CELLULAR IMMUNITY IN VITRO

I. Immunologically Mediated Enhancement of Macrophage Bactericidal Capacity

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The relationship between classical delayed hypersensitivity, as exemplified by the tuberculin-type skin test, and cellular immunity, here defined as an acquired enhancement of resistance to infection with intracellular microorganisms, has long been a subject of investigation. Recently the delayed hypersensitivity reaction has been opened to renewed study because in vitro models such as lymphocyte transformation and macrophage migration inhibitory factor (MIF)[1] production have been developed (1). The work of Mackaness and his group (2) has greatly expanded our understanding of the mechanism of cellular immunity, largely through in vivo studies.

The present studies describe a quantitative in vitro model of cellular immunity in the guinea pig. Oil-induced peritoneal exudate cells from immune and control guinea pigs were cultured overnight with and without three different protein antigens. The cultures were then washed, removing lymphocytes and antigen, and the remaining macrophage monolayers were infected with *Listeria monocytogenes*. Macrophage bactericidal capacity was evaluated both by sequential visual counting and by quantitative culture of intracellular bacteria. Both techniques demonstrated that peritoneal exudate macrophages from immune animals had a greatly enhanced bacterical capacity when the exudate had been cultured with antigen before infection. Macrophage migration was also inhibited under these conditions.

Materials and Methods

Animals.—Inbred strain 2 guinea pigs of both sexes weighing 200–500 g were used. For the initial studies the guinea pigs were immunized with complete Freund's adjuvant (CFA) containing 5 mg/ml of *Mycobacterium tuberculosis* H37Ra (Difco Laboratories, Inc., Detroit,

[1] *Abbreviations used in this paper:* BGG, bovine gamma globulin; BHI, brain–heart infusion; CFA, complete Freund's adjuvant; MEM, Eagle's minimal essential medium; MI, migration index; MIF, migration inhibitory factor; MP, macrophages; PEC, peritoneal exudate cells; Pic-HSA, picrylated human serum albumin; PPD, purified protein derivative; RMI, relative migration index.

Mich.); each animal received 0.5 ml divided between the footpads and neck. Control animals were injected with incomplete adjuvant emulsified with saline. Skin tests with 5 μg of tuberculin purified protein derivative (PPD) (Parke, Davis & Co., Detroit, Mich.) were uniformly positive in the experimental animals and negative in the controls.

For subsequent studies strain 2 guinea pigs were immunized with 0.125 mg of bovine gamma globulin (BGG) (Pentex Biochemical, Kankakee, Ill.) in 0.5 ml of commercial CFA (Difco), or with 0.125 mg of picrylated human serum albumin (Pic-HSA) (kindly provided by Dr. John S. Johnson, Laboratory of Clinical Investigation, National Institutes of Health, Bethesda, Md.) in 0.5 ml of CFA. Control animals received CFA alone. The animals were boosted in a similar fashion 1–6 wk before each was studied. Skin tests with 10 μg of BGG were uniformly positive in the BGG-immune animals and negative in the other groups; skin tests with 10 μg of Pic-HSA were uniformly positive in the Pic-HSA–immune animals and negative in the other groups.

Bacteria.—Listeria monocytogenes (American Type Culture Collection 19115) was maintained in semisolid media with monthly passage through brain–heart infusion (BHI) broth. Antibiotic sensitivity patterns and guinea pig virulence remained constant throughout the study period. Bacterial inocula were prepared from an overnight culture in BHI broth and were quantitated by determining the optical density of a saline suspension at 650 nm. Each inoculum was confirmed by quantitative pour plates. BHI agar was used for the pour plates, and all cultures were performed in 5% CO_2 at 37°C.

Peritoneal Exudate Cells (PEC).—Peritoneal exudates were induced by intraperitoneal injection of 30 ml of sterile Bayol F (Esso Chemical Co., Inc., New York). 3 days later the cells were harvested from CO_2 narcotized animals by peritoneal lavage with heparinized (5 units/ml) Eagle's minimal essential medium (MEM) (Microbiological Associates, Inc., Bethesda, Md.) The cells were centrifuged at 900 rpm for 10 min and resuspended in MEM. Differential cell counts and preparations incubated with 0.2% neutral red revealed an average of 70% macrophages (MP), 15% lymphocytes, and 15% granulocytes. PEC were > 95% viable by vital dye (nigrosine) exclusion. For most experiments PEC from two immune and two control animals were harvested simultaneously and pooled.

Cell cultures were performed in MEM enriched with 1 ml of 40% glucose, 1 ml of 3% glutamine, 1 ml of 100 mM pyruvate (Microbiological Associates, Inc.), and 1 ml of nonessential amino acids (100 X) (Grand Island Biological, Grand Island, N. Y.), per 100 ml. The final culture medium contained 20% pooled normal Hartley strain guinea pig serum which had been heat inactivated at 56°C for 30 min, sterilized by millipore filtration, and frozen until use. The serum did not agglutinate *Listeria*, although it did contain opsonins, and the final tissue culture medium had no listericidal properties.

Exudate cells were adjusted to a concentration of 2×10^6/ml of enriched MEM and 2 ml were placed in 35 mm plastic tissue culture dishes (Falcon Plastics, Los Angeles, Calif.) which were incubated overnight at 37°C in 5% CO_2. Cells from immune and control animals were cultured both in the presence and absence of the appropriate antigen: preservative-free PPD (kindly supplied by Dr. H. B. Devlin, Parke, Davis & Co.) was used at a concentration of 10 μg/ml; BGG was used at a concentration of 50 μg/ml; and Pic-HSA was used at a concentration of 1 μg/ml.

After overnight culture, all plates were washed with MEM to remove nonadherent cells and antigen. Random plates from each group were evaluated by Gram staining, Wright staining, incubation with neutral red, incubation with 1.0 μ of polystyrene particles (Dow Chemical Co., Midland, Mich.), and estimation of viability by vital dye exclusion. These studies showed that > 95% of the cells present after overnight incubation and washing were viable; > 99% of the viable cells were morphologically consistant with macrophages and were avidly phagocytic. Visual microscopic evaluation of stained plates from each of the four experimental groups with the aid of an eyepiece grid showed that the numbers of MP on

individual culture dishes were comparable. In addition, direct cell counts were performed after removing the MP by scraping with a rubber policeman after incubation for 8 min in phosphate-buffered saline containing 0.01% ethylenediaminetetraacetate (EDTA) and 0.1% trypsin. Cultures from immune animals incubated with antigen contained an average of 1.71×10^6 MP/culture dish on eight determinations; those from immune animals cultured without antigen contained an average of 1.63 10^6 MP (six determinations); those from control animals cultured with antigen contained an average of 1.97×10^6 MP (six determinations); those from control animals cultured without antigen contained an average of 1.70×10^6 MP (five determinations). These counts are not significantly different from each other (P at least > 0.10 by the two-sample t test) suggesting that variations in MP numbers did not influence the listericidal assay system.

Bactericidal Assay.—Approximately 5×10^7 *Listeria* in 2 ml of enriched MEM containing 20% heated Hartley guinea pig serum was added to each culture dish. After incubation for 30 min at 37°C in 5% CO_2, the dishes were washed with MEM and reincubated with enriched MEM containing 20% Hartley serum and 1.2 μg of sodium penicillin/ml. This concentration of penicillin was three times the measured minimal inhibitory concentration for the *Listeria* used in these studies; as a result, the culture fluid was sterilized and further phagocytosis of bacteria released from damaged macrophages was prevented. To insure that the penicillin did not penetrate MP and alter the results of the bactericidal assay, a parallel experiment was performed without the antibiotic.

After incubation for 1½ and 5 hr after infection in initial studies and for 4 hr in subsequent studies, the MP cultures were thoroughly washed with MEM to remove all penicillin and the cells were lysed by exposure to distilled water for 15 min and scraping with a rubber policeman. This procedure did not alter bacterial viability. Serial dilutions and quantitative pour plates were made from each culture plate and colonies were counted after 36 hr. For each experiment the experimental and control groups were processed simultaneously under identical conditions. The bactericidal assay was performed on three or four different plates from each cell group.

Phagocytic Index and Monolayer Morphology.—In the studies of BGG and Pic-HSA–immunized animals, randomly selected MP culture dishes from each group were washed and Gram stained 10 min, 30 min, 4 hr, and 24 hr after infection with *Listeria*. The dishes were randomized and coded, and the number of bacteria in 50 cells in each dish was counted by two observers. The morphology of the MP monolayer was also evaluated.

MIF Assay.—Inhibition of peritoneal exudate macrophage migration was evaluated by the method of David et al. (3). PEC from BGG-immune and control guinea pigs were harvested and washed as described above; these were then divided into samples for the bactericidal and MIF assays. For the MIF studies, 50×10^6 PEC were suspended in 1 ml of MEM and drawn up in capillary tubes. One end of each tube was heat-sealed. The tubes were spun at 1000 rpm for 5–10 min and broken exactly at the cell–medium interface. Two tube segments containing packed PEC were put in each Sykes-Moore chamber and were held in place with sterile silicone grease. The chambers were sealed and filled with 1 ml of enriched MEM containing 20% Hartley guinea pig serum with or without BGG (50 μg/ml).

The chambers were incubated at 37°C in 5% CO_2 for 20–24 hr, at which time the fan of migrating cells was projected, 20 times enlarged, on a screen and traced on paper. The area covered by the migrating cells was measured by a planimeter and expressed in planimeter units. Four tubes, in two chambers, were measured for each group and the mean area determined.

The migration index (MI) was calculated from the formula:

$$\text{MI} = \frac{\text{mean area of migration with BGG}}{\text{mean area of migration without BGG}} \times 100$$

To compensate for any nonspecific effect of BGG alone, the relative migration index (RMI) was determined from the formula:

$$\text{RMI} = \frac{\text{MI of immune cells}}{\text{MI of control cells}} \times 100$$

RESULTS

Animals Immunized with Tubercle Bacilli.—In this initial series of experiments, peritoneal exudate cells from guinea pigs hypersensitive to tuberculin

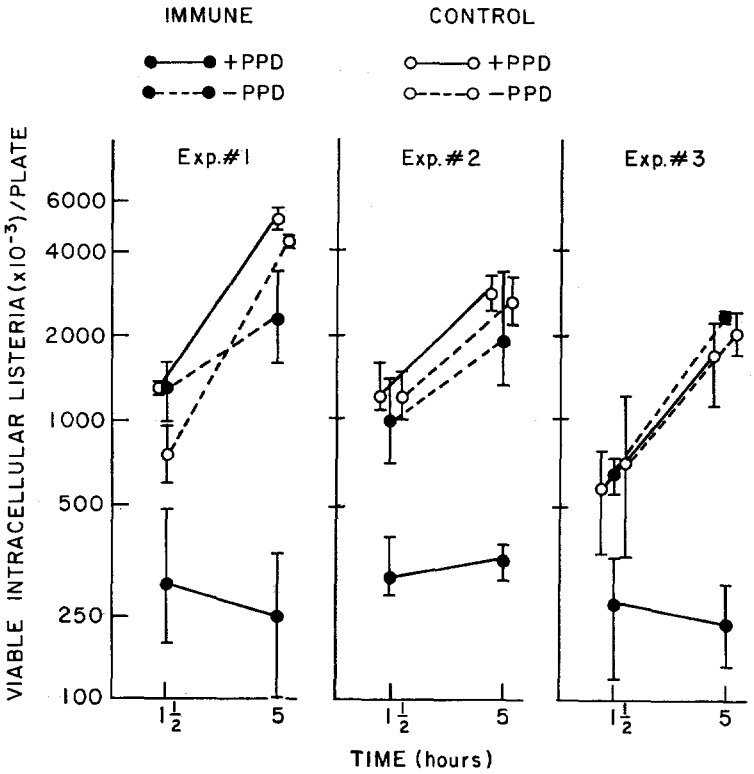

FIG. 1. Effect of prior incubation with PPD on the growth of *Listeria* in macrophages from PPD-immune and nonimmune guinea pigs.

were compared with PPD-negative controls, which had received incomplete adjuvant. PEC from both groups were cultured overnight with and without PPD (10 μg/ml). 1½ and 5 hr after *Listeria* infection, the MP were lysed and the number of intracellular bacteria determined. By 1½ hr (Fig. 1) the cells from immunized animals precultured with PPD contained many fewer bacteria than cells from the same animals cultured without antigen ($P < 0.005$, t test, in all three experiments). Addition of PPD had no effect on the cells from unim-

munized animals. Cells from immune animals cultured without antigen were no more bactericidal than control cells. Even more striking was the observation that the number of bacteria increased markedly over the next 3½ hr in all control groups, while the immune cells precultured with antigen held the infection in check. Thus, the differences noted at 1½ hr were magnified at 5 hr.

Animals Immunized with Pic-HSA in CFA.—The bactericidal capacity of peritoneal exudate MP from Pic-HSA–immune and control animals was evaluated by quantitative culture of viable intracellular organisms 4 hr after *Listeria* infection. This single point in time was chosen to simplify the technical procedure. Table I (experiment 1) illustrates the striking enhancement of the

TABLE I

Quantitative Assay of Viable Intracellular Listeria 4 Hr after Infection of Immune or Control Guinea Pig Macrophages Cultured with or without Specific Antigen before Infection

Experiment	Antigen	Viable intracellular *Listeria* ($\times 10^{-3}$) at 4 hr from			
		Immune cells		Control cells	
		+ Antigen	− Antigen	+ Antigen	− Antigen
1. Pic-HSA–immune cells vs. control	Pic-HSA (1 μg/ml)	75 (54–98)*	3147 (2020–4260)	1641 (1400–1980)	2252 (2180–2440)
2. BGG-immune cells vs. control	BGG (50 μg/ml)	105 (74–136)	3722 (2840–5000)	4197 (3820–4700)	4906 (4000–5920)
3. BGG- and PPD-immune cells vs. control	BGG (50 μg/ml)	85 (52–168)	2844 (1100–4820)‡	4977 (4440–5340)	4648 (4020–5080)‡
	PPD (10 μg/ml)	305 (92–650)	2844 (1100–4820)‡	5700 (4140–6740)	4648 (4020–5080)‡

* Geometric mean (range) of three or four cell cultures.

‡ Cells cultured without antigen are the same controls for both BGG and PPD cell groups.

bactericidal activity of MP from the immune animals cultured with 1 μg/ml of Pic-HSA for 24 hr before infection. Once again the experimental group was significantly ($P < 0.001$) different from controls. In this case there was a slight nonspecific effect of Pic-HSA itself in the control cultures ($P < 0.05$) which was not seen in our other antigen systems.

These same MP cultures were also evaluated by counting intracellular organisms on Gram-stained tissue culture dishes as the infection progressed. Table II (experiment 1) shows that at 10 min the MP from Pic-HSA–immune animals had the same number of intracellular bacteria whether or not antigen had added to the culture medium. The control cells cultured with Pic-HSA had a similar number of *Listeria* per cell at 10 min, but for unknown reasons the control cells cultured without antigen had a smaller number. By 30 min those

relationships had changed so that the immune cells cultured with antigen had the fewest numbers of bacteria. During this first 30 min period, bacteria remain in the MP culture media, so that these counts reflect a balance between continued phagocytosis and bacterial killing and degradation. Because extracellular organisms are then removed by washing and by the addition of penicillin, subsequent counts reflect killing alone. At 4 hr the visual counts paralleled the quantitative cultures, as the experimental cells contained far fewer *Listeria* than the controls (Table II, experiment 1). The decline in the number of intracellular bacteria observed in the stimulated MP in the period between 30 min and 4 hr suggests that the in vitro exposure to antigen has activated a bactericidal, rather than a bacteriostatic, mechanism. This finding was consistent in all

TABLE II

Microscopic Determination of Average Number of Listeria Per Macrophage and Condition of MP Monolayer at Various Times after Infection

Experiment	Cells	Antigen	Intracellular *Listeria*/MP at			Condition of monolayer at 24 hr
			10 min	30 min	4 hr	
1. Pic-HSA-immune cells vs. control	Pic-HSA-immune	Pic-HSA	4.38 ± 0.47*	7.28 ± 0.67	0.66 ± 0.18	I‡
	Pic-HSA-immune	None	4.56 ± 0.44	14.43 ± 1.29	26.0 ± 3.19	D‡
	Control	Pic-HSA	5.30 ± 0.57	20.04 ± 2.16	22.80 ± 2.70	D
	Control	None	2.92 ± 0.45	17.36 ± 1.63	27.04 ± 3.50	D
2. BGG-immune cells vs. control	BGG-immune	BGG	2.97 ± 0.24	5.22 ± 0.28	1.94 ± 0.20	I
	BGG-immune	None	4.26 ± 0.46	9.76 ± 0.39	28.62 ± 1.72	D
	Control	BGG	3.36 ± 0.39	10.26 ± 0.69	21.72 ± 2.01	D
	Control	None	3.76 ± 0.42	7.58 ± 0.77	21.90 ± 1.14	D

* Mean ± SE of 50 cells counted for each value.

† I = Intact with very few or no bacteria present; D = Destroyed.

experiments. To be emphasized is the fact that the immune cells cultured with antigen survived the infection and were culturally sterile and morphologically normal at 24 hr, while all control cell monolayers were badly damaged by 4 hr and completely destroyed in 24 hr. The photographs in Fig. 2 illustrate this difference between immune MP cultured with antigen and the control monolayers at various points in time.

Animals Immunized with BGG in CFA.—Identical results were obtained when cells from BGG-immune and control animals were cultured with and without 50 μg/ml of BGG for 24 hr before infection. The quantitative culture results shown in Table I (experiment 2) again show that the cells cultured with

FIG. 2. Morphology of MP monolayers from Pic-HSA-immune animals after *Listeria* infection. Pic-HSA was added to the monolayers in column *a* for 24 hr before infection. Monolayers in column *b* were cultured without antigen. *1* = 10 min; *2* = 30 min; *3* = 4 hr (all × 800); *4* = 24 hr (×80).

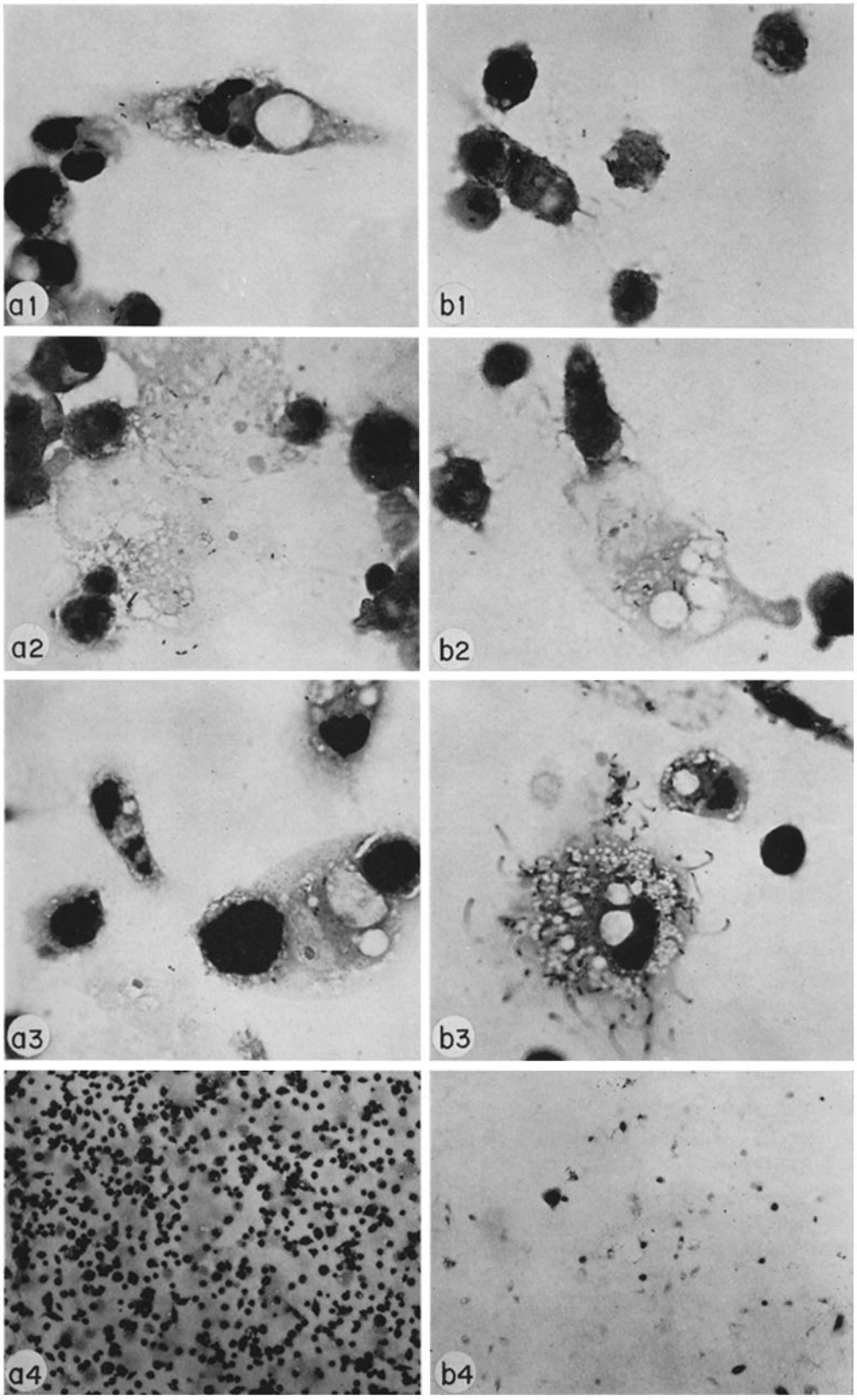

FIG. 2

antigen were significantly ($P < 0.001$) more bactericidal than the three control groups, which are statistically indistinguishable. Table II (experiment 2) illustrates the same phenomena in terms of visual counts of the number of bacteria per cell. In this experiment the immune MP cultured with BGG contained fewer bacteria per cell than the immune MP without BGG at 10 min, however that same group (immune MP with BGG) did not differ significantly from either group of control MP. At 30 min and 4 hr, the immune MP cultured with antigen contain significantly ($P < 0.01$) fewer bacteria than control cells.

Because these animals were immunized with BGG in CFA, they had positive skin tests to both BGG and PPD. An experiment was performed to see if their exudate cells could respond to either antigen with enhanced macrophage bactericidal activity. Table I (experiment 3) demonstrates that culture of the PEC

TABLE III

Effect of Presence or Absence of Penicillin on Bactericidal Assay System. Viable Intracellular Listeria Counted 4 Hr after Infection of Immune or Control MP Monolayers Cultured with or without Antigen

Experimental groups	Antigen	Penicillin	Viable intracellular *Listeria* ($\times 10^{-3}$) at 4 hr recovered from			
			Immune cells		Control cells	
			+ Antigen	− Antigen	+ Antigen	− Antigen
BGG-immune cells vs. control, with penicillin	BGG	+	140 (106–204)*	2580 (2040–3480)	3821 (3500–4660)	3551 (2540–4520)
BGG-immune cells vs. control, without penicillin	BGG	−	285 (210–352)	4520 (3980–5320)	6084 (5160–6820)	7002 (6640–7200)

* Geometric mean (range) of three cell cultures.

with either BGG or PPD did elicit this response. Although the MP cultured with BGG were found to have lower bacterial counts than those cultured with PPD, the groups were not significantly different ($P > 0.1$); however, both groups were significantly ($P < 0.05$) more bactericidal than all four controls, which are themselves statistically inseparable.

To emphasize the immunologic specificity of this system, it should be noted that while the addition of BGG greatly enhanced the bactericidal capacity of MP from BGG immune animals, it had no effect on the cells from animals immunized with Pic-HSA or with CFA alone.

Effect of Penicillin.—As noted in the Material and Methods section, 30 min after *Listeria* infection, the MP monolayers were washed and reincubated in fresh media containing 1.2 μg of penicillin/ml. The antibiotic killed all extracellular bacteria including those released from damaged infected MP, thus preventing continued phagocytosis. Although penicillin is not believed to penetrate cells (4), an experiment was performed to evaluate what effect its presence played in our assay system. Table III shows that when the BGG experi-

ment was simultaneously performed with and without penicillin, the cultures reincubated without penicillin had consistently higher bacterial counts; nevertheless within each group, the BGG-immune cells cultured with BGG were strikingly more bactericidal than controls ($P < 0.001$). These results show that the presence of penicillin does not account for the markedly enhanced MP bactericidal capacity demonstrated in these studies.

Varying Bacterial Inoculum.—The previously described experiments were all performed using a standard inoculum of 5×10^7 *Listeria* per MP culture dish. In this experiment the BGG-immune cells cultured with BGG were challenged with inocula of 50×10^7 and 5×10^7, while the control cells were infected

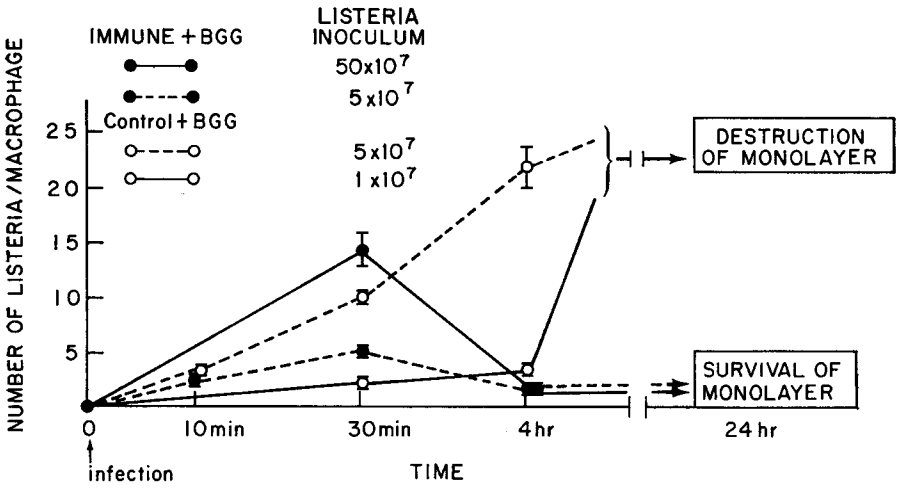

FIG. 3. Effect of varying the *Listeria* inocula on the number of bacteria phagocytized by immune and control MP and on survival of the monolayers.

with 5×10^7 and 1×10^7 *Listeria* per culture plate. The morphological observations recorded in Fig. 3 show that at 30 min the immune cells cultured with BGG which received the high inoculum contained over five times more bacteria per cell than the control cells infected with the low inoculum. Nevertheless by 4 hr the immune MP cleared most of the bacteria and actually had slightly fewer organisms than the control cells. By 24 hr the control monolayers were destroyed while the immune cells survived a 50 times larger infecting inoculum.

These studies demonstrate that differences in the numbers of bacteria initially phagocytized by the MP cannot account for the results described in the previous experiments. That is, a very small number of intracellular *Listeria*, resulting from a low inoculum, is still sufficient to kill control cells, while a much larger number of intracellular organisms, resulting from a large inoculum, is readily controlled by the immunologically stimulated MP.

Inhibition of Macrophage Migration.—Table IV shows that in three different

experiments, PEC from BGG-immune animals showed marked inhibition of MP migration when cultured with 50 μg/ml of BGG; MI ranged from 10 to 26. The RMI ranged from 12 to 31, indicating that despite some nonspecific inhibition by BGG alone in experiments 2 and 3 (Table IV), marked migration inhibition occurred in the immune system. The MIF-positive cells were from the same population as the MP exhibiting enhanced bactericidal activity, showing that the in vitro conditions resulting in enhanced bactericidal activity were also productive of MIF.

TABLE IV

Inhibition of Macrophage Migration of Immune vs. Nonimmune Guinea Pig Peritoneal Exudate Cells in Three Different Experiments

Experiment	Guinea pig	Antigen	MIF results		
			Mean area (± SE)*	MI	RMI
1	BGG-immune	BGG	115.3 ± 14.8	26	20
		None	436.8 ± 8.9		
	Control	BGG	258.0 ± 15.3	131	
		None	196.0 ± 33.0		
2	BGG-immune	BGG	13.3 ± 1.8	10	12
		None	135.0 ± 33.6		
	Control	BGG	184.8 ± 70.3	87	
		None	211.5 ± 34.8		
3	BGG-immune	BGG	46.8 ± 15.6	22	31
		None	208.5 ± 28.0		
	Control	BGG	96.8 ± 16.8	70	
		None	138.8 ± 31.2		

* Area in planimeter units; mean is of four tubes (two chambers) for each group.

DISCUSSION

In a series of elegant experiments, Mackaness and co-workers (2) have demonstrated that tuberculous (BCG) infection in the mouse produced markedly enhanced resistance to *Listeria* infection, that this phenomenon paralleled temporally the development of the delayed hypersensitivity reaction, and that the enhanced listericidal activity could be recalled rapidly by reexposure to BCG. Further, they showed that while serum could not passively transfer *Listeria* resistance to normal recipients under any conditions, immunity could be transferred by lymphoid cells from *Listeria*-immune mice (5). In addition, lymphocytes from BCG-immune mice could transfer resistance to *Listeria* only when the normal recipient also received an eliciting dose of BCG (5).

The present studies examine similar phenomena in an in vitro guinea pig system. In these experiments macrophages from guinea pigs with established

delayed hypersensitivity reactions to PPD, BGG, and Pic-HSA display markedly enhanced bactericidal capacity for an immunologically unrelated bacterium, *Listeria monocytogenes*, when the mixed population of peritoneal exudate cells have been incubated with specific antigen before infection. Like Mackaness' data, these findings suggest that there are at least two components of cellular immunity: an immunologically specific antigen recognition system and an immunologically nonspecific bactericidal or effector mechanism.

The peritoneal exudates used in these studies are composed of macrophages, lymphocytes, and granulocytes. The granulocytes are not viable in this culture system and are not presumed to play a role. The monolayers were washed after overnight incubation so that at the time of *Listeria* infection they were composed of only glass adherent, phagocytic, mononuclear cells. Thus, the macrophage alone is sufficient to account for the immunologically unselective enhanced bacterial killing observed. In terms of their bactericidal capacity, these cells would appear functionally similar to the activated macrophages described by Cohn (6), Dannenberg (7), and others; morphological and metabolic studies to explore this possibility are now in progress.

It is assumed that antibacterial antibody and complement played no role in our experiments. First, the only serum used in the culture media was from Hartley strain guinea pigs which had not been exposed either to *Listeria* or to the other antigens used in these studies. This pooled serum was heat-inactivated, thus eliminating a possible contribution of exogenous complement. Opsonizing activity was present, but the serum did not agglutinate or kill *Listeria*. Finally, serum from the same pool was used in the experimental and control groups in each study.

The PEC were washed before being put in culture; hence preformed circulating antibody was not added to the cultures. We cannot eliminate the possibilities that cytophilic antibody was bound to the PEC or that the cells from immune animals synthesized antibody while in culture. In any case, there is no known antigenic cross-reactivity between PPD, BGG, Pic-HSA, and *Listeria*, making it most unlikely that cytophilic or newly synthesized antibody would have bactericidal activity. If antibody were present, immune complexes might have been formed, and such complexes could conceivably influence macrophage function. This possibility seems remote but has not been ruled out.

The recognition system responsible for the production of enhanced MP bactericidal capacity cannot be further dissected from the present studies alone. However, other systems make it tempting to speculate that specific antigen interacts first with the sensitized lymphocyte which then influences the macrophage to become a more bactericidal cell. If this is the case, sensitized lymphocytes should, in the presence of antigen, enhance the bactericidal capacity of normal macrophages. Preliminary studies in our laboratory suggest that this does occur. Antigen-stimulated lymphocytes could affect macro-

phages through production of a lymphokine like MIF, by means of cytophilic antibody, through direct cell-to-cell interaction, or through other presently unknown mechanisms. In our system, the conditions which resulted in enhanced bactericidal activity also led to MIF production. The coincidence of these phenomena is suggestive but by no means establishes their identity.

Supporting the concept of lymphocyte–macrophage interaction are the previously cited findings of Mackaness that cellular immunity could be established in normal animals through passive transfer of sensitized lymphocytes (5). In addition, Patterson and Youmans (8) recently reported that normal mouse peritoneal macrophages suppress the multiplication of tubercle bacilli in vitro when cultured with splenic lymphocytes from tuberculin-immune mice or with ribonucleic acid (RNA) extracts from these cells. Moreover, they found that cell-free supernatant fluids from tuberculin-sensitive lymphocytes cultured with tubercle bacilli resulted in a somewhat greater suppressive effect when added to normal macrophages than did supernates from immune lymphocytes cultured without antigen or from normal lymphocytes with or without antigen.

Another study by Goihman-Yahr et al. (9) which utilized in vitro *Listeria* infection of guinea pig macrophages found enhanced bactericidal activity related to tuberculin hypersensitivity but not to graft rejection or allergic contact dermatitis. The methodological approach employed in this study differs greatly from ours, and our data do not support their conclusion that bacterial allergy is a unique stimulus to macrophage activation. Finally, Dodd (10) showed in vivo that guinea pigs immunized with BGG in CFA and given BGG before *Listeria* infection clear *Listeria* from the spleen more effectively than control animals. These results correlate well with our own findings in vitro that a simple protein antigen and a haptenated protein are as effective as bacterial antigens in the production of cellular immunity.

SUMMARY

An in vitro model of cellular immunity in the guinea pig was established. Animals were immunized with tubercle bacilli, bovine gamma globulin, or picrylated human serum albumin in complete Freund's adjuvant. Oil-induced peritoneal exudates from immune and control animals were cultured overnight with and without specific antigen. The cultures were washed and the macrophage monolayers were infected with *Listeria monocytogenes*. At intervals the monolayers were lysed and the numbers of viable intracellular bacteria were quantitated by pour plate cultures. Random monolayers were also evaluated in sequence by visually counting the intracellular bacteria on Gram-stained plates. Both methods demonstrated that the macrophages from immune animals had markedly enhanced listericidal activity when the peritoneal exudates were cultured with antigen before infection. Macrophage migration inhibition was also demonstrated under these conditions.

The experiments reported here describe an in vitro model of cellular immunity which will allow separation and recombination of cell types and direct assay of cell products in efforts to elucidate further the mechanisms of the immunologically mediated enhancement of macrophage bactericidal capacity.

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