INFECTION AND IMMUNITY, June 1982, p. 1096-1101
0019-9567/82/061096-06$02.00/0

Vol. 36, No. 3

Macrophages in Resistance to Rickettsial Infection: Strains of Mice Susceptible to the Lethal Effects of *Rickettsia akari* Show Defective Macrophage Rickettsicidal Activity In Vitro

CAROL A. NACY[1]* AND MONTE S. MELTZER[2]

Department of Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20012,[1] and Laboratory of Immunobiology, National Cancer Institute, Bethesda, Maryland 20205[2]

Received 4 December 1981/Accepted 26 February 1982

Activation of macrophages was assessed in strains of mice inoculated intraperitoneally with 1,000 times the 50% lethal dose of *Rickettsia akari*. Macrophages from mice resistant to *R. akari* infection (C3H/HeN, C57BL/10J, and BALB/cN) were nonspecifically tumoricidal 2 to 4 days after rickettsial inoculation. Moreover, these macrophages were microbial for *R. akari* in vitro; cells were resistant to infection with the bacterium and were capable of killing intracellular rickettsiae. In contrast, macrophages from strains of mice susceptible to *R. akari* (C3H/HeJ, C57BL/10SnCR, and A/J) failed to develop nonspecific tumoricidal activity over the course of lethal disease and became infected with *R. akari* in vivo within 2 days of rickettsial inoculation. Macrophages from uninfected mice of strains susceptible to *R. akari* also could not be activated for rickettsicidal or tumoricidal activities by treatment with macrophage-activating agents (*Mycobacterium bovis* BCG) in vivo or by treatment with lymphokines in vitro.

Activated macrophages, defined as mononuclear phagocytes with antigenically nonspecific tumoricidal and microbicidal properties, develop during the course of host immune responses against a broad spectrum of infectious agents (8, 10, 12, 14, 15, 18, 19). These cytotoxic effector cells have been implicated as prime constituents of natural resistance to both infection and malignancy. We have identified certain mouse strains with genetic defects in the development of macrophage-mediated cytotoxic activity against tumor cells (2). Mouse strains (C3H/HeJ and C57BL10/ScN) with a defective *Lps*^{d} gene (a gene that controls cell responsiveness to the lipid A region of bacterial endotoxic lipopolysaccharides) fail to develop tumoricidal macrophages after treatment in vivo or in vitro with macrophage-activating agents, whereas macrophages from counterpart strains (C3H/HeN and C57BL/10J) with the normal *Lps*^{n} gene are fully responsive (16, 17). Mouse strains derived from A mice (A/J and A/HeN) show phenotypically similar defects in macrophage tumoricidal activity, yet genetic control of the A/J defect is unrelated to the *Lps* gene (3).

The recent observations that these two groups of mice (C3H/HeJ and A/J) are highly sensitive to the lethal effects of *Rickettsia akari* infection raised the possibility that activated macrophages play a determinative role in the host-rickettsiae interaction (9). Moreover, the induction and expression of cytotoxic effector function by macrophages against rickettsiae and tumor cells may be regulated by mechanisms under common genetic control. We pursued this hypothesis by examining macrophage-mediated microbicidal activity against *R. akari* in mouse strains with macrophage tumoricidal defects.

MATERIALS AND METHODS

Animals. Mice were obtained from specific pathogen-free stocks maintained at the National Institutes of Health or purchased from the Jackson Laboratories, Bar Harbor, Maine. Male C3H/HeJ, C3H/HeN, C57BL/10SnCR, C57BL/10J, A/J, and BALB/cN animals, 6 to 12 weeks old, were used.

Rickettsiae. *R. akari* strain Kaplan (ATCC VR-148, lot 7) was purchased from the American Type Culture Collection, Rockville, Md. The rickettsiae were propagated in irradiated L-929 cell monolayers and quantified by methods previously described (13). In brief, irradiated L cell monolayers were infected with reconstituted *R. akari* and incubated for 5 to 7 days at 34°C in 5% CO_2 and moist air. The cells were suspended by vigorous shaking and pooled. The infected cell suspension was centrifuged at 400 × *g* for 10 min, and the cell pellet was suspended in a small volume of medium 199 (GIBCO Laboratories, Grand Island, N.Y.) with 10% fetal bovine serum (FBS; Flow Laboratories, Inc., Rockville, Md.) The cells were disrupted by blending, and samples were stored at −70°C until use. Rickettsiae were quantified by plaque assay in irradiated L-929 cells by the exposure of monolayers to various dilutions of rickettsial suspensions and the application of an agar feeder overlay (medium 199, 10% FBS, 0.5% agarose) to the monolayer. Cultures were incubated

for 7 to 8 days at 34°C in 5% CO_2 in moist air. A staining overlay (medium 199, 10% FBS, 0.5% agarose. 1/10,000 dilution of neutral red) was applied to the infected monolayers 24 h before the assay was read. The number of plaques on at least five plates at each dilution was determined, and PFU per milliliter were calculated as: average number of plaques × reciprocal of dilution × 10 (0.1 ml of suspension was plated per dilution).

Macrophage activation agents. *Mycobacterium bovis* strain BCG (Phipps substrain 1029) was obtained from the Trudeau Institute, Saranac Lake, N.Y. Mice were infected intraperitoneally with 10^6 viable *M. bovis* BCG 8 to 12 days before macrophages were harvested.

Inoculation of mice with rickettsiae. Four to six mice of each strain were inoculated intraperitoneally with 10^3 PFU of *R. akari* on 9 successive days. On day 10, macrophages were harvested from all infected and control (uninfected) animals. Generally, mice susceptible to this rickettsial infection (C3H/HeJ, A/J, and C57BL/10ScCR) died within 9 days of infection. One set each of infected resistant mice (C3H/HeN, BALB/c), and C57BL/10J) was observed for 30 days; no deaths occurred in these groups.

Peritoneal cells. Peritoneal cells (PC) were harvested from 4 to 6 mice by the injection of 8 to 10 ml of RPMI 1640 (MA Bioproducts, Bethesda, Md.), 20% FBS (Flow Laboratories, Inc.), and 50 μg of gentamicin per ml (MA Bioproducts). The pooled cells were washed, adjusted to 10^6 macrophages per ml in RPMI 1640 (MA Bioproducts) and 20% FBS (Flow Laboratories, Inc.), and distributed in 0.5-ml amounts into polypropylene tubes (no. 2063; Falcon Plastics, Oxnard, Calif.) for the rickettsicidal assay or into 16-mm culture wells (Cluster[24]; Costar, Cambridge, Mass.) for the tumor cytotoxicity assay. The PC were incubated for 2 h at 37°C in 5% CO_2 and moist air.

Rickettsicidal assay. PC were exposed to 5 PFU of tissue culture-propagated *R. akari* strain Kaplan per macrophage for 1 h at room temperature with occasional shaking. The cells were washed once at 400 × *g* and were suspended in medium or in a 1/10 dilution of lymphokines. Cultures were incubated at 34°C for 24 h (13). Gimenez-stained cell smears were examined microscopically for percent infected macrophages, and the rickettsicidal activity of macrophages treated with *M. bovis* BCG in vivo or with lymphokines in vitro was determined by the following formula: 100 × (% infected control macrophages − % infected treated macrophages)/(% infected control macrophages). The number of infected macrophages in control cultures after 1 h of exposure to rickettsiae varied between 30 and 40% for all mouse strains.

Tumor cytotoxicity assay. Nonadherent PC were removed from the macrophage monolayers by repeated washes of the culture wells, and adherent PC (more than 95% macrophages) were incubated in medium or in dilutions of lymphokines for 4 h at 37°C in 5% CO_2 and moist air. The cultures were washed, and [^{3}H]thymidine ([^{3}H]TdR)-labeled mKSA-TU-5 tumor target cells were added (4×10^4 target cells per well). The PC-tumor cell cultures were incubated for 48 h. Total incorporated counts were determined by digestion of the labeled tumor cell monolayers with 0.5% sodium dodecyl sulfate in water. Macrophage-mediated tumor cytotoxicity was estimated by measurement of the [^{3}H]TdR released into culture supernatants at 48 h and was expressed as percent total incorporated counts.

TABLE 1. Rickettsicidal activity of macrophages from resistant strains of mice infected with *R. akari*

Mouse strain	Treatment in vivo	Macrophage rickettsicidal activity[a] (%) at:	
		1 h	24 h
C3H/HeN	*R. akari* infection[b]	32	74
	None	0	0
C57BL/10J	*R. akari* infection	34	72
	None	0	0
BALB/cN	*R. akari* infection	22	58
	None	0	0

[a] Macrophages were exposed to 5 PFU of *R. akari* for 1 h at 24°C, washed, and sampled at 1 h and again at 24 h. Cells were examined microscopically; percent macrophages infected was determined by the examination of 400 to 800 macrophages in triplicate cultures. The rickettsicidal activity was determined by the formula described in the text.

[b] Mice were inoculated with 1,000 times the 50% lethal dose (10^3 PFU) of *R. akari* 7 days before macrophages were harvested.

Lymphokine supernatants. Mice were immunized intradermally with 10^6 viable *M. bovis* BCG and were sacrificed at 3 to 6 weeks after immunization. Spleens were aseptically removed and passed through 50-mesh stainless steel sieves into tissue culture medium (RPMI 1640; GIBCO Laboratories). Single cell suspensions, obtained by serial aspirations through 19- and 23-gauge needles, were treated with NH_4Cl lysis buffer to remove erythrocytes. The spleen cells were centrifuged at 250 × *g* for 10 min at 4°C and suspended to a concentration of 5×10^6 viable cells per ml in medium with 5% heat-inactivated FBS (GIBCO Laboratories). Samples (20 ml) of spleen cell suspension with 50 to 100 μg of purified protein derivative (Connaught Laboratories, Toronto, Canada) per ml were incubated in 75-cm^2 plastic tissue culture flasks (no. 3023, Falcon Plastics) for 48 to 60 h at 37°C. Suspensions of mouse spleen cells cultured with 5 μg of concanavalin A per ml for 48 h were also used as lymphokine sources. Concanavalin A was adsorbed by 10 μg of Sephadex G-10 per ml before storage. The supernatant fluids from replicate cultures were pooled, centrifuged at 450 × *g* for 15 min at 4°C, and divided into aliquots. The aliquots were stored at 4°C until use.

RESULTS

Macrophage activation during *R. akari* infection in resistant and susceptible strains of mice. Activated macrophages that are both tumoricidal and rickettsicidal can be recovered from peritoneal cavities of mice with regressing *Rickettsia tsutsugamushi* infections (10, 12). In this study, we examined the development of these cytotoxic effector functions by macrophages from mice infected with *R. akari*. Peritoneal

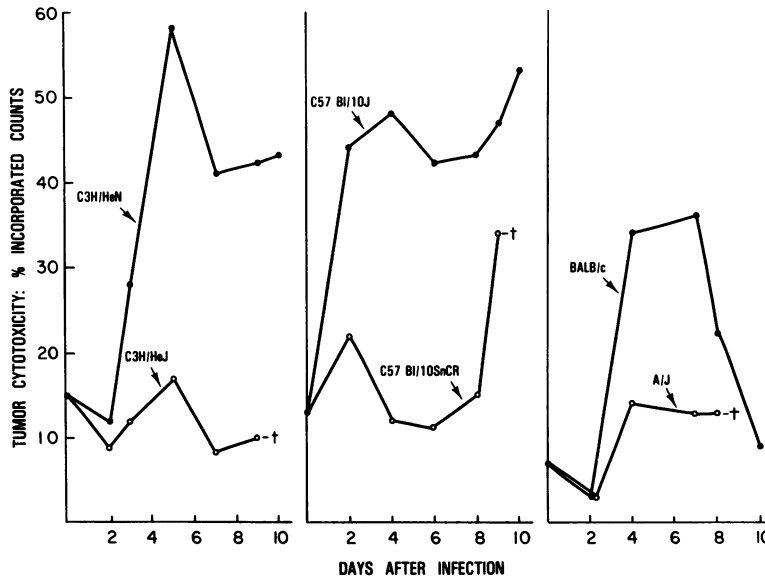

FIG. 1. Nonspecific tumor cytotoxicity of macrophages from *R. akari*-infected mice. Macrophages were harvested from mice inoculated with 1,000 times the 50% lethal dose (10^3 PFU) of *R. akari* for 1 to 10 days. The macrophages were incubated with [^{3}H]TdR-labeled mKSA-TU-5 tumor cells at an effector-to-target cell ratio of 10:1. Tumor cytotoxicity was estimated by measurement of the [^{3}H]TdR released into culture supernatants at 48 h. Results are expressed as percent total incorporated counts.

macrophages were harvested from strains of mice resistant to the lethal effects of *R. akari* infection (C3H/HeN, C57BL/10J, and BALB/cN) and from closely related but susceptible strains (C3H/HeJ, C57BL/10SnCR, and A/J). Macrophages from resistant strains of mice developed significant levels of tumoricidal and rickettsicidal activities 2 to 4 days after intraperitoneal inoculation of rickettsiae (Table 1, Fig. 1). By day 4, activated macrophages from resistant strains were substantially free of intracellular rickettsiae; cells from this peritoneal population were strongly tumoricidal and rickettsicidal in vitro. In contrast, macrophages from susceptible strains of mice failed to develop tumoricidal activity after intraperitoneal infection (Fig. 1). All susceptible mice died of rickettsial disease 8 to 10 days after infection. (Cells from *R. akari*-infected C57BL/10SnCR mice harvested as the mice died [day 9] were tumoricidal; this observation was reproducible, but occurred only in moribund mice.) PC of susceptible mouse strains contained viable intracellular rickettsiae within 2 days of inoculation; since background levels of infected macrophages in these strains were 20 to 40% of the total cell population, the assessment of macrophage rickettsicidal activity (by further in vitro exposure to viable organisms) was technically impossible.

Lymphokine-induced macrophage rickettsicidal activity. Previous analysis of lymphokine-induced macrophage activation for rickettsial killing suggested two distinct mechanisms by which macrophages remain free of infection: lymphokine-activated macrophages develop increased resistance to rickettsial infection, and activated cells that become infected acquire an enhanced ability to kill the intracytoplasmic bacterium (11). Macrophages from susceptible and resistant strains of mice were exposed to medium or lymphokines for 4 h, washed, and infected with *R. akari* (Table 2). In certain instances, cultures were also exposed to lymphokines after infection. Lymphokine-treated macrophages from resistant strains of mice developed both increased resistance to infection (quantified by pretreatment of macrophages with lymphokines and counting of numbers of infected cells 1 h after the introduction of rickettsiae) and increased intracellular killing (quantified by treatment of macrophages with lymphokines after infection and counting of numbers of infected cells 24 h later). In contrast, lymphokine-treated macrophages from susceptible mouse strains showed no increase in resistance to infection. Moreover, the levels of intracellular killing exhibited by these cells at 24 h were only 1/3 to 1/10 of those exhibited by cells from resistant mouse strains. Macrophages from C3H/HeJ, C57BL/10SnCR, or A/J mice showed little or no development of rickettsicidal activity

TABLE 2. Treatment of macrophages with lymphokines in vitro: rickettsicidal activity of macrophages from resistant and susceptible mouse strains

Mouse strain	Macrophages treated with lymphokines:		Macrophage rickettsicidal activity[a] (%) at:	
	Before infection	After infection	1 h	24 h
Resistant to infection				
C3H/HeN	+[b]	−	35	30
	−	+	0	39
	+	+	35	48
C57BL/10J	+	−	35	42
	−	+	0	55
	+	+	35	70
BALB/cN	+	−	29	37
	−	+	0	70
	+	+	29	78
Susceptible to infection				
C3H/HeJ	+	−	0	4
	−	+	0	11
	+	+	0	24
C57BL/10SnCr	+	−	0	7
	−	+	0	20
	+	+	0	26
A/J	+	−	0	15
	−	+	0	8
	+	+	0	14

[a] Macrophages were harvested from four to six mice of each strain, adjusted to 10^6 macrophages per ml, and incubated in medium or lymphokines (1/10 dilution) for 4 h. The treated macrophages were exposed to 5 PFU of *R. akari* for 1 h, washed, and incubated in medium or lymphokines (1/10 dilution) for an additional 24 h. Duplicate samples were removed immediately after rickettsial adsorption or at 24 h and were examined microscopically for percent infected macrophages (400 macrophages observed per culture). The variation (standard error of the mean) between samples was ≤8% of the mean for each group. The rickettsicidal activity was determined by the formula described in the text.

[b] +, Treated; −, not treated.

after treatment with lymphokines derived from either antigen- (tuberculin-stimulated *M. bovis* BCG-immune) or mitogen-stimulated spleen cell cultures (data not shown).

Development of macrophage rickettsicidal activity during *M. bovis* BCG infection. Peritoneal macrophages were collected from groups of mice that had been inoculated with viable *M. bovis* BCG 10 days previously. Macrophages from BCG-infected C3H/HeN, C57BL/10J, and BALB/cN mice (strains resistant to *R. akari* infection) developed strong tumoricidal and rickettsicidal activities (Table 3). In contrast, macrophages from *M. bovis* BCG-infected C57BL/10SnCR, C3H/HeJ, and A/J mice (strains susceptible to *R. akari* infection) were not cytotoxic for tumor cells in vitro, did not develop increased resistance to rickettsial infection, and showed levels of intracellular killing that were 1/3 to 1/5 of those shown by cells from *M. bovis* BCG-infected resistant mice. A similar pattern of response among these mouse strains was observed after treatment with killed *Corynebacterium parvum* (data not shown).

DISCUSSION

Macrophages occupy a central and critical position in natural host resistance against rickettsial infection. An analysis of this position, however, presents an apparent paradox. Many of the rickettsial species (e.g., *Coxiella burnetii, Rickettsia mooseri, R. tsutsugamushi*, and *R. akari*) naturally infect and replicate within mononuclear phagocytes during the course of rickettsial disease (4, 7, 10, 12). Yet very effective macrophage-mediated antirickettsial mechanisms have been documented in vitro (4, 11). The role of any of these in vitro effector mechanisms in natural resistance against rickettsiae, however, is unknown. An examination of *R. tsutsugamushi* infection provides an illustrative example. *R. tsutsugamushi*, the etiological agent of human scrub typhus, causes lethal infections in certain strains of mice (5). Susceptibility to the lethal effects of *R. tsutsugamushi* is controlled by a single gene, *Ric*, on chromosome 5 (6). Is the cellular basis for this control mediated by macrophages? We have recently demonstrated potent macrophage microbicidal activity against *R. tsutsugamushi* in vitro (11). Macrophages activated in vivo by immune responses to *M. bovis* BCG or to *C. parvum*, or cells activated in vitro by lymphokine supernatants, developed significant rickettsicidal activity. An analysis of macrophage activation during *R. tsutsugamushi* infection, however, revealed no consistent differences between resistant and susceptible mouse strains (10). Thus, while the gene product of the *Ric* locus and the molecular basis of its control are not yet known, the capacity of macrophages to be activated during infection appears to be intact. Indeed, susceptible (Ric^s) strains of mice die with activated peritoneal macrophages (10).

The development of activated macrophages, however, may be important for natural resist-

TABLE 3. Rickettsicidal and tumoricidal activity of macrophages from mice resistant or susceptible to *R. akari* infection

Mouse strain	Treatment of macrophages:		Macrophage rickettsicidal activity[a] (%) at:		Macrophage tumoricidal activity[b]
	in vivo	in vitro	1 h	24 h	
Resistant to infection					
C3H/HeN	None	None	0	0	600 ± 90 (8)
	None	Lymphokines[c]	26	69	3,480 ± 60 (46)
	BCG[d]	None	32	89	4,160 ± 90 (55)
C57BL/10J	None	None	0	0	450 ± 100 (6)
	None	Lymphokines	33	70	3,170 ± 80 (42)
	BCG	None	35	76	3,510 ± 100 (46)
BALB/cN	None	None	0	0	380 ± 20 (5)
	None	Lymphokines	27	62	3,180 ± 40 (42)
	BCG	None	30	72	3,060 ± 110 (40)
Susceptible to infection					
C3H/HeJ	None	None	0	0	530 ± 30 (7)
	None	Lymphokines	0	26	620 ± 40 (8)
	BCG	None	0	32	830 ± 60 (11)
C57BL/10SnCR	None	None	0	0	610 ± 10 (8)
	None	Lymphokines	0	17	680 ± 50 (9)
	BCG	None	0	28	1,060 ± 40 (14)
A/J	None	None	0	0	620 ± 50 (8)
	None	Lymphokines	0	10	540 ± 20 (7)
	BCG	None	0	14	610 ± 20 (8)

[a] Macrophages were exposed to 5 PFU of *R. akari* for 1 h at 24°C and washed, and duplicate cultures were sampled at 1 h and again at 24 h. Macrophage rickettsicidal activity was determined by the formula described in the text. At least 400 macrophages were observed per treatment at each time of sampling; variation between samples was ≤10% of the mean for each group.

[b] Release of [^{3}H]TdR expressed as mean counts per mintue ± standard error of the mean for triplicate cultures at 48 h and as percent total incorporated counts (shown within parentheses). Total incorporated counts were estimated by the digestion of [^{3}H]TdR-labeled tumor cell monolayers with 0.5% sodium dodecyl sulfate; total incorporated counts in this experiment were 7,560 ± 130 cpm.

[c] Macrophages were treated with medium or with a 1/10 dilution of lymphokines 4 h before the addition of rickettsiae or tumor cells. Lymphokines were added to certain cultures after rickettsial adsorption for the analysis of macrophage intracellular killing.

[d] Mice were inoculated with 10^6 viable *M. bovis* BCG 10 days before macrophages were harvested.

ance against *R. akari*. Among mouse strains susceptible to *R. akari*, C3H/HeJ, C57BL/10ScN, and A/J mice all have defects in the development of nonspecific tumoricidal activity by activated macrophages (9). We have shown in this report that the development of rickettsicidal activity was also defective. Little or no rickettsicidal activity was detected with macrophages from these susceptible strains of mice at any time during *R. akari* infection. Moreover, treatment of macrophages in vitro with lymphokines or in vivo by the effects of *M. bovis* BCG infection did not induce destruction of rickettsiae by these cells. Thus, over a wide range of experimental conditions with both in vitro and in vivo activation stimuli, macrophages from mice susceptible to *R. akari* failed to develop microbicidal activity. Susceptibility to the lethal effects of *R. akari* in mice is also under genetic control (1). It should be emphasized, however, that unlike *R. tsutsugamushi*, control of *R. akari* infection is completely unrelated to the *Ric* gene. In fact, genetic control of susceptibility to *R. akari* is determined by more than one genetic locus. Mouse strains with the *Lps^d* gene on either the C3H/He or the C57BL/10 genetic background are susceptible; counterpart strains with the *Lps^n* gene are resistant. However, A/J mice, which have the *Lps^n* gene, are also susceptible. Preliminary evidence suggests that the

defect in resistance in A/J mice is also mediated by a single autosomal gene, but not at the *Lps* locus. That two strains of mice, each with a genetically distinct defect in the development of nonspecific microbicidal activity by activated macrophages, are susceptible to *R. akari* infection suggests that the activated macrophage plays a determinative role in this infection. The nature of this role and the molecular basis of the microbicidal defect are not yet known. However, the identification of these genetic models should provide a valuable resource for the future analysis of rickettsial disease.

LITERATURE CITED

1. **Anderson, G. W., Jr., and J. V. Osterman.** 1980. Host defenses in experimental rickettsialpox: genetics of natural resistance to infection. Infect. Immun. **28**:132–136.
2. **Boraschi, D., and M. S. Meltzer.** 1979. Macrophage activation for tumor cytotoxicity: genetic variation in macrophage tumoricidal capacity among mouse strains. Cell. Immunol. **45**:188–194.
3. **Boraschi, D., and M. S. Meltzer.** 1979. Defective tumoricidal capacity of macrophages from A/J mice. II. Comparison of the macrophage cytotoxic defect of A/J mice with that of lipid A-unresponsive C3H/HeJ mice. J. Immunol. **122**:1592–1597.
4. **Catanzaro, P. J., A. Shirai, P. K. Hildebrandt, and J. V. Osterman.** 1976. Host defenses in experimental scrub typhus: histopathological correlates. Infect. Immun. **13**:861–875.
5. **Groves, M. G., and J. V. Osterman.** 1978. Host defenses in experimental scrub typhus: genetics of natural resistance to infection. Infect. Immun. **19**:583–588.
6. **Groves, M. G., D. L. Rosenstreich, B. T. Taylor, and J. V. Osterman.** 1980. Host defenses in experimental scrub typhus: mapping the gene that controls natural resistance in mice. J. Immunol. **125**:1395–1399.
7. **Hinrichs, D. J., and T. R. Jerrells.** 1976. *In vitro* evaluation of immunity to *Coxiella burnetii*. J. Immunol. **117**:998–1003.
8. **Mackaness, G. B.** 1962. Cellular resistance to infection. J. Exp. Med. **116**:381–417.
9. **Meltzer, M. S., and C. A. Nacy.** 1980. Macrophages in resistance to rickettsial infection: susceptibility to lethal effects of *Rickettsia akari* infection in mouse strains with defective macrophage function. Cell. Immunol. **54**:487–490.
10. **Nacy, C. A., and M. G. Groves.** 1981. Macrophages in resistance to rickettsial infections: early host defense mechanisms in experimental scrub typhus. Infect. Immun. **31**:1239–1250.
11. **Nacy, C. A., and M. S. Meltzer.** 1979. Macrophages in resistance to rickettsial infections: activation of macrophages *in vitro* for killing of *Rickettsia tsutsugamushi*. J. Immunol. **123**:2544–2549.
12. **Nacy, C. A., and J. V. Osterman.** 1979. Host defenses in experimental scrub typhus: role of normal and activated macrophages. Infect. Immun. **26**:744–750.
13. **Oaks, S. C., Jr., J. V. Osterman, and F. M. Hetrick.** 1977. Plaque assay and cloning of scrub typhus rickettsiae in irradiated L-929 cells. J. Clin. Microbiol. **6**:76–80.
14. **Patel, P. J., and M. J. Lefford.** 1978. Induction of cell-mediated immunity to *Mycobacterium leprae* in mice. Infect. Immun. **19**:87–93.
15. **Remington, J. S., J. L. Krahenbuhl, and J. W. Mendenhall.** 1972. A role for activated macrophages in resistance to infection with *Toxoplasma*. Infect. Immun. **6**:829–834.
16. **Ruco, L. P., and M. S. Meltzer.** 1978. Defective tumoricidal capacity of macrophages from C3H/HeJ mice. J. Immunol. **120**:329–334.
17. **Ruco, L. P., M. S. Meltzer, and D. L. Rosenstreich.** 1978. Macrophage activation for tumor cytotoxicity: control of macrophage tumoricidal capacity by the *Lps* gene. J. Immunol. **121**:543–548.
18. **Schwartzberg, J. E., J. L. Krahenbuhl, and J. S. Remington.** 1975. Dichotomy between macrophage activation and degree of protection against *Listeria monocytogenes* and *Toxoplasma gondii* in mice stimulated with *Corynebacterium parvum*. Infect. Immun. **12**:1037–1043.
19. **Williams, D. M., S. Sawyer, and J. S. Remington.** 1976. Role of activated macrophages in resistance of mice to infection with *Trypanosoma cruzi*. J. Infect. Dis. **134**:610–614.