2.5% Brewer thioglycolate medium or 4 days after intraperitoneal injection with 2.5 ml of sterile (1.2%) sodium caseinate (Difco Laboratories, Detroit, Mich.) in isotonic saline.

Preparation of macrophage subpopulations. Macrophages were separated according to their buoyant densities on discontinuous Percoll (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) gradients by a modification of the methods of Walker (52) and Serio et al. (45). Percoll is a polyvinylpyrrolidone-coated silica of low osmolality and viscosity and allows for the rapid separation and recovery of viable and functionally active cells. The gradient medium was diluted in calcium- and magnesium-free phosphate-buffered saline at pH 7.2 to 7.4 to approximately 8 to 4% Percoll. The concentrations were adjusted according to their respective refractive indices by using an Abbe refractometer to final densities of 1.075, 1.064, 1.054, 1.038, and 1.029 at 26°C, respectively.

Peritoneal macrophages were washed in calcium- and magnesium-free phosphate-buffered saline and counted, and cell viability was determined by trypan blue exclusion. Approximately 1×10^7 to 2×10^7 cells were pelleted and suspended in 3.0 ml of 8% Percoll in a 15-ml Corex (Corning Glass Works, Corning, N.Y.) centrifuge tube. With the aid of a sterile Pasteur pipette, we overlaid 2.0 ml of the 7 to 4% Percoll solutions on the 8% cell solution, and the tube was centrifuged vertically at $490 \times g$ for 40 min at 4°C with a Sorvall RC-2 centrifuge equipped with a no. 302 adaptor. Macrophage subpopulations were collected by aspiration of the interface bands, washed, and adjusted to contain 10^6 morphologically distinct macrophages per ml in Dulbecco modified Eagle medium. The serum was deleted from the medium because it was determined to adversely affect the expression of antibacterial activity of immunologically activated macrophages. Each macrophage subpopulation was allowed to attach to a 16-mm plastic dish (Costar, Cambridge, Mass.) for 2 h in a 10% CO_2 atmosphere at 37°C. Nonadherent cells were withdrawn, and the monolayers were reincubated for an additional 15 h to ensure the removal of any residual adherent polymorphonuclear leukocytes and contaminating lymphocytes. Kinetic studies of antibacterial activity by *Listeria*-immune antigen-elicited macrophages indicate that bactericidal activity is retained for at least 22 h, at which time the ability to kill intracellular *Listeria* declines with further incubation (M. S. Wilder, unpublished data). Histological examination of cover slips stained with a Diff-Quik system (Harleco, Gibbstown, N.J.) revealed a rapid development of confluent monolayers consisting of morphologically distinct macrophages. The viability of the adherent population was routinely confirmed by dye exclusion. It was determined that macrophages cultivated in Dulbecco modified Eagle medium for 72 h in the absence of serum remain actively phagocytic and adherent and do not release appreciable levels of lactic dehydrogenase.

Infection and enumeration of cultures. Procedures for ascertaining the postphagocytic fate of *L. monocytogenes* in cultured macrophages have been detailed elsewhere (19). Briefly, 10^6 macrophages were pulsed at different multiplicities of infection (MOI), with log-phase cultures of bacteria opsonized in pooled homologous serum. After 40 min of phagocytosis, the monolayer was washed and incubated for 30 min with an antibiotic mixture consisting of 100 μg of streptomycin and 100 U of penicillin per ml to sterilize residual extracellular bacteria. The cultures were washed with fresh Dulbecco modified Eagle medium to remove the pulsed drug, and zero-time determinations of viable intracellular bacteria were determined by plate counts of lysates of macrophages fractured by being scraped with no. 10 glass beads. In replicate cultures, the medium was changed at 0.5-h intervals for 6 h, at which time a final intracellular count was performed. The assay describes the intracellular fate of *Listeria* between the period after the termination of phagocytosis and the ensuing 6 h. The kinetics of the host-parasite relationship during the initial period of phagocytosis will be the subject of a subsequent communication. Data on the listericidal capacity of unfractionated macrophage populations have recently been presented (19).

Peroxidase cytochemistry. Macrophage fractions were examined for evidence of peroxidase activity by the method of Kaplow (22). The cover slips were fixed with ethanolic Formalin and were stained briefly with benzidine dihydrochloride and hydrogen peroxide. The cells were counterstained in Diff-Quik for differentiation between macrophages, lymphocytes, and neutrophils.

Phagocytosis. The phagocytic efficiency of macrophage subpopulations was assessed with polystyrene latex particles (2 μm in diameter) by the procedure of Gordon et al. (16). Macrophages were incubated for 45 min at 37°C with 5×10^9 washed latex particles suspended in sterile phosphate-buffered saline. Monolayers were washed five times in sterile Dulbecco modified Eagle medium, and phagocytosis was quantitated by counting the number of macrophages in 100 40× fields which had ingested a minimum of three latex beads.

Secretion of plasminogen activator. Plasminogen-dependent fibrinolysis was assayed in the following manner. Wells 3 mm in diameter were cut into fibrin-impregnated agar plates (43). After a 24-h incubation of macrophage cultures in the presence or absence of latex particles, 80 μl of culture supernatant medium was added to duplicate wells, and then the plates were incubated at 37°C in a humidified atmosphere for 18 h. Diffusion zones were measured in at least two diameters, and the activity of the samples was read from a reference curve by using streptokinase (Nutritional Biochemicals Corp., Cleveland, Ohio) as a standard and was expressed as plough units. Heat-denatured fibrinogen plates were utilized to screen for the presence of nonspecific proteolytic enzymes. Human urine, which contains two activators of the plasminogen-plasmin system (17), was used as a positive control.

RESULTS

When suspensions of murine peritoneal macrophages were centrifuged on discontinuous gradients of Percoll, the cells separated into density subpopulations located at the interfaces of different Percoll solutions. These locations were used to define the subpopulations used here synonymously with subsets or subclasses; e.g., subpopulations 4 to 5% consisted of cells at the 4 to 5% interface. Macrophages in all bands were adherent and viable (greater than 95%) as measured by vital staining with trypan blue. The total cell recovery and cell distribution patterns varied with the state and disposition of the macrophages. Differential counts of the individual subsets after in vitro cultivation indicated that the adherent monolayer consisted of well-spread macrophages. The few polymorphonuclear leukocytes, lymphocytes, and erythrocytes were removed after overnight cultivation.

The intracellular fate of *L. monocytogenes* in cultivated CD-1 peritoneal macrophages obtained from the gradients is presented in Table 1. Bacteria were added to monolayers at infectivity ratios of 0.08 and 0.8 bacteria per macrophage

TABLE 1. Intracellular fate of *L. monocytogenes* in resident CD-1 peritoneal macrophage subpopulations

Density band (%)[a]	MOI	Recovered cells (%)[b]	Viable intracellular bacteria[c]	
			Zero time	6 h
4+	0.08	13	248 (40)	306 (40)
	0.8	9	1,352 (49)	3,191 (238)
4–5	0.08	14	398 (22)	986 (84)
	0.8	21	2,445 (84)	6,649 (76)
5–6	0.08	20	643 (69)	826 (176)
	0.8	16	3,888 (246)	6,295 (145)
6–7	0.08	22	823 (79)	1,254 (24)
	0.8	15	3,634 (227)	4,018 (217)
7–8	0.08	8	213 (12)	200 (8)
	0.8	5	5,209 (574)	12,824 (1,377)

[a] Macrophage subpopulations separated on Percoll gradients.
[b] Percent distribution of cells recovered from the gradient.
[c] Intracellular determinations of bacteria for each subpopulation represent the average and standard deviation (parentheses) of duplicate determinations performed on four separate macrophage cultures. Experiments employing different MOIs were performed on separate days.

and examined for growth, stasis, or killing between zero time and 6 h. At an MOI of 0.08, approximately 41% of the resident macrophages representing bands 4+, 5 to 6, and 7 to 8% were capable of inhibiting intracellular *Listeria* replication, whereas significant growth was observed in all cell fractions when the MOI was increased 10-fold. However, growth was marginal in the 6 to 7% density population, whereas cells in the other interface bands supported a two- to three-fold increase in bacterial numbers.

Subpopulations derived from caseinate-elicited macrophages exhibited functional heterogeneity with respect to their ability to control the intracellular growth of *Listeria* (Table 2). Compared with the low- and high-density bands, the macrophage subsets contained in the 4 to 5 and 5 to 6% bands were best able to curtail the growth of phagocytized bacteria. Although none of the caseinate-elicited fractions exhibited overt killing, bacteriostatic activity was evident in macrophages obtained from the 5 to 6% interface, whereas those derived from the 4+ and 7 to 8% density bands allowed for approximately 5- and 10-fold growth, respectively, during the 6-h incubation period. In contrast to the previous observations with resident macrophages (Table 1), the intracellular behavior was not markedly influenced by the infectivity ratio.

Thioglycolate-elicited macrophage subsets displayed a variability in their ability to control the intracellular replication of *Listeria* (Table 3). With the exception of cells derived from the low-density 4+% band, which exhibited listeriostatic action, all other thioglycolate subsets supported the growth of interiorized bacteria. Macrophages isolated from the bands at the 6 to 7 and the 7 to 8% interface appeared to allow for unrestricted growth of the microorganisms, whereas populations positioned in the 4 to 5 and the 5 to 6% bands were intermediate in their ability to curtail the rate of proliferation of phagocytized *Listeria*.

The data presented in Table 4 present evidence that immune C57BL/6 macrophages are functionally heterogeneous with respect to their ability to express antibacterial activity. Compared with resident or elicited cells (Tables 2 and 3), significant bactericidal action was demonstrated in macrophages cultivated from the 4+% band. Listericidal activity was less evident in macrophages contained in the 4 to 5% interface during this same interval. Macrophages in the 5 to 6% fraction of the Percoll gradient were bacteriostatic, whereas *Listeria* replicated in cells pelleted with the high-density fraction. All resident C57BL/6 peritoneal macrophage subpopulations with the exception of the cell fraction found at the 7 to 8% interface, which reduced the intracellular level of bacteria by 50%, supported bacterial growth.

We previously reported (19) that macrophages from *Listeria*-immune mice contain a small percentage of peroxidase-positive mononuclear cells. Accordingly, it was of interest to investigate whether enzyme activity might be associated with those subsets displaying antibacterial capability. The data presented in Table 4 indicate that the peroxidase-positive population was distributed between the 4 to 5% density band and represented 24% of the population. Although this fraction was moderately listericidal, essentially peroxidase-negative macrophages, which banded in the 4+% Percoll interface,

TABLE 2. Intracellular fate of *L. monocytogenes* in caseinate-elicited CD-1 macrophage subpopulations

Density band (%)[a]	MOI	Recovered cells (%)[b]	Viable intracellular bacteria[c]	
			Zero time	6 h
4+	0.1	27	255 (24)	1,130 (25)
	0.4	32	4,945 (22)	21,080 (1,439)
	0.8	31	6,503 (1,815)	30,488 (916)
4–5	0.1	26	795 (37)	1,358 (48)
	0.4	28	5,710 (536)	10,060 (1,089)
	0.8	17	5,688 (193)	10,410 (369)
5–6	0.1	15	843 (32)	1,803 (35)
	0.4	8	1,095 (151)	1,935 (123)
	0.8	12	1,215 (188)	1,715 (97)
6–7	0.1	11	228 (17)	1,335 (137)
	0.4	13	1,276 (131)	7,355 (487)
	0.8	6	2,020 (71)	16,750 (1,923)
7–8	0.1	7	63 (18)	775 (63)
	0.4	7	1,320 (90)	13,472 (1,453)
	0.8	4	1,028 (61)	15,800 (1,376)

[a–c] See footnotes of Table 1.

exhibited maximal intracellular bacterial killing.

Activated macrophages generally exhibit enhanced capacities for phagocytosis (2, 7, 36, 47) and for secretion of plasminogen activator (16, 18, 48, 50). Experiments were performed to determine whether these activities correlate with each other as well as with expression of antimicrobial resistance within distinct subpopulations of peritoneal macrophages. The results of these studies (Tables 4 and 5) indicated that bactericidal subsets derived from *Listeria*-immune mice were not consistently associated with a capacity for secretion of high levels of plasminogen activator. For example, mononuclear cells positioned in the 6 to 8% density band secreted high levels of plasminogen activator, but exhibited a minimal capacity for intracellular killing of *Listeria*. In this regard, populations of caseinate- or thioglycolate-elicited macrophages, which secreted maximal levels of enzyme, were similarly devoid of antilisterial activity. The relationship is not an inverse one since subpopulations contained in the 4 to 5% band secreted 40 plough units of enzyme and were capable of inhibiting bacterial growth.

The Percoll separation procedure resulted in the isolation of macrophage subsets which exhibited differential capacities for the engulfment of latex. The findings, however, did not reveal any consistent pattern of phagocytosis among populations of resident, elicited, and immunologically activated macrophages. Although the low-density band containing bactericidal C57BL/6 macrophages was least able to interiorize latex, there did not appear to be a negative correlation between these two events. In this regard, the two high-density macrophage populations displayed a wide variation in the ingestion of latex, but were equally incapable of killing *Listeria*. With the exception of resident cells derived from the 7 to 8% interface, there was similarly no correlation between phagocytosis and secretion of plasminogen activator.

DISCUSSION

The data presented in this report document significant differences among mouse peritoneal subpopulations with regard to antilisterial activi-

TABLE 3. Intracellular fate of *L. monocytogenes* in thioglycolate-elicited CD-1 macrophage subpopulations

Density band (%)[a]	MOI	Recovered cells (%)[b]	Viable intracellular bacteria[c]	
			Zero time	6 h
4+	0.8	18	1,691 (81)	2,065 (254)
4–5	0.8	48	1,438 (168)	5,060 (187)
5–6	0.8	21	2,839 (202)	6,648 (287)
6–7	0.8	6	1,546 (73)	9,672 (98)
7–8	0.8	6	2,928 (446)	39,796 (1,812)

[a–c] See footnotes of Table 1.

TABLE 4. Peroxidase cytochemistry and intracellular fate of *L. monocytogenes* in resident and immune C57BL/6 macrophage populations

Density band (%)[a]	Macrophage	MOI	Recovered cells (%)[b]	Peroxidase[c] (%)	Viable intracellular bacteria[d]	
					Zero time	6 h
4+	*Listeria*-immune[e]					
	Expt 1	0.8	17	1 (1)	10,700 (1,238)	3,230 (170)
	Expt 2	0.8	29		10,005 (427)	3,240 (171)
	Resident	0.8	18	1 (1)	896 (64)	2,073 (374)
4–5	*Listeria*-immune					
	Expt 1	0.8	24	9 (2)	3,878 (132)	2,050 (129)
	Expt 2	0.8	17		3,063 (337)	2,168 (34)
	Resident	0.8	14	1 (1)	2,193 (122)	4,553 (344)
5–6	*Listeria*-immune					
	Expt 1	0.8	7	1 (1)	2,228 (193)	2,133 (306)
	Expt 2	0.8	6		2,208 (148)	2,033 (270)
	Resident	0.8	15	1 (1)	2,147 (197)	5,973 (297)
6–7	*Listeria*-immune					
	Expt 1	0.8	7	1 (1)	978 (57)	2,313 (152)
	Expt 2	0.8	2		868 (74)	2,145 (38)
	Resident	0.8	8	1 (1)	920 (115)	3,067 (743)
7–8	*Listeria*-immune					
	Expt 1	0.8	4	1 (1)	556 (128)	926 (102)
	Expt 2	0.8	1		410 (22)	1,283 (28)
	Resident	0.8	2	1 (1)	900 (254)	426 (30)

[a] Macrophage subpopulations separated on Percoll gradients.

[b] Percent distribution of cells recovered from the gradient.

[c] The percentage of peroxidase-positive macrophages on cover slips in Costar chambers represents the average and standard deviation (parentheses) of triplicate determinations, each involving the observation of 300 macrophages.

[d] Intracellular determinations of bacteria for each subpopulation represent the average and standard deviation (parentheses) of duplicate determinations performed on four separate macrophage cultures. Experiments employing different MOIs were performed on separate days.

[e] Macrophages were collected 18 h after the elicitation of 7-day *Listeria*-immune animals with an intraperitoneal injection of 10^7 heat-killed homologous organisms.

ty. Although *L. monocytogenes* was not killed by any of the fractionated resident CD-1 macrophages during the 6-h interval after phagocytosis, there were distinct differences in the postphagocytic fate of the microorganisms within the various subpopulations (Table 1). Whereas some subsets controlled intracellular growth by stasis, others allowed for a two- and threefold increment in bacterial replication. A subsequent experiment (Table 4) revealed the expression of bactericidal action by a single high-density subpopulation derived from resident C57BL/6 mice. The failure to observe parallel antibacterial behavior in cell fractions obtained from these mouse strains most likely reflects the genetic differences in the distribution and functional capacities of their respective macrophage subpopulations. These observations are consistent with a previous report (10) demonstrating differential capacities for resident cells from inbred mouse strains to retard the growth of *Listeria* during the initial stages of infection. In experiments with resident cells, a reduction in the MOI was accompanied by a corresponding augmentation in the expression of macrophage antibacterial activity. Baughn and Bonventre (3) observed a similar relationship between the MOI and the kinetics of intracellular inactivation of *Staphylococcus aureus*. The diminished rate and extent of killing at the high infectivity ratio were thought to be consequences of macrophage depletion of bactericidal capabilities. An alternative explanation might be that the lower phagocytic load selects for macrophages with enhanced capacity for ingestion and restriction of intracellular growth. In these experiments, low levels of infectivity were employed to prevent toxic manifestations and to establish a situation wherein individual macrophages would not have to contend with large numbers of intracellular bacteria. However, it is important to note that such inocula lead to the infection of a relatively small percentage of the macrophages, which may result in phagocytosis by a

TABLE 5. Phagocytosis and secretion of plasminogen activator by mouse peritoneal macrophage subpopulations

Mouse strain	Macrophages[a]	Density band (%)[b]	Phagocytosis (%)[c]	Plasminogen activator[d]
CD-1	Resident	4+	32	5
		4–5	45	5
		5–6	37	5
		6–7	26	5
		7–8	85	50
CD-1	Caseinate	4+	16	5
		4–5	21	5
		5–6	56	50
		6–7	59	80
		7–8	27	5
CD-1	Thioglycolate	4+	44	5
		4–5	27	35
		5–6	42	20
		6–7	55	5
		7–8	79	5
CD-1	*Listeria*-immune	4+	21	5
		4–5	17	5
		5–6	38	50
		6–7	43	35
		7–8	35	35
C57BL/6	*Listeria*-immune	4+	13	5
		4–5	10	40
		5–6	44	5
		6–7	67	50
		7–8	36	65

[a] See text for preparation of elicited and activated macrophages.
[b] Macrophage subpopulations separated on Percoll gradients.
[c] Percentage of macrophages ingesting three or more latex beads.
[d] Plasminogen-dependent fibrinolysis after latex phagocytosis expressed as plough units with streptokinase as standard.

subclass of cells within the larger subpopulation. Optimal conditions would require the infection of the majority of the cells within the subpopulation without initiation of artifactual injury produced by the presence of excessive bacteria and their products.

Density gradient-separated caseinate and thioglycolate-elicited macrophage populations differed in their abilities to restrict the multiplication of bacteria (Tables 2 and 3). Although none of these populations expressed listericidal activity, there was wide variation in the level to which elicited cells supported postphagocytic growth of the pathogen. These findings confirm and extend previous observations (6, 35, 46) regarding the absence of bactericidal action against facultative intracellular parasites by nonspecifically stimulated cells. The relatively bacteriostatic fraction derived from the 5 to 6% band of the caseinate-stimulated cells (Table 2) seems to represent an intermediate level of activation which, compared with resident cells, may be less vulnerable to exhaustion of residual antimicrobial activity.

We recently reported that immunologically activated macrophages display bactericidal activity towards *L. monocytogenes* only when the animals received an injection of heat-killed bacteria 18 h before the harvest of the peritoneal cells (19). The data presented in this manuscript establish the heterogeneity of immune-elicited macrophages by identifying distinct populations of cells which differ in their ability to inhibit bacterial growth. While some subsets were strongly or moderately bactericidal, a fraction derived from a high-density band was deficient in the killing of bacteria. The heterogeneity observed among immune macrophages may depend, at least in part, on their ability to respond to lymphocyte signals. This idea is consistent with the recent demonstration of subpopulations of human monocytes exhibiting differential responses to various chemotaxins, including lymphocyte-derived chemotactic factor (13). More-

over, the magnitude of the responses of murine macrophages to lymphokine for the expression of antitumor activity varies with cell size (26). Functional heterogeneity may also involve prostaglandin regulation of intracellular cyclic AMP. In this regard, prostaglandin E_1 increases cellular cyclic AMP in peritoneal macrophages and human monocytes, and this activity is associated with the prevention of tumoricidal activity (42) and the decreased killing of *S. aureus* (37), respectively. Additionally, peripheral blood monocytes subjected to discontinuous density gradient fractionation demonstrate significant differences in the synthesis of prostaglandins (15), which can serve as a feedback mediator capable of modulating macrophage metabolism (38).

Macrophage populations display heterogeneity in their expression of Ia molecules (11, 12), and it is only the Ia-bearing subsets that are responsible for antigen interaction with T cells (5, 12, 44). Beller et al. (4) recently reported that infection and elicitation of mice with live and heat-killed *L. monocytogenes* results in an enrichment in the peritoneal cavity of Ia-positive macrophages. This phenomenon was antigen specific and required an immune stimulus, since exudates induced by other eliciting agents failed to generate an increase in the absolute number of Ia-positive cells. Because gene products of the I region of the major histocompatibility complex appear to play a critical role in the interaction of macrophages and lymphocytes, it would be useful to determine whether Ia-positive subsets represent the predominant macrophage population responsible for intracellular killing of bacteria.

The results of this study confirm our previous observation that macrophage bactericidal activity is not associated with a population of peroxidase-positive cells (19). The percentage of cells stainable for peroxidase was relatively meager and was confined to a single fraction, which was dissociated from macrophages exhibiting maximal bactericidal activity. These observations bear upon the recent report of Lee et al. (26), who successfully separated immature peroxidase-positive macrophages from the large, mature peroxidase-negative subsets by using velocity sedimentation. The subpopulation most responsive to activation by endotoxin and lymphokine to produce interleukin 1 and to express tumoricidal activity consisted of the more mature cells and was not exclusive to peroxidase-positive macrophages as previously indicated (29, 40).

Peroxidase activity has generally been taken as a marker for macrophage differentiation. Promonocytes and monocytes abundantly endowed with lysosomal myeloperoxidase lose this granule enzyme during maturation to the mature, macrophagic form (34, 49). The data presented in Table 4 indicate that immune macrophage fractions expressing a wide variation in bactericidal activity display comparable levels of peroxidase content. This observation supports the idea that macrophage heterogeneity as seen in these experiments is not a reflection of the properties of cells in various stages of maturation.

The expression of bactericidal activity by subpopulations of immune cells derived from C57BL/6 animals appears to vary somewhat inversely with phagocytosis of latex particles. Conversely, macrophages displaying elevated levels of intracellular *L. monocytogenes* immediately after the period allotted for phagocytosis were best able to control the growth of the microorganism. To date, there is no clear-cut correlation between phagocytosis and macrophage activation. Whereas some investigators have found that activation is accompanied by an enhanced phagocytic capacity (2, 7, 36), others have found no change (23, 47) or a depression in phagocytosis (1, 33, 41). Such conflictual observations may be technical with respect to the assay, to the nature of the particle, or to the state of activation. Additional kinetic studies on the phagocytosis of bacteria and other particles are necessary before any definitive conclusions can be drawn regarding the above observations.

It should be noted that a significant percentage of cells in each macrophage fraction did not internalize latex beads (Table 5). Rice and Fishman (39) similarly reported that phagocytosis of latex particles among macrophage subsets ranged from 5 to 70%. It cannot be concluded from these studies that large segments of the population consist of nonphagocytic cells, and it seems more likely that optimal dose and time conditions for phagocytosis may not have been obtained. In this regard, Nathan and Terry (33) determined that macrophages had to be exposed to large particle doses for extended periods of time before latex uptake reached a plateau. Additionally, these workers identified and characterized a small subpopulation of esterase-positive nonphagocytic-adherent peritoneal cells, which increased threefold upon immunization with BCG (31). This level of nonphagocytic cells in the population at large, however, would be of insufficient magnitude to account for the percentage of cells which were negative for interiorized latex. We cannot rule out the possibility that early manipulations, used to fractionate the macrophages, induced alterations in the phagocytic process. In this regard, Serio et al. (45) have shown that although Percoll solutions do not affect cell viability or cytolytic activity, there is an inhibitory effect on phagocytosis.

Additionally, the foregoing experiments have demonstrated that plasminogen activator secretion can be dissociated from antibacterial activity. Plasminogen activator was measurable in supernatants derived from cultures of resident and elicited cells which did not display bactericidal activity. In the case of immunologically activated macrophages, subpopulations exhibiting maximal listericidal action were not associated with copious secretion of enzyme. Similar observations to those recorded here were made by Noguiera et al. (35), who noted that although increased fibrinolytic and trypanocidal activity have a parallel time course, these two properties are not related. Thioglycolate-activated macrophages secreted large amounts of plasminogen activator, but did not display any capacity to inhibit the intracellular growth of the trypomastigotes. In the experiments described herein, secretory activity was characterized in three cell populations of thioglycolate-elicited cells, each of which failed to inhibit the replication of *Listeria*. The findings support the view that plasminogen activator represents a nonspecific activity of elicited or immunologically activated macrophages, which occurs independently of the development of bactericidal capabilities.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service research grant AI-15853 from the National Institute of Allergy and Infectious Diseases.

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