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Cultures of *Chlamydia trachomatis* in Mouse Peritoneal Macrophages: Factors Affecting Organism Growth

CHO-CHOU KUO

Department of Pathobiology, School of Public Health and Community Medicine, University of Washington, Seattle, Washington 98195

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Growth of *Chlamydia trachomatis* B/TW-5/OT and L_2/434/Bu strains in cultures of thioglycolate-activated mouse peritoneal macrophages was studied. Both strains grew to a limited extent in the macrophages, but lymphogranuloma venereum (LGV) grew better than trachoma. Growth was enhanced by centrifugation of the inoculum onto the macrophage cell layer and inhibited by pretreatment of macrophages for 2 h with 100 μg of concanavalin A per ml. No significant effect was observed by pretreatment of macrophages with diethylaminoethyl-dextran (30 μg/ml, 30 min), cytochalasin B (20 μg/ml, 1 h), and cyclophosphamide (200 μg/ml, overnight) or by treatment with hydrocortisone (1 and 10 μg/ml, overnight before inoculation and during a 2-day incubation after inoculation). Resistance to intracellular growth of the two organisms was not increased in macrophages obtained from mice immunized with the organisms compared with macrophages from nonimmunized mice unless they were pretreated in vitro with penicillin (100 U/ml, overnight). The yields of LGV organisms from the penicillin-pretreated macrophages of LGV-immunized mice were 100-fold less than from the pretreated macrophages of nonimmunized control mice. At the same time, the yields of LGV organisms from penicillin-pretreated macrophages of mice immunized with trachoma, gonococcus, and HeLa cells were not different from those obtained in pretreated macrophages of nonimmunized control mice.

Chlamydiae are obligate intracellular parasites. Whereas trachoma organisms infect mainly the epithelial cells of ocular and genital mucous membranes, lymphogranuloma venereum (LGV) and psittacosis organisms have tropism for mononuclear phagocytic cells. Chlamydiae can now be readily cultivated in chicken embryo and mammalian cell cultures. This ease of culture has contributed greatly to our present understanding of the diseases they cause (8). In vitro growth of chlamydiae in mononuclear phagocytes (macrophages) has been reported (2, 3, 17). Since macrophages have supported only limited growth of chlamydiae, few sustained efforts have been made to explore the use of this system for studies of cellular immunity, intracellular surviving mechanisms of the organisms, and pathogenesis.

In this report we describe: a method of mouse peritoneal macrophage culture for growing *Chlamydia trachomatis*; studies on factors affecting growth of the organisms in macrophages; and some applications of this system for the study of cellular immunity.

MATERIALS AND METHODS

***C. trachomatis* strains.** The strains used were trachoma B/TW-5/OT and LGV L_2/434/Bu (13). Chlamydiae were propagated in HeLa 229 cells (13). Infected cells were harvested with glass beads, sonically treated, and then submitted to one cycle of differential centrifugation. The organisms were resuspended in 2.5 ml of sucrose-phosphate-glutamate medium (13) per 32-oz. (about 960-ml) culture bottle and frozen at −65°C. The infectivity titers were usually 10^8 to 10^9 inclusion-forming units (IFU)/ml for trachoma and 10^9 to 10^{10} IFU/ml for LGV.

Culture of mouse peritoneal macrophages. Male Swiss Webster mice, 6 to 8 weeks old, were used for peritoneal macrophages. The methods of obtaining and culturing the macrophages were according to Lin and Steward (14). Briefly, 1.5 ml of thioglycolate was injected intraperitoneally (i.p.). On day 3, mice were sacrificed by neck dislocation. Peritoneal exudate cells were obtained by injecting i.p. 5 ml of Hanks balanced salt solution (HBSS) containing 10 U of heparin per ml. The abdomen was massaged, and the fluid was withdrawn with a needle and syringe. Peritoneal exudate cells were pelleted and washed once with HBSS. The final cell pellet was resuspended in alpha-medium (Flow Laboratories, Rockville, Md.) containing 10% fetal calf serum, 5% horse serum, 10% L-cell conditioned medium, and 100 μg each of streptomycin and vancomycin per ml. Cell concentration was adjusted to 10^6/ml and plated 1 ml/vial in a 1-dram (4-ml) vial shell containing a 12-mm circular cover slip. The culture vials were loosely capped and incubated in a CO_2 incubator at 37°C in an atmosphere of 5% CO_2 in air saturated with water vapor. The cell suspension was

kept in the cold after removal from the peritoneal cavity until incubation to minimize the loss due to adhesion of macrophages to the glassware.

Inoculation of macrophage cultures with *C. trachomatis*. One-day-old macrophage monolayers were used for inoculation. The medium was removed, the cell layer was washed once with HBSS, and then 0.1 ml of inoculum was added. Adsorption was done for 1 h in the CO_2 incubator. After adsorption, the inoculum was removed, and culture medium was added and incubated for 2 days. Five culture vials were usually inoculated with each suspension of test organism. Two vials were Giemsa stained for morphological study, and the remaining three vials were used for infectivity assay. For infectivity assay, macrophages were harvested in 0.5 ml of sucrose-phosphate-glutamate medium per culture vial by scraping off the cells from the cover slip surface with a capillary pipette. The harvested material was sonically treated and frozen at −65°C.

Evaluation of *C. trachomatis* growth in macrophage cultures. Growth of *C. trachomatis* in macrophage cultures was evaluated by titrating the harvested materials in HeLa 229 cells (12). One-day-old HeLa cells grown in the same culture vials with a round cover slip were used for infectivity assay. HeLa cells were pretreated with diethylaminoethyl (DEAE)-dextran (30 μg/ml for 30 min) before inoculation. One-tenth milliliter of diluted organism suspension was inoculated, and the vials were centrifuged at 900 × *g* for 60 min at 22°C. After centrifugation, the inoculum was removed and culture medium was added. The vials were incubated for 3 days at 35°C for the trachoma strain and for 2 days at 37°C for the LGV strain. The cover slips were fixed with methanol and stained with May-Greenwald-Giemsa. Inclusions were counted in 30 fields at ×400 magnification with the aid of a micrometer. The average of counts in four tubes was determined. The infectivity titers were expressed as IFU per milliliter.

Methods used for treating macrophage cultures. (i) DEAE-dextran. Before inoculation, the medium was replaced with 1 ml of HBSS containing 30 μg of DEAE-dextran per ml (molecular weight, 5 × 10^5; Pharmacia, Uppsala, Sweden) and incubated for 30 min in the CO_2 incubator. The controls were treated with HBSS only.

(ii) Centrifugation. After inoculation, vials were centrifuged at 900 × *g* for 60 min at room temperature. The residual inoculum was removed after centrifugation. Temperatures varied from 22°C at the beginning to 32°C at the end of centrifugation. The controls were left in the CO_2 incubator.

(iii) ConA. Before inoculation, the medium was replaced with freshly prepared medium containing 100 μg of concanavalin A (ConA) per ml (Calbiochem, La Jolla, Calif.) and incubated for 2 h in the CO_2 incubator. The controls received fresh medium without ConA.

(iv) Cytochalasin B. Before inoculation, the medium was replaced with freshly prepared medium containing 20 μg of cytochalasin B per ml (Calbiochem, La Jolla, Calif.) and incubated for 1 h in the CO_2 incubator. The controls received fresh medium without cytochalasin B.

(v) Cyclophosphamide. Macrophage cell layers were incubated overnight with medium containing 200 μg of cyclophosphamide per ml (Cytoxan, Mead Johnson Laboratories, Evansville, Ind.) before inoculation. The controls were incubated with medium only.

(vi) Hydrocortisone. Hydrocortisone (Solu-Cortef, The Upjohn Co., Kalamazoo, Mich.) at various concentrations was incorporated in the culture medium for preincubation overnight of macrophage cell layers before inoculation and for incubation after inoculation. The control cultures were incubated with medium only.

The concentrations of the above agents chosen were based on our work and that of others on cell culture studies with chlamydiae and other microorganisms (1, 5, 6, 12, 13, 23).

Immunization of mice. Chlamydial antigens used for immunization and testing of mice were grown in HeLa 229 cell culture as described above. Control antigen was the harvest of uninfected HeLa cells processed as that of infected cell culture. Mice were immunized intravenously through the tail vein with 0.5 ml of antigen on days 0 and 7 and sacrificed on day 12 to obtain peritoneal macrophages. The antigens used were live organisms at the following concentrations: trachoma-TW-5, 10^6 IFU; LGV-434, 10^6 IFU; and *Neisseria gonorrhea* strain 9, 10^9 colony-forming units. Animals from the same group of mice, either nonimmunized or injected with HeLa cell material, served as controls. Serum antibodies were determined by the micro-immunofluorescence test (22). Delayed hypersensitivity was tested on day 12 by the footpad test of Gray and Jennings (7) as follows: heat-inactivated (56°C for 10 min) organisms at trachoma-TW-5 and LGV-434 concentrations of 10^8 and 10^9 IFU, respectively, were injected intradermally into the footpad of one hind leg, and heat-inactivated HeLa cell material was injected into the other leg; footpad thickness was measured with a sliding caliper at 24 and 48 h.

RESULTS

Determination of optimal culture conditions. (i) Optimal time of harvesting infected macrophages. Macrophages were infected with 2 IFU of trachoma-TW-5 and 1 IFU of LGV-434 per cell. Cultures were stained, and the infectivity was assayed daily from day 1 to day 9. Typical intracytoplasmic inclusions in the macrophages were seen by Giemsa stain with both organisms, although the numbers were not large. Infectious organisms in the culture supernatant and in the cells were assayed separately. With trachoma-TW-5, infectious organisms were detected from days 2 to 4, all in the cells and none in the culture supernatant. With LGV-434, infectious organisms could be detected from days 1 to 9, with more than 99% in the cells and less than 1% in the culture supernatant. The highest infectivity titers were obtained on day 2 with both organisms. In the remaining experiments, only cells were harvested for infectivity assay.

(ii) Optimal inoculum concentrations. Serial 10-fold dilutions of organisms were inoculated, starting with 10^0 (30 IFU/cell) of trachoma-TW-5 and 10^{-1} (100 IFU/cell) of LGV-434. Results of one of the repeated experiments are presented in Table 1. Inocula at concentrations of 30 IFU of trachoma-TW-5 and 100 IFU of LGV-434 per cell were toxic, and most of the cells were destroyed. No gross toxicity was observed with inocula of less than 3 IFU of trachoma-TW-5 and 10 IFU of LGV-434 per cell. The optimal inoculum concentrations were between 0.1 and 1 IFU/cell. Recoveries of infectious organisms from the 2-day cultures as calculated from the number of IFU found in the culture vial divided by the number of IFU inoculated were less than 4% with trachoma-TW-5 and less than 50% with LGV-434. Inoculum concentrations of 1 to 2 IFU/cell with trachoma-TW-5 and 0.2 to 0.5 IFU/cell with LGV-434 were subsequently used for the studies.

Activated versus nonactivated macrophages. Growth of *C. trachomatis* in thioglycolate-activated macrophages and resident macrophages was compared. The latter were obtained by washing out peritoneal cells with HBSS without prior i.p. injections of stimulant. The yields of infectious organisms from the cultures were three to five times greater with activated than with nonactivated macrophages for both trachoma-TW-5 and LGV-434.

Serial passages of *C. trachomatis* in macrophage cultures. Serial passages in macrophages were carried out by inoculating the harvest from infected macrophages. Trachoma-TW-5 failed to pass in two trials and passed one time only in two other trials. LGV-434 was successfully passed three times in each of three trials. In one of these trials the titer did not fall, but in the other two attempts the titers fell 1 to 2 logs by the third passage.

Some factors affecting the growth of *C. trachomatis* in macrophage cultures. Tables 2 and 3 show effect of treatment of macrophages with several agents on growth of trachoma-TW-5 and LGV-434. The results were similar with both organisms. No significant effect was observed with DEAE-dextran, which enhances at-

TABLE 1. *Determination of optimal inoculum concentrations of C. trachomatis for infecting mouse peritoneal macrophage culture*

Infecting strain	Infectivity assay[a]	Yields				
		10^0[b]	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Trachoma B/TW-5/OT	Inclusion counts[c]	Toxic	5	7	0+	
	Infectious organisms[d]		6.5×10^3	1.0×10^4	1.3×10^3	
	Recovery (% of input)		0.1	2	4	
LGV L_2/434/Bu	Inclusion counts		Toxic	18	18	2
	Infectious organisms			1.2×10^6	5.5×10^5	8.9×10^4
	Recovery (% of input)			6	30	50

[a] 48 h after inoculation.
[b] Inoculum dilution. Inoculum concentration (10°): TW-5, 30 IFU/cell; 434, 1,000 IFU/cell.
[c] Number of inclusions/30 micrometer fields at ×400 magnification; 0+, inclusion positive by scanning.
[d] IFU per milliliter as titrated in HeLa 229 cells when resuspended in 0.5 ml of macrophage culture per tube.

TABLE 2. *Effects of several different agents on the growth of trachoma-TW-5 in the mouse peritoneal macrophage culture*

Treatment[a]	Expt no.	Yield of infectious organisms (IFU/ml)[b]		
		Control	Test	Ratio (test/control)
DEAE-dextran, 30 μg/ml for 30 min	1	3.9×10^4	4.0×10^4	1.08
	2	2.0×10^5	1.7×10^5	0.84
Centrifugation, 900 × *g* for 1 h	1	6.2×10^4	1.6×10^5	2.50
	2	3.3×10^3	5.2×10^3	1.60
ConA, 100 μg/ml for 2 h	1	1.6×10^4	2.0×10^3	0.12
	2	1.1×10^5	1.2×10^4	0.10
Cytochalasin B, 20 μg/ml for 1 h	1	4.2×10^4	2.7×10^4	0.64
	2	1.7×10^4	1.2×10^4	0.71
Cyclophosphamide, 200 μg/ml overnight	1	1.4×10^4	1.2×10^4	0.81
	2	3.1×10^4	5.4×10^4	1.76

[a] See text for procedures.
[b] 48 h after inoculation; assayed by titration in HeLa 229 cells.

tachment and penetration of trachoma but not LGV in HeLa cells (12); cytochalasin B, which inhibits phagocytosis and plasma membrane mobility of macrophages (1); and cyclophosphamide, an alkylating and immunosuppressive agent. Centrifugation, which enhances attachment and penetration of trachoma and LGV in HeLa cells (12), gave slight enhancement of infection (1.6- to 2.5-fold) with trachoma-TW-5 and a greater enhancement (three- to four-fold) with LGV-434. ConA, which affects pinocytic activity and membrane function of macrophages (4, 5), had an inhibitory effect on growth of both organisms. None of the treatments caused any apparent toxicity to the macrophages.

Hydrocortisone, an anti-inflammatory and immunosuppressive drug, had no effect on growth of both organisms at 1- and 10-μg/ml concentrations (Table 4), while a concentration of 100 μg/ml was toxic.

Immune versus nonimmune macrophages. Good antibody responses were seen in the immunized mice. The micro-immunofluorescence serum antibody titers were: to trachoma-TW-5, 1:64 to 1:256; to LGV-434, 1:128 to 1:1,024; and to *N. gonorrhea* (strain 9), 1:1,024 to 1:2,048. Antibodies to trachoma-TW-5, LGV-434, and *N. gonorrhea* strain 9 were not detected in the sera of control mice.

Six immunized and control mice were tested for delayed hypersensitivity by the footpad test. Only a very weak reaction was observed in some mice. The average increase in footpad thickness was 0.25 mm (range, 0 to 0.5 mm) with trachoma-TW-5-immunized mice tested with TW-5 antigen and 0.3 mm (range, 0 to 0.5 mm) with LGV-434-immunized mice tested with 434 antigen at 24 h. Visible swelling was seen in two of the TW-5- and three of the 434-immunized mice that had an increase of 0.4 mm or more. The reaction subsided by 48 h. No increase in footpad thickness was seen with the control mice (average, 0 mm; range, 0 to 0.1 mm).

Cultures of macrophages obtained from trachoma-TW-5- or LGV-434-immunized mice and from nonimmunized control mice were alternately infected with trachoma-TW-5 and LGV-434. There was no difference in the yields of infectious organisms between the cultures of macrophages of immunized and nonimmunized mice (Table 5). In another set of experiments, penicillin-pretreated immune and nonimmune

TABLE 3. *Effects of several different agents on the growth of LGV-434 in the mouse peritoneal macrophage culture*

Treatment[a]	Expt no.	Yield of infectious organisms (IFU/ml)[b]		
		Control	Test	Ratio (test/control)
DEAE-dextran, 30 μg/ml for 30 min	1	4.4×10^5	5.3×10^5	1.20
	2	1.4×10^6	1.2×10^6	0.88
Centrifugation, 900 × *g* for 1 h	1	1.4×10^6	4.3×10^6	3.10
	2	9.2×10^4	3.9×10^5	4.21
ConA, 100 μg/ml for 2 h	1	1.3×10^6	2.2×10^5	0.16
	2	2.4×10^6	6.8×10^5	0.29
Cytochalasin B, 20 μg/ml for 1 h	1	1.6×10^6	1.9×10^6	1.19
	2	9.8×10^5	1.1×10^6	1.12
Cyclophosphamide, 200 μg/ml overnight	1	3.9×10^5	4.6×10^5	1.15
	2	4.3×10^5	5.4×10^4	1.25

[a] See text for procedures.
[b] 48 h after inoculation; assayed by titration in HeLa 229 cells.

TABLE 4. *Effect of hydrocortisone on the growth of C. trachomatis in mouse peritoneal macrophage culture*

Strain	Expt no.	Yield of infectious organisms (IFU/ml)[a]		
		0[b]	1	10
Trachoma	1	8.0×10^4	7.1×10^4 (0.9)[c]	7.3×10^4 (0.9)
B/TW-5/OT	2	6.3×10^4	3.8×10^4 (0.6)	5.7×10^4 (0.9)
LGV	1	1.2×10^6	1.7×10^6 (1.4)	7.3×10^5 (0.6)
L_2/434/Bu	2	2.6×10^6	3.7×10^6 (1.4)	3.7×10^6 (1.4)

[a] 48 h after inoculation; assayed by titration in HeLa 229 cells.
[b] Concentration (in micrograms per milliliter) of hydrocortisone. See text for procedure; 100-μg/ml concentration was toxic.
[c] Ratio: cortisone treated/control.

TABLE 5. *Comparative growth of C. trachomatis in the culture of peritoneal macrophages from normal and immunized mice*

Expt	Immunizing strain	Infecting strain	No. of expt	Differential yields of organisms[a] (normal minus immunized macrophages)	
				Individual test	Avg
I	B/TW-5/OT	B/TW-5/OT	3	0.34, 0, 0.38	0.24
	B/TW-5/OT	L_2/434/Bu	3	0.24, −0.06, −0.13	0.02
	L_2/434/Bu	L_2/434/Bu	3	0.41, 0.28, −0.01	0.23
	L_2/434/Bu	B/TW-5/OT	3	0, 0.37, 0.30	0.22
II[b]	L_2/434/Bu	L_2/434/Bu	4	3.04, 2.32, 1.07, 1.70	2.03
	B/TW-5/OT	L_2/434/Bu	4	−0.10, −0.39, 0.82, 0.40	0.18
	N. gonorrhoea	L_2/434/Bu	3	−1.84, 0.04, 0.67	−0.38
	HeLa cells	L_2/434/Bu	3	−0.29, −0.53, −0.22	−0.35

[a] Log_{10} as titrated in HeLa 229 cells.

[b] Penicillin-pretreated macrophages were used. Macrophages were preincubated overnight with 100 U of penicillin per ml. Before inoculation, cells were washed twice with HBSS. After inoculation, cells were cultured with a medium containing no penicillin. Greater multiplicity of infection of 434 was used for inoculation (1 IFU/cell in experiment I and 100 IFU/cell in experiment II).

macrophages were compared (Table 5). Penicillin (100 U/ml) was incorporated in the culture medium at the time of preparation of the macrophage cell layer. Before inoculation, the cell layers were washed twice with HBSS to remove extracellular penicillin. After infection, the infected macrophages were cultured in the absence of penicillin. Since penicillin partially inhibited chlamydial growth and the penicillin-pretreated macrophages yielded low infectivity titers, a large inoculum dose (100 IFU of 434/cell) had to be used. Gross toxicity was not seen with this dose. Yields of LGV-434 from penicillin-pretreated macrophages of mice immunized with LGV-434 averaged 2 logs less than yields from penicillin-pretreated macrophages of nonimmunized mice. The yields of LGV-434 from penicillin-pretreated macrophages of mice immunized with trachoma-TW-5, *N. gonorrhea* strain 9, and HeLa cell material were the same as those from penicillin-pretreated macrophages of nonimmunized control mice. Growth of trachoma-TW-5 in penicillin-pretreated immune and nonimmune macrophages was also compared. However, the infectivity titers of trachoma-TW-5 were too low to obtain comparative results.

DISCUSSION

This study showed that thioglycolate-activated mouse peritoneal macrophages can support the growth of trachoma and LGV organisms to a limited extent in in vitro culture. The growth of LGV was better than that of trachoma, as seen previously with cultures of established mammalian cell lines (13).

Technically, the use of small vials as compared with the conventional use of petri dishes for cultures eased the handling, reduced the chance of contamination, economized the number of macrophages used, and increased the number of cultures that could be handled at one time. This improved macrophage culture system has allowed us to do many experiments in a manner comparable to the use of ordinary cell cultures.

We did not compare the growth of *C. trachomatis* in macrophages cultured with and without L-cell conditioned medium, and therefore the effect of colony-stimulating factor contained in the L-cell conditioned medium on the growth of chlamydiae is unknown. However, according to Lin and Steward (14), colony formation of mouse peritoneal exudate cells was not apparent until 2 to 3 weeks after initiation of the culture. In our study, macrophages were inoculated on day 2 and the cells were harvested on day 4 of the culture. Division of macrophages during this period was probably insignificant.

The frequency of inclusion bodies seen by Giemsa stain in macrophages and the yields of infectious organisms from macrophage cultures were very poor, suggesting that most of the phagocytized organisms were unable to grow and develop. Since the infectivity assay of macrophage cultures was done at 48 h postinoculation, only infectious organisms from the first cycle of development were assayed. However, the efforts to pass harvested organisms to new macrophage cultures showed that trachoma organisms could grow only one developmental cycle, whereas LGV organisms were able to start a second and probably more cycles in new macrophage cells.

High multiplicity of infection of *C. trachomatis* was toxic to macrophages. This phenomenon has been reported by the others with macrophages (11, 21; M. Gardner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, D6, p. 70; P. B. Wyrick, B. Brownridge, and B. E. Ivins, Abstr.

Annu. Meet. Am. Soc. Microbiol. 1977, D5, p. 70) and mouse L-cells (16) using other chlamydial strains. Our studies on the cytotoxicity of *C. trachomatis* organisms for mouse peritoneal macrophages will be reported in a separate paper.

The growth of *C. trachomatis* in activated macrophages was better than in nonactivated macrophages. This was probably due to more infecting organisms being phagocytosed with activated macrophages (5). That application of centrifugal force during the adsorption period was able to increase the infectivity titers with both trachoma and LGV organisms also suggests that enhanced phagocytosis promotes growth.

Cytochalasin B, which is known to reversibly inhibit cellular microfilaments and to stop cell movement and cytoplasmic streaming, and phagocytic ability (1, 23) did not affect the growth of *C. trachomatis* in macrophages. This is compatible with the observation of W. W. Gregory and G. I. Byrne (Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, D2, p. 70), who showed that preincubation of L-cells with 10^{-6} M cytochalasin B for 1.5 h failed to inhibit the uptake of *C. psittaci* strain 6BC by L cells. It is probable that chlamydiae can still induce phagocytosis (10) to overcome the weak inhibitory action of cytochalasin B.

Corticosteriods are known to aggravate and induce relapse of trachoma eye infection (18). Cytologically, steroids stabilize lysosomal membranes and affect phagolysosome fusion and endocytosis (9, 19). The lack of effect by hydrocortisone on the growth of trachoma and LGV in macrophages in vitro indicates that cellular resistance against *Chlamydia* does not depend on macrophages alone.

The lack of effect of cyclophosphamide is not unexpected. Cyclophosphamide must be activated in vivo by hepatic enzymes to exert its biological effect (20). We have also tested macrophages obtained from mice 3 days after subcutaneous injection of 0.25 or 0.1 mg of cyclophosphamide per g of body weight. However, we had difficulty in culturing the macrophages because of poor yield of macrophages per mouse, poor survival of macrophages in the culture, and frequent bacterial and fungal contamination due to depressed host resistance by the drug. Pathways other than through hepatic enzymes appear to be available to activate cyclophosphamide in vitro and the effects are dependent on both the cell type and the medium in which the cells are grown (6). We were not able to confirm this finding because of the negative result of our study.

Inhibition of the growth of trachoma and LGV in macrophages pretreated with ConA is an interesting observation. ConA has been shown to enhance the pinocytic rate of mouse peritoneal macrophages for horseradish peroxidase (4, 5). It also causes a vacuolation response (4, 19). ConA binds to the plasma membrane and causes reorganization and clustering of the ConA receptor, mannose. The interiorized ConA coalesces to form vacuoles that fail to fuse with preexisting lysosome. Studies on attachment of chlamydiae to ConA-treated macrophages or observation under the electron microscope of the phagocytosis of chlamydiae by ConA-treated macrophages may lead to an understanding of the mechanism of inhibition.

Benedict and McFarland (2) demonstrated that peritoneal macrophages obtained from guinea pigs infected with meningopneumonitis were able to suppress the growth of meningopneumonitis in in vitro culture. In this study, resistance in intracellular growth of trachoma and LGV was not increased in macrophages obtained from mice immunized with the organisms. Also, substantial delayed hypersensitivity was not demonstrated in the immunized mice by the footpad test. In the experiments of Benedict and McFarland (2), guinea pigs were infected with the psittacosis agent. However, trachoma and LGV do not infect mice by the intravenous route. Our findings may reflect an inadequate stimulus of the immunization, since it has been well demonstrated by Mackaness (15) that cellular resistance to intracellular bacteria is best induced when the hosts are actively infected.

The mechanism of the specific resistance against LGV by the penicillin-pretreated LGV-immune macrophages is not clear. Although it must be due to the immunization, what is not clear is why the effect can only be seen with penicillin-pretreated macrophages and not in the experiments comparing immunized and controls without pretreatment. Chlamydial growth was partially inhibited by residual penicillin because a higher inoculum dose was required to infect penicillin-pretreated than nontreated macrophages. However, it is unlikely that pinocytic activity for penicillin was enhanced only with the LGV-immune but not with the trachoma- and gonococcus-immune macrophage.

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