THE INTERACTION IN VITRO BETWEEN POLYMORPHONUCLEAR LEUKOCYTES AND MYCOPLASMA*

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Mycoplasmas are the smallest known free-living microorganisms. Members of this genus cause disease in plants, rodents, fowl, and domestic animals (1–4). These organisms are also part of the normal flora of man, and at least one species, *Mycoplasma pneumoniae*, is a proven pathogen for humans. The role of other species of mycoplasma in diseases of man has yet to be established. A voluminous literature has appeared pertaining to both humoral and cellular aspects of host defense with regard to other microbes. Thus, numerous in vitro studies have been carried out of interactions between bacteria and various types of phagocytic cells, but few such investigations have dealt with mycoplasma. Among the latter are morphological observations on the interaction in vitro between various types of human cells with *M. gallisepticum* and *M. neurolyticum* (5, 6), and a very recent morphological study of ingestion of *M. pulmonis* by cultured mouse macrophages (7).

This report deals with the interaction between human or rabbit polymorphonuclear leukocytes and *M. hominis* or *arthritidis*. We have examined the ability of the leukocytes to sequester and kill mycoplasma, to mount a number of metabolic responses known to occur during phagocytosis, and the effect of mycoplasma on the leukocytes' capability to kill *Escherichia coli*.

Materials and Methods

Granulocytes.—Rabbit granulocytes were collected from sterile peritoneal exudates produced by intraperitoneal injection of 0.1% glycogen in normal saline. Differential counts showed that the cell suspensions contained 95% or more polymorphonuclear leukocytes. Human peripheral blood leukocytes were prepared from heparinized blood obtained by venipuncture. After mixing the blood with one-fifth vol 6% dextran 75 (Travenol Lab. Inc., Morton Grove, Ill.), the erythrocytes were allowed to sediment by stationary incubation at 37°C and the leukocyte-rich plasma was then harvested. Differential counts of these cells showed that 60–70% were polymorphonuclear leukocytes. The rabbit or human leukocytes were sedimented by centrifugation at 50 *g* for 10 min, washed, and resuspended in Eagle's

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minimal essential medium (MEM)[1] or medium 199 (Grand Island Biological Co., Grand Island, N.Y.) at the desired concentration. Concentrated homogenates of granulocytes were prepared in distilled water using a motor-driven teflon pestle. The homogenates were then diluted to the desired concentration in MEM or medium 199.

Culture and Incubation Procedures.—

Mycoplasmas (pleuropneumonia-like organisms [PPLO]): Mycoplasmas were grown and maintained in broth or agar media. The broth contained 70% PPLO broth (Difco Laboratories, Inc., Detroit, Mich.), 20% unheated horse serum, 10% yeast extract, 0.5% arginine hydrochloride, and 1000 units of penicillin/ml. Agar plates contained the above plus 1% Bacto-agar (Difco Lab.). *M. hominis* and *M. arthritidis* strains grown overnight in broth medium were sedimented by centrifugation at 10,000 *g* for 15 min, washed, and resuspended in broth medium or MEM. Species-specific antisera directed at the mycoplasma used in these experiments were prepared by serial inoculation of rabbits with whole, heat-killed organisms emulsified in Freund's complete adjuvant. Specificity of the sera was determined by growth-inhibition tests (8) against homologous and heterologous mycoplasma species. Incubation mixtures consisted of mycoplasma at various concentrations of viable organisms, 4×10^7 granulocytes/ml or comparable concentrations of granulocyte homogenate, type-specific antiserum, or normal rabbit serum in a final concentration of 1/200, all in a total volume of 5 ml of MEM or medium 199. Incubation was carried out in a rotary shaker at 37°C. Viability of the mycoplasmas was determined in duplicate aliquots of incubation mixtures taken at various time intervals, serially diluted in PPLO broth, and plated on agar. Colony-forming units were enumerated after incubation of these plates at 37°C for at least 72 hr when the colonies could be seen without magnification. Aliquots of some mycoplasma suspensions were also counted directly in a hemacytometer using a phase-contrast microscope.

Labeling of PPLO Lipids with Palmitate-1-^{14}C.—

M. hominis: Lipids of *M. hominis* were labeled during growth overnight with palmitate-1-^{14}C (2.5 μCi; specific activity 55 mCi/mmole, Amersham-Searle Corp., Arlington Heights, Ill.) complexed to bovine serum albumin (9). Lipids of suspensions consisting of PPLO alone or with leukocytes were extracted by the method of Bligh and Dyer (10). Total radioactivity of the lipid extracts and the distribution of the radioactivity among major lipid fractions, separated by thin-layer chromatography, were determined as previously described (11).

E. coli: The K-12 strain of *E. coli* was grown and maintained in heart infusion broth or on agar slants. Phagocytosis experiments were performed with 4-hr cultures of *E. coli* sedimented by centrifugation at 3000 *g*, washed, and resuspended in MEM at the desired concentration before addition to incubation mixtures with granulocytes and mycoplasmas as indicated. At intervals, duplicate aliquots of the bacterial suspensions were taken, diluted in heart infusion broth, and 0.1 ml of each dilution was incorporated into pour plates. Colonies were enumerated after incubation at 37°C overnight.

Glucose-1-^{14}C metabolism: Hexose monophosphate shunt activity of the leukocytes was measured by the conversion of glucose-1-^{14}C to $^{14}CO_2$. Suspensions of leukocytes (2×10^7 cells) in MEM were placed in 10 ml Erlenmeyer flasks that contained 0.1 ml of fresh frozen rabbit serum, 0.075 μCi of glucose-1-^{14}C (260 μCi/mg), PPLO in the concentrations indicated, and/or 0.1 ml of polystyrene particles (1.1 μ diameter, 3.6×10^9 particles, Dow Chemical Company, Midland, Mich.) in physiological saline. The total volume of 0.5 ml contained glucose in a final concentration of 80 mg per cent. Evolved $^{14}CO_2$ was collected in polyethylene cups suspended from rubber stoppers (Kontes Glass Co., Vineland, N.J.). At the indicated

[1] *Abbreviations used in paper:* MEM, Eagle's minimal essential medium; PPLO, pleuropneumonia-like organisms.

time intervals the reaction was stopped by injecting 0.2 ml of 10 N H_2SO_4 through the rubber stopper into the main chamber. Hyamine (0.4 ml) was added to the collection cups and incubation at 37°C was continued for 1 hr. At this time the cups were removed, placed in counting vials, and shaken well with 12 ml of toluene-BBOT (2,5-bis-2-(5-*tert*-butylbenzoxazolyl)-thiophene, Packard Instrument Co., Downers Grove, Ill.) scintillation mixture. Counting in a Packard liquid scintillation spectrometer was carried out immediately after addition of scintillation mixture and cooling of the vials. Under these circumstances quenching was minimal. Radioactivity of portions of glucose-1-^{14}C was determined also in the presence of hyamine and thus provided an internal standard. In some experiments, as indicated in the text, 0.075 μCi of glucose-6-^{14}C was used in place of glucose-1-^{14}C.

Determination of the Conversion of Lysolecithin-^{32}P to Lecithin by Granulocytes.—This procedure has been described in detail elsewhere (12–15). In brief: Biosynthetically prepared lecithin-^{32}P was degraded to lysolecithin-^{32}P by treatment with snake venom phospholipase

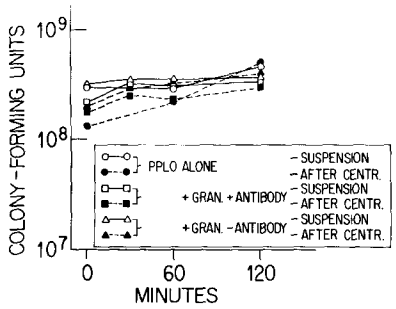

FIG. 1. Viability of *M. hominis* (PPLO) incubated with or without rabbit granulocytes and specific antiserum. Individual points represent the mean of duplicate viable counts determined on aliquots taken at the indicated times of incubation. The open symbols represent aliquots of the whole suspension and the closed symbols aliquots of supernatant fluids prepared by centrifugation of the suspension at 50 *g* for 10 min. Incubation mixtures were prepared as described in Materials and Methods.

A2. The labeled lysolecithin was complexed to bovine serum albumin (4 g/100 ml) in Hanks' solution (14). Incubation mixtures contained 5×10^7 rabbit granulocytes in Hanks' solution, 0.1 ml of lysolecithin-^{32}P–albumin solution, approximately 5×10^9 PPLO (as determined by viable count) in 0.2 ml of growth medium or medium 199, and 0.1 ml of polystyrene particles suspended in physiological saline, all in a total volume of 0.5 ml. Mixtures devoid of PPLO or polystyrene particles were supplemented with the appropriate medium alone. The concentration of lysolecithin in the final volume of 0.5 ml of each incubation mixture in different experiments ranged from 20 to 50 nmoles. Conversion of lysolecithin to lecithin was determined by radiochemical assay, separating the labeled compounds in lipid extracts of the incubation mixtures by thin-layer chromatography as previously described (12–15).

RESULTS

Effects of Human or Rabbit Granulocytes Incubated with PPLO on Viability of the Microorganisms.—In marked contrast to rapid killing of *E. coli* and other bacteria by rabbit granulocytes in vitro, the viability of *M. hominis* incubated under similar conditions was unaffected by the presence of granulocytes (Fig. 1).

Colony counts of aliquots taken over a 2-hr period were virtually the same in suspensions of PPLO alone, in suspensions of PPLO and granulocytes, and in the supernatant fluids obtained after the leukocytes had been sedimented by centrifugation. Addition of antiserum directed at mycoplasma also had no effect on viability.

When the ratio of PPLO to leukocytes was varied from 1 to 100, no decreased viability was observed at any concentration. Fig. 2 shows such an experiment

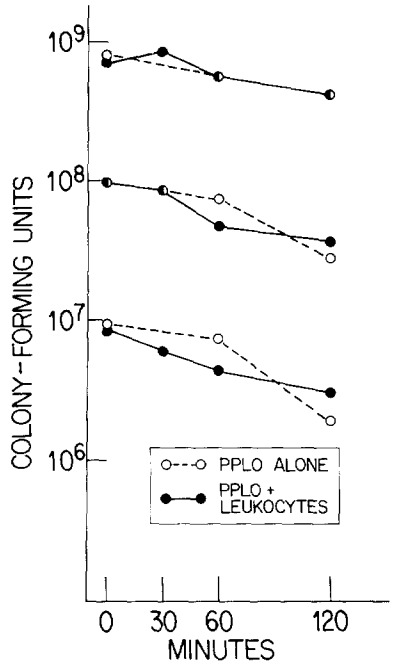

FIG. 2. Viability of *M. hominis* (PPLO) incubated with human peripheral blood leukocytes in the presence of antiserum at varying ratios of organisms to cells. The experiment was carried out as described in legend of Fig. 1.

carried out with human leukocytes. Similar results were obtained in experiments with rabbit granulocytes. Moreover, at none of the three concentrations examined was there evidence of adherence of the mycoplasma to the phagocytes, since medium after sedimentation of the granulocytes and whole suspensions contained comparable numbers of viable organisms (Fig. 3). Thus, failure to detect appreciable killing of PPLO appears not because of an excess of the organisms in the suspension. Modification of the incubation mixture by substituting medium 199 for MEM failed to uncover mycoplasmacidal activity.

Jones and Hirsch, in their studies of phagocytosis by mouse peritoneal macrophages of *M. pulmonis*, demonstrated ingestion and digestion using 20% heat-

inactivated fetal calf serum (7). As shown in Fig. 4, viable counts of *M. hominis* incubated with human leukocytes were the same at three different human serum concentrations. Substitution of heat-inactivated fetal calf serum for the human serum in the incubation mixture also did not alter the results.

Extracts of leukocytes have been shown to possess potent mycoplasmacidal activity at low pH towards *M. pneumoniae*, *M. salivarium* and *M. fermentans* (16), and *M. hominis* (17). Fig. 5 shows one of four virtually identical experi-

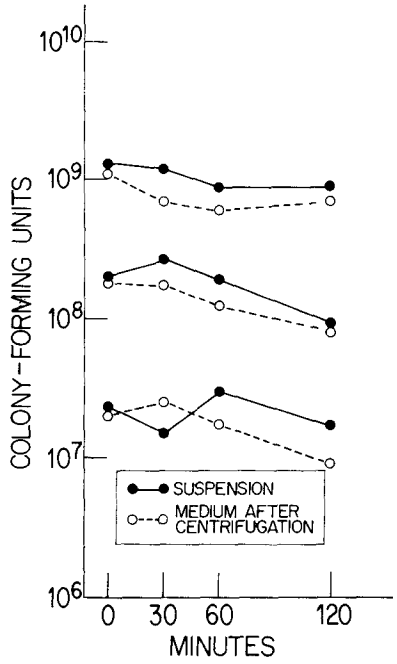

FIG. 3. Viability of *M. hominis* (PPLO) incubated with rabbit granulocytes and antiserum at varying ratios of organisms to cells (see legend to Fig. 1). Open symbols represent aliquots of the whole suspension and the closed symbols aliquots of supernatant fluids prepared by centrifugation of the suspension at 50 *g* for 10 min.

ments in which the effect of incubation with intact or homogenized leukocytes on viability of *M. hominis* was compared. It is evident that whole leukocyte homogenates also contain mycoplasmacidal activity towards *M. hominis*. The lag of 2 hr before killing of mycoplasma became manifest was also observed by Dajani and Ayoub (16), when the leukocyte extract was diluted.

Differences in apparent potency between the extracts prepared by Dajani and Ayoub and the homogenates used in our experiments probably reflect the fact that we tested mycoplasmacidal activity at pH 7.4 (rather than at its optimal pH of 4.7 [16]), so that the effect of homogenized cells could be compared with that of intact cells.

Since estimates of the total number of PPLO in the suspensions were based on the capacity of the organisms to form colonies, it is conceivable that the actual number of mycoplasma particles in a suspension greatly exceeded the number of colony-forming units. This did not seem likely since counting of aliquots of mycoplasma suspensions under the phase-contrast microscope gave values that corresponded to the number of colony-forming units. However, an additional measure of uptake of PPLO was deemed necessary. Radioisotopically-labeled PPLO were prepared by incubating *M. hominis* in rapid growth phase with palmitate-1-^{14}C. This results in extensive labeling of the organisms' phospholipids. After washing of the labeled PPLO, more than 95% of the radioactivity was in phospholipids and less than 3% remained as free fatty acid. Incubation

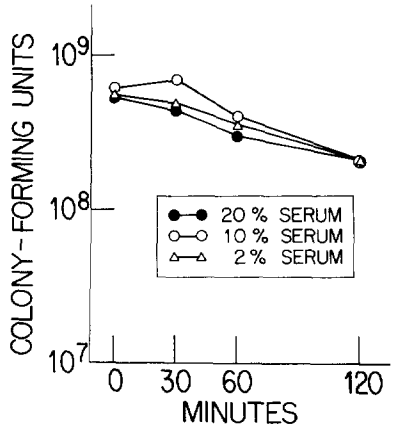

FIG. 4. Viability of *M. hominis* (PPLO) incubated with human peripheral blood leukocytes, antiserum, and normal human serum at the indicated concentrations.

of the labeled PPLO at two concentrations with rabbit granulocytes revealed that practically the same amount of radioactivity remained in the supernatant fraction of suspensions of PPLO alone and of PPLO plus granulocytes (Fig. 6), confirming the evidence obtained in the preceding experiments on viability.

At the lower concentration of labeled PPLO it appears that somewhat more radioactivity sedimented with the leukocytes after incubation for 2 hr. In four additional experiments, carried out for 3 hr, no evidence was obtained that the granulocytes sequestered more PPLO at longer time intervals, either at high or at low PPLO to leukocyte ratios. Fractionation of the lipids extracted from suspensions of PPLO and of PPLO incubated with granulocytes for periods of up to 3 hr, showed no release of labeled free fatty acid or other breakdown products of the PPLO phospholipids. Thus, we obtained no evidence of degradation of PPLO lipids. These findings contrast with those obtained in similar experiments with another microorganism (partially) devoid of the cell wall,

namely spheroplasts prepared from palmitate-1-^{14}C-labeled *E. coli*, which undergoes rapid and extensive degradation when exposed to granulocytes (unpublished observation). These experiments indicated not only that PPLO is not killed to any extent during incubation with leukocytes for 2 hr, but also that

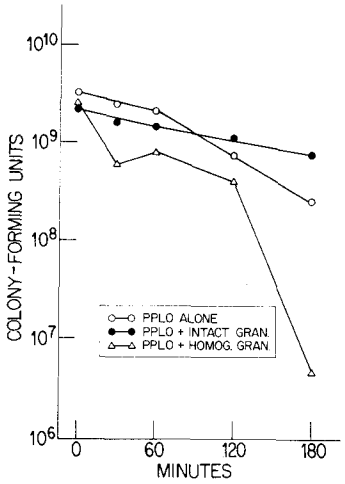

FIG. 5. Viability of *M. hominis* (PPLO) incubated with intact or homogenized rabbit granulocytes and specific antiserum.

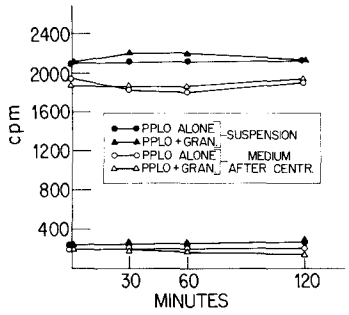

FIG. 6. Lack of removal of palmitate-1-^{14}C-labeled *M. hominis* by rabbit granulocytes. The experiment was carried out as described in the legend of Fig. 1, except that aliquots of the whole suspension or of the supernatant fluid obtained after centrifugation at 50 *g* for 10 min were extracted for lipids. Labeled PPLO were added in ratios of 100 or 10 organisms to one granulocyte.

no appreciable tight adsorption onto the phagocytic cells could have taken place.

Effect of PPLO on Several Metabolic Functions of Rabbit and Human Leukocytes.—Phagocytosis of various types of particles and microorganisms is accompanied by marked stimulation of O_2 uptake and formation of $^{14}CO_2$ from glucose-1-^{14}C (in this cell type a reasonably accurate index of hexose mono-

phosphate shunt [HMS] activity). Despite the apparent inability of the leukocytes to remove PPLO from the suspension, $^{14}CO_2$ production from glucose-1-^{14}C added to the medium is markedly enhanced (Fig. 7). Production of $^{14}CO_2$ by granulocytes incubated with PPLO or with polystyrene particles was compared. Polystyrene particles elicited the familiar almost 10-fold stimulation of $^{14}CO_2$ production from glucose-1-^{14}C. In 10 experiments conversion of glucose-1-^{14}C to $^{14}CO_2$ by 2×10^7 leukocytes incubated with polystyrene amounted to 100 ± 16 nmoles (mean $\pm$ SEM) in 30 min. Comparable stimulation was seen when PPLO in the presence of antiserum in a ratio of 10 organisms:1 granulocyte were added to the suspension. An even greater stimulation occurred when the ratio was 100:1. In the absence of antiserum the stimulation was approximately half of that observed in its presence. Appropriate controls showed no $^{14}CO_2$ production from glucose-1-^{14}C by *M. hominis* or *M. arthritidis* incubated

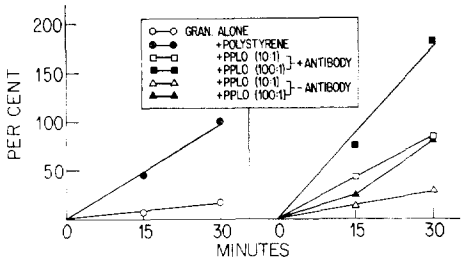

FIG. 7. Production of $^{14}CO_2$ from glucose-1-^{14}C by rabbit granulocytes. The results are expressed as per cent of the $^{14}CO_2$ produced by rabbit granulocytes incubated with polystyrene beads for 30 min. *M. arthritidis* (PPLO) and specific antiserum were added as indicated.

in the absence of polymorphonuclear leukocytes. Antiserum alone had no effect on $^{14}CO_2$ production by leukocytes. Aerobic glycolysis, as measured by the conversion of glucose-6-^{14}C to $^{14}CO_2$, was modestly stimulated by polystyrene particles, but did not increase when mycoplasma was added (not shown), excluding the possibility of a nonspecific stimulation of glucose metabolism through increased entry of glucose because of a change in permeability.

The effect of various antisera on $^{14}CO_2$ production from glucose-1-^{14}C by granulocytes incubated with either *M. hominis* or *M. arthritidis* is shown in Table I. The antisera were species specific in their capacity to inhibit growth of mycoplasma. While heterologous antisera enhanced $^{14}CO_2$ production by granulocytes in the presence of PPLO, type-specific antisera, even when diluted, were consistently more stimulatory. Heat-inactivated PPLO evoked the same antiserum-dependent metabolic response as live organisms (Table II).

Previous studies in our laboratory have indicated that phagocytosis of polystyrene particles by granulocytes is associated with a marked stimulation of the

conversion of medium lysolecithin to cellular lecithin (15, 18). This stimulation of acylation of the lysocompound is not observed in presence of PPLO (Table III). Further, acylation is less when both polystyrene particles and PPLO are present than with polystyrene particles alone.

TABLE I

Effect of Specific and Nonspecific Antiserum on Stimulation of $^{14}CO_2$ from Glucose-1-^{14}C by Rabbit Granulocytes Incubated with PPLO

Incubation (min)	*M. arthritidis*		*M. hominis*	
	15	30	15	30
		nmoles		
Normal rabbit serum	8	15	20	49
Anti-*M. arthritidis*	47	110	48	85
Anti-*M. hominis* undiluted	—	—	68	105
Anti-*M. hominis* 1:10	—	—	69	122
Anti-*M. hominis* 1:100	—	—	34	81
Anti-*M. salivarium*	18	39	40	90
Anti-*M. neurolyticum*	18	33	36	78

The experiment was carried out as described in Materials and Methods. Mycoplasma was added in a ratio of 100 organisms to 1 granulocyte. All sera, undiluted or diluted as indicated were added in an amount of 10 μl/reaction mixture of 0.5 ml. Results are given as nmoles of glucose oxidized.

TABLE II

Comparison of Effect of Live and Heat-Killed PPLO on $^{14}CO_2$ Production from Glucose-1-^{14}C by Granulocytes

Incubation (min)	15	30
	nmoles	
Live PPLO + NRS*	8	16
Live PPLO + antiserum	41	81
Dead PPLO + NRS*	9	18
Dead PPLO + antiserum	43	87

Comparison of the effect of live and heat-killed *M. hominis* on $^{14}CO_2$ production from glucose-1-^{14}C by rabbit granulocytes. The experiment was carried out as described in Materials and Methods and in the legend of Table I. *M. hominis* or heat-killed *M. hominis* were added to the granulocytes in a ratio of 100 organisms to 1 granulocyte. Normal rabbit serum or specific antiserum were added in an amount of 10 μl/incubation flask.

* NRS, normal rabbit serum.

Effect of PPLO on the Killing of E. coli by Granulocytes.—The findings described so far suggested that PPLO induces alterations in leukocyte function without being ingested to any great extent. In the following experiments we examined the possibility that PPLO affected the granulocyte's ability to carry out phagocytosis of other microorganisms. The effect of PPLO added at various

TABLE III

Lecithin Synthesis from Lysolecithin-^{32}P by Granulocytes Incubated with or Without PPLO in the Presence or Absence of Polystyrene Particles

Incubation time	Granulocytes − PPLO		Granulocytes + PPLO	
	− Polyst.	+ Polyst.	− Polyst.	+ Polyst.
min				
15	33.5 ± 3.7	61.8 ± 2.0	35.3 ± 2.9	45.9 ± 3.9
30	58.3 ± 3.8	100	53.5 ± 2.5	73.6 ± 5.9

Incorporation of lysolecithin-^{32}P complexed to albumin into granulocyte lecithin was determined as described in Materials and Methods and elsewhere (18).

Results of five experiments, carried out in duplicate, was given as per cent (mean ± standard error of the mean) of the value for lecithin synthesis at 30 min. The differences between lecithin synthesis by granulocytes incubated with polystyrene beads in the absence and presence of PPLO were statistically significant. (At 15 min $P < 0.005$ and at 30 min $P < 0.02$). Suspensions of PPLO did not convert lysolecithin to lecithin.

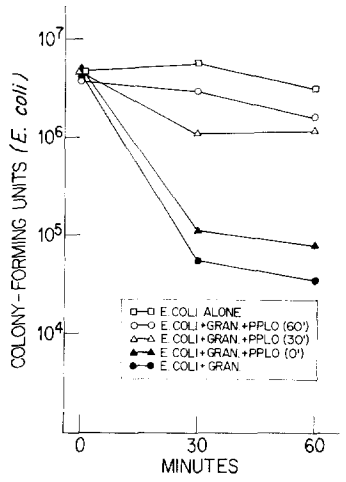

FIG. 8. Effect of preincubation of human leukocytes with *M. hominis* on bactericidal activity against *E. coli*. Leukocytes, *M. hominis* (100 organisms/leukocyte), and specific antiserum were incubated for the indicated periods of time before *E. coli* were added to the mixtures. Individual points represent viable counts of *E. coli* derived from aliquots of the suspensions taken at the indicated times of incubation.

times before addition of *E. coli* on bacterial killing by granulocytes is shown in Fig. 8. Preincubation of the granulocytes with PPLO for 30 or 60 min results in marked impairment of bacterial killing. When PPLO and *E. coli*, both in a ratio of 100 microorganisms:1 granulocyte, are added simultaneously, killing of *E. coli* was usually only little inhibited, suggesting that any effect of PPLO on

the leukocyte's bactericidal activity was not simply because of dilution of *E. coli* as ingestible material.

Fig. 9 depicts the protective effect of PPLO against killing of *E. coli* by granulocytes, as a function of the number of *E. coli* in the suspension. The fewer the *E. coli* the more dramatic the inhibition of killing.

DISCUSSION

The findings we have presented here indicate that two species of mycoplasma, *M. hominis* and *M. arthritidis*, are not effectively ingested and killed by human and rabbit leukocytes under conditions that permit the rapid phagocytosis of *E. coli*. The main evidence for this conclusion rests on studies of viability of

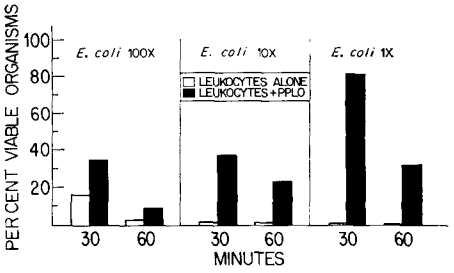

FIG. 9. Effect of preincubation of human leukocytes with *M. hominis* (100 organisms/leukocyte) for 30 min on subsequent killing of *E. coli* added at various ratios of bacteria to leukocytes. Incubation was carried out as described in the legend of Fig. 8. The bars represent the per cent of the viable *E. coli* count at time 0 (mean of duplicate determinations).

PPLO incubated with granulocytes, as well as on experiments with labeled PPLO. The latter showed in addition that PPLO apparently do not tightly adhere to the leukocytes since little or no radioactive material sedimented with the phagocytes. This finding is surprising because it contrasts with morphological observations by others that indicate a striking tendency on the part of PPLO to adhere to surfaces of mammalian cells (5–7) including polymorphonuclear leukocytes (5). However, neither the tightness of binding, nor the ability of the species of mycoplasma used in our studies to adhere to cells other than leukocytes, has been examined to our knowledge.

Further evidence that mycoplasma is not readily engulfed by granulocytes, even in the presence of species-specific antiserum, is provided by electron-microscopic observations[2] (not shown), that revealed only rare intracellular PPLO at a ratio of 10:1, and few intracellular organisms at a ratio of 100 PPLO: 1 granulocyte.

[2] We are indebted to Dr. D. Zucker-Franklin who performed the electron microscopy.

Even though few mycoplasmas could be demonstrated intracellularly in these experiments, stimulation of $^{14}CO_2$ production from glucose-1-^{14}C was at least as great after addition of PPLO as in the presence of polystyrene particles (thin sections of such granulocytes contained from 15 to 25 latex particles). It appears less likely that this metabolic response is because of occasional ingested mycoplasmas, since up to 10-fold stimulation of $^{14}CO_2$ production was seen under circumstances where virtually no intracellular PPLO were seen. However, stimulation of $^{14}CO_2$ production by granulocytes in the absence of phagocytosis can be demonstrated in response to numerous chemical and biological agents (19–22). Most of these have detergent properties and affect the cell surface. We propose therefore that the effect of PPLO on granulocyte metabolism reflects a perturbation of the cell surface and quite possibly damage to the cells. Support for this hypothesis may be found in the inhibitory effects of PPLO on killing of *E. coli* and on the stimulation of acylation of lysolecithin that normally accompanies phagocytosis of polystyrene and other particles (15, 18). The concept that mycoplasma may cause cell damage, especially in conjunction with antibody and complement, has been put forth previously (23).

The strikingly different results of our studies on granulocytes and of those of Jones and Hirsch (7) on peritoneal macrophages may perhaps be explained in this context. Jones and Hirsch found specific antiserum-dependent ingestion and digestion of *M. pulmonis* by mouse peritoneal macrophages in culture. The ability of the macrophages but not of cultured fibroblasts to engulf adherent *M. pulmonis* was triggered within minutes after addition of anti-mycoplasma antibody and exhibited all the morphological characteristics of effective phagocytosis. It is possible that differences in the origins of the cells and in incubation procedure account for the different behavior of the rabbit or human granulocytes and the mouse macrophages. On the other hand, our experiments were carried out under conditions that permit rapid phagocytosis and killing of a wide variety of microorganisms. It seems more likely therefore that the apparent inability of the granulocyte to ingest *M. hominis* or *M. arthritidis* in vitro represents a specific feature of this type of phagocyte. For example, it is conceivable that the granulocyte possesses a sensitivity to mycoplasma or its product(s) that distinguishes this phagocyte from others.

While our data seem consistent with a damaging effect of mycoplasma on the leukocytes, the manner in which this action is exerted remains to be determined. In preliminary experiments we have noted that medium (MEM) in which PPLO had been incubated for varying periods of time before removal of the organism by sedimentation at 20,000 *g* for 20 min affects $^{14}CO_2$ production by granulocytes. This finding suggests release of substance(s) that modify the granulocyte's membranes and/or metabolism. However, thus far effects of medium prepared in this manner have been variable and at no time as marked as those of whole PPLO suspensions. Another aspect that requires further

study is the effect of PPLO on phagocytosis and killing of microorganisms other than *E. coli*. Although viability of *E. coli* alone is not altered by PPLO under the conditions of these experiments, diminished bactericidal activity of the leukocytes might be because of an effect on the organism rather than on the phagocyte. Such effects might differ with different microbial species.

SUMMARY

The interaction between mycoplasma (PPLO) and human or rabbit leukocytes was examined in vitro.

Upon incubation of *M. hominis* or *M. arthritidis* for 2 hr with rabbit peritoneal exudate granulocytes or leukocytes from human peripheral blood, no killing of mycoplasma was observed either in the presence or absence of type-specific antiserum. However, $^{14}CO_2$ production from glucose-1-^{14}C was stimulated up to 10-fold in the presence of live or heat-killed PPLO. The extent of stimulation depended upon the number of organisms and the presence of type-specific antiserum. The stimulation of $^{14}CO_2$ production seems not because of tight adherence of PPLO to the leukocytes, since PPLO were quantitatively recovered in the medium after sedimenting the granulocytes.

The enhanced conversion of medium lysolecithin to cellular lecithin that accompanies phagocytosis of polystyrene particles was significantly reduced when PPLO were also present. Mycoplasma alone elicited no stimulation of lecithin formation.

Killing of *E. coli*, a microorganism readily engulfed and killed by leukocytes in vitro, was diminished when the leukocytes were preincubated with mycoplasma.

These findings indicate that *M. hominis* and *M. arthritidis* are not ingested by granulocytes to any detectable extent, but that these organisms affect the leukocytes' metabolism and also impair phagocytosis of *E. coli*.

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