

Effect of Alveolar Macrophages on *Toxoplasma gondii*

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As pulmonary involvement can occur in disseminated toxoplasmosis in immunosuppressed patients, studies were initiated to define local mechanisms of resistance of the lung to *Toxoplasma gondii*. Alveolar macrophages were obtained from normal mice and mice chronically infected with *T. gondii* by bronchopulmonary lavage and cultured in vitro. Although normal alveolar macrophages were difficult to infect with *Toxoplasma*, they supported intracellular multiplication of this organism. When exposed to *Toxoplasma* that had been pretreated with heat-inactivated serum containing specific antibody, the number of intracellular organisms increased remarkably, and the macrophages destroyed the coated parasites. After development of chronic infection with *Toxoplasma*, there was a transient period during which a striking increase in numbers of alveolar macrophages was observed in lavage specimens. These macrophages differed from those of normal alveolar macrophages. There was a greater percentage of large cells, a greater tendency to spread on glass, and an increased number of intracellular *Toxoplasma*, and the cells were activated to kill or inhibit multiplication of the parasite. During the period when activated macrophages were demonstrable in bronchopulmonary washings, histological changes in the lungs revealed a marked mononuclear cell infiltrate. These studies support a role for the activated alveolar macrophage as an effector in resistance of the lung to infection with *Toxoplasma*.

Toxoplasma gondii is an obligate intracellular parasite that is gaining wider recognition as an opportunistic pathogen producing fulminant disseminated infection in the compromised host (29). Because lung involvement can occur during disseminated toxoplasmosis in immunosuppressed patients (13, 31), we considered it of interest to study the host response to pulmonary infection with this parasite and to attempt to define the factors, both local and systemic, that are important to resistance of the lung to *Toxoplasma*.

The demonstration that cell-mediated immunity is of major importance in resistance to *Toxoplasma* (10) and that macrophages of mice (18, 25) and hamsters (15) and peripheral blood monocytes (3) and monocyte-derived macrophages (2) of humans in their activated state may be important effectors of cell-mediated immunity in *Toxoplasma* infection suggests that alveolar macrophages might also serve as a barrier to this infection or to its recrudescence. As the alveolar macrophage appears to play a major role in resistance of the lung to a variety of organisms (20), it seemed important to determine the effect of this cell on *Toxoplasma*. The apparent dichotomy in some experimental

models between the cell-mediated immune reaction in the lungs versus other organs (23, 30) precludes extrapolation of data derived from studies of macrophage function in extrapulmonary sites directly to what might occur in the lung. The studies described here were performed by using a murine model to determine whether normal alveolar macrophages and alveolar macrophages of mice infected with *Toxoplasma* have the ability to kill or inhibit the multiplication of this parasite.

MATERIALS AND METHODS

Animals. Mice were outbred females of the Swiss Webster strain and weighed 20 to 24 g (obtained from Simonsen Laboratories, Gilroy, Calif.).

Infection of animals and collection of immune serum. Chronic infection was established by treatment with sulfadiazine (40 mg/100 ml of drinking water) for 21 days beginning 72 h after intraperitoneal inoculation of 10^5 trophozoites of the C56 strain of *T. gondii* as described previously (12). Immunity of these chronically infected mice was evaluated at 4 weeks of infection by challenging them intraperitoneally with 5×10^5 trophozoites of the virulent RH strain; all mice infected with the C56 strain survived, whereas all uninfected control mice died on day 6 or 7 after challenge.

Samples of blood were collected from chronically

infected mice by severing the axillary artery. Sera were pooled, stored at 4°C, and used within 36 h after collection as a source of antibody to coat *Toxoplasma*. The pool of sera obtained from these mice is referred to as *Toxoplasma* immune serum. A pool of sera from normal uninfected mice was prepared in the same manner.

Antibody determination. The Sabin-Feldman dye test was performed as described by Frenkel and Jacobs (11).

Collection of macrophages. Mice were sacrificed by injecting air into a tail vein. Peritoneal cells were collected as described previously (28). The abdominal aorta and inferior vena cava were then severed to drain as much blood as possible from the intravascular space, and bronchoalveolar cells from the same mouse were harvested. The trachea was exposed and dissected free of connective tissue, and the thorax was opened to allow for full expansion of the lungs. The inferior vena cava was clamped with a hemostat to prevent a backflow of fluid through this vessel. The pulmonary vasculature was flushed by injecting 5 ml of cold (4°C) heparinized Hanks balanced salt solution (HBSS; 10 U of heparin per ml) into the right ventricle after making an incision through the left atrium.

A sterile 18-gauge needle, modified by removing the beveled tip, was attached to a three-way stopcock (Becton, Dickinson & Co., Rutherford, N.J.) and inserted into the trachea through a transverse incision. The lungs were lavaged with cold (4°C) heparinized HBSS with a 12-ml plastic syringe. Initial experiments using HBSS with and without Ca²⁺ and Mg²⁺ revealed no difference in cell yield. The fluid was injected into the lungs until they were fully distended (0.8 to 1.0 ml) and then aspirated into a receiving syringe. The aspirated fluid was expressed into 40-ml siliconized centrifuge tubes kept on ice. This process was repeated until a total of 10 ml was collected.

Cultures of macrophages. Bronchoalveolar and peritoneal cells were processed in a similar manner. After centrifugation of the cell suspensions at 225 × *g* for 15 min at 4°C, the cells were suspended in cold (4°C) tissue culture medium 199 (M199; Grand Island Biological Co., Grand Island, N.Y.) containing antibiotics (100 U of penicillin per ml, 100 µg of streptomycin per ml) and 20% heat-inactivated (56°C for 30 min) dye test-negative fetal calf serum (M199-20% FCS). The volumes of different cell suspensions were adjusted to equalize concentrations of viable macrophages.

By using an Oxford sampler (Oxford Laboratories, San Mateo, Calif.), 0.05 ml of each cell suspension was placed in the center of each chamber of two-chambered tissue culture slides (Lab-Tek Products, Naperville, Ill.). The slides were incubated for 1 h at 37°C in a moist atmosphere containing 5% CO₂ to allow for cell adherence. At that time, 1 ml of warm (37°C) M199-20% FCS was added to each tissue culture chamber, and the slides were incubated for an additional 3 h. Nonadherent cells were then removed by washing the monolayers with warm HBSS.

Macrophages are defined as mononuclear cells that adhere to glass and phagocytize heat-killed *Candida*. Alveolar macrophages from control (uninfected) mice are referred to as normal alveolar macrophages

(NAM) and those from mice chronically infected with *T. gondii* are referred to as *Toxoplasma* alveolar macrophages (TAM). Peritoneal macrophages are designated NPM for normal peritoneal macrophages and TPM for *Toxoplasma* peritoneal macrophages.

Cell counts and viability. Total cell counts were done with a Neubauer hemacytometer.

Bronchoalveolar cell suspensions were pelleted on glass slides with a Shandon-Elliott cytocentrifuge (Shandon Southern Instruments, Inc., Sewickly, Pa.) immediately after pulmonary lavage. After fixation in methanol, the cells were stained with Giemsa stain. Additional preparations were stained for esterase (33) to facilitate differentiation of lymphocytes from small macrophages and peroxidase (21). Monolayers of bronchoalveolar cells were similarly fixed and stained after the initial 4 h of incubation and washing to remove nonadherent cells. All preparations were examined for cell morphology, and differential counts were done on 200 cells. Cell viability in the monolayers was assessed by the exclusion of trypan blue dye.

Preparations of lung tissue for histological examination. The lungs of normal mice and mice chronically infected with *T. gondii* were inflated with buffered Formalin, and the tracheas were tied with the lungs fully distended. The lungs were immersed in buffered Formalin for 24 h before sectioning and staining with either Giemsa stain, periodic acid-Schiff, or hematoxylin and eosin.

Preparation of *Toxoplasma* challenge. *Toxoplasma* trophozoites of the RH strain were obtained from the peritoneal fluid of 2-day-infected mice as previously described (25). The organisms were counted in a hemacytometer, centrifuged at 600 × *g* for 15 min, and resuspended in cold (4°C) M199-20% FCS. The suspension was brought to room temperature just prior to the time of challenge of the monolayers.

Infection of macrophages. Each chamber containing the spot (8 to 9 mm in diameter) of adherent bronchoalveolar cells was challenged with 5 × 10⁶ *Toxoplasma* suspended in 1 ml of M199-20% FCS. Chambers containing peritoneal macrophages were challenged with 1 × 10⁶ to 5 × 10⁶ trophozoites. After 60 min, the monolayers were washed with warm (37°C) HBSS to remove extracellular trophozoites.

The wash fluid used to free the monolayers of extracellular trophozoites, as well as supernatants recovered from each monolayer at varying time periods, was collected in separate siliconized glass centrifuge tubes, and samples of each were cytocentrifuged to determine whether *Toxoplasma*-infected cells had detached from the slides during washing or during the incubation period. Only rarely was a *Toxoplasma*-infected cell identified. After 60 min of incubation with *Toxoplasma* (zero time) and at various intervals thereafter, the monolayers were washed with warm HBSS, fixed in 0.4% aminoacridine hydrochloride in 50% ethanol, and stained with Giemsa stain. Intracellular organisms were easily distinguished from extracellular organisms because of their presence in vacuoles when examined by light and phase microscopy (2, 19). Observations were made of the staining characteristics and morphology of the intracellular parasites, as well as the number of organisms per vacuole.

Infection of macrophages with antibody-coated *Toxoplasma*. A suspension of 3×10^7 organisms per ml of cold (4°C) M199 was added to an equal volume of heat-inactivated (56°C for 30 min) dye test-positive (titer, 1:1,024) or dye test-negative mouse serum and mixed continuously for 20 min, and the concentration of serum was adjusted to 20% as described previously (1). There was no difference in viability of *Toxoplasma* exposed to immune or non-immune serum as assessed by criteria employed previously (1).

To evaluate whether the organisms were coated with antibodies, portions of the suspensions were examined after exposure of the parasites to a fluorescein-conjugated antiserum to mouse immunoglobulin G (IgG). After incubation with either immune or control serum, the organisms were washed with M199 and then pelleted on glass slides with a cytocentrifuge. *Toxoplasma* were exposed to the fluorescein-conjugated IgG antiserum (Meloy Labs, Inc., Springfield, Va., lot no. C40533831) and evaluated by fluorescence microscopy as described previously (14).

NAM were incubated for 60 min with 10^6 antibody-coated trophozoites or 5×10^6 noncoated *Toxoplasma*. At various time intervals the monolayers were washed with warm (37°C) HBSS, fixed in 0.4% aminoacridine in 50% ethanol, and then stained with Giemsa stain. Observations were made on the number of macrophages containing intracellular *Toxoplasma* and the morphology and staining characteristics of these organisms, as well as the number of trophozoites per vacuole.

RESULTS

Cell yield and morphology. The total cell yield and differential counts of bronchoalveolar cells from normal mice and mice chronically infected with *Toxoplasma* are depicted in Table 1. Bronchoalveolar lavage of normal mice produced 1.7×10^5 to 2.7×10^5 (mean \pm standard error of the mean [SEM] = $2.2 \pm 0.2 \times 10^5$) cells per mouse. The cells contained 83 to 95% ($83 \pm 3\%$) macrophages and 6 to 16% ($10 \pm 2\%$) lymphocytes.

The cell yield and morphology in bronchoalveolar lavage specimens of mice infected 25, 90, and 168 days earlier with the C56 strain of *T. gondii* were similar to those of uninfected mice (Table 1). However, at 42 to 70 days earlier, lavage produced 5×10^5 to 2.0×10^6 (1.2 ± 0.4

$\times 10^6$) cells per mouse. Cytocentrifuge preparations of these latter cells revealed 55 to 69% (60 ± 3) macrophages and 30 to 45% (40 ± 3) lymphocytes.

When examined in cell culture, glass-adherent bronchoalveolar cells of uninfected mice were present as single cells or small aggregates, with little variation in morphology. Most had a single nucleus located in an eccentric position and greater than 95% phagocytized heat-killed *Candida* and stained with esterase stain. Only 2 to 6% ($4 \pm 1\%$) exceeded a cell diameter of 20 μ m; 47 to 53% ($50 \pm 1\%$) had a diameter of 10 to 14 μ m, and 42 to 50% ($46 \pm 2\%$) were intermediate in size with a diameter of 15 to 19 μ m. Approximately 20% contained one or more vacuoles generally located at the periphery. An equal number of cells contained large, dark-staining granules that were peroxidase negative. Rarely, a very large, highly vacuolated cell was identified.

Of adherent bronchoalveolar cells from mice infected with *Toxoplasma*, greater than 90% were macrophages. These cells were indistinguishable from NAM, except when they were obtained from mice that had been infected with *Toxoplasma* 42 to 70 days earlier. During this time period, there was a greater percentage of large macrophages; 26 to 34% ($30 \pm 4\%$) exceeded 20 μ m in diameter compared with only 2 to 6% ($4 \pm 1\%$) of macrophages from uninfected mice. Of these macrophages that had attached to glass, 33 to 50% ($42 \pm 9\%$) had spread 4 h after plating, whereas only 4 to 9% ($7 \pm 3\%$) of adherent macrophages from uninfected mice had spread by this time. In addition, there were more macrophage aggregates, and occasionally nonphagocytic, esterase-negative cells with a diameter in the 7- to 9- μ m range were observed in contact with single macrophages usually within one of these aggregates.

Gross and histological alterations of lung architecture related to the time after infection with *Toxoplasma*. The gross appearance of the lung was normal 25 days after primary infection with *Toxoplasma*. Microscopic examination at this time revealed normal architecture

TABLE 1. Number and differential count of bronchoalveolar cells obtained by pulmonary lavage of normal mice (NAM) and mice chronically infected with *Toxoplasma* (TAM)

Macro-phages	No. of days after infection with <i>Toxoplasma</i> ^a	No. of bronchoalveolar cells per mouse	Range	Differential (%)	
				Macro	Lymph
NAM		$2.2 \pm 0.2 \times 10^{5b}$	1.7×10^5 to 2.7×10^5	88 ± 3^b	10 ± 2^b
TAM	25, 90, 168	$2.0 \pm 0.1 \times 10^5$	1.8×10^5 to 2.2×10^5	91 ± 2	7 ± 2
	40, 56, 70	$1.2 \pm 0.4 \times 10^6$	5×10^5 to 20×10^5	60 ± 3	40 ± 3

^a C56 strain.

^b Mean \pm 1 SEM of six separate experiments. Cells from 10 mice were pooled for each experiment. All experiments were run in duplicate.

except for occasional small foci of mononuclear cell infiltrates. There was a dramatic change in the appearance of the lungs of mice infected with *Toxoplasma* 42 to 70 days earlier. Many small nodules were grossly visible on the pleural surface of the lungs. On histological examination, these nodules were composed of mononuclear cells with dark-staining nuclei occasionally intermingled with larger mononuclear cells with vacuolated cytoplasm. These mononuclear cell aggregates were observed throughout the lung parenchyma, around bronchi and blood vessels. In addition, there was an interstitial infiltrate of mononuclear cells, and many more intraalveolar cells were present than in uninfected mice or in *Toxoplasma*-infected mice examined at 25 days after infection. Many of these intraalveolar cells were larger and more vacuolated than their counterparts in normal mice, often forming what appeared to be a syncytium within the alveolus. At 90 days after infection, the mononuclear cell infiltrates were reduced in size and extent, and the alveolar architecture had returned to normal in most areas of the lung.

In vitro infection of macrophages with *Toxoplasma*. The results of in vitro infection of NAM, NPM, TAM, and TPM are shown in Table 2. Despite incubation of monolayers of NAM with 5×10^6 trophozoites, only 17 to 33% ($29 \pm 3\%$) contained 1.3 to 1.5 (1.4 ± 0.1) *Toxoplasma* per macrophage. Incubation of NAM with *Toxoplasma* for longer periods of time did not significantly increase the percentage of infected cells. A substantial increase in the number of macrophages containing *Toxoplasma* was noted when TAM from mice infected 42 to 70 days earlier were incubated with the same concentration of *Toxoplasma* used to infect NAM. Of these macrophages, 56 to 64% ($60 \pm 2\%$) were

infected with 1.9 to 3.3 (2.8 ± 0.3) *Toxoplasma*. In contrast, only 29 to 34% ($32 \pm 1\%$) of TAM obtained from mice infected with *Toxoplasma* 25, 90, and 168 days earlier were infected with 1.1 to 1.4 (1.3 ± 0.1) *Toxoplasma*; thus, the percentage of infected macrophages was comparable to NAM. The difference in the mean rates of infection and the mean number of *Toxoplasma* per macrophage of NAM and TAM (from mice infected 42 to 70 days earlier) could not be explained by differences in the cell densities of the monolayers as the number of cells per unit area for TAM was either comparable to or greater than that for NAM.

In parallel experiments in which densities of the two monolayers were comparable, NPM were much easier to infect with *Toxoplasma* than were NAM. After a 60-min incubation period with 1×10^5 to 5×10^5 trophozoites (a log reduction from that used for NAM), 47 to 60% ($53 \pm 3\%$) of NPM were infected with 1.6 to 2.0 (1.8 ± 0.1) *Toxoplasma* per macrophage. When the same *Toxoplasma* inoculum was used, 55 to 79% ($67 \pm 5\%$) of TPM contained 2.0 to 2.4 (2.2 ± 0.2) *Toxoplasma* per macrophage.

Multiplication of *Toxoplasma* (RH strain) in NAM. NAM and NPM supported the intracellular multiplication of *Toxoplasma* (Table 3). In these experiments peritoneal and bronchoalveolar cells were maintained in culture 4 h before the chambers were washed; the adherent cells were then incubated with *Toxoplasma* for 60 min. All intracellular organisms were contained in individual cytoplasmic vacuoles immediately after incubation with *Toxoplasma*. Only a minority of these parasites had multiplied by 8 h (Table 3). However, in both groups by 18 h after infection, over 95% of vacuoles contained *Toxoplasma* that had multiplied. Thus, there were similarities in the intracellular multiplication of *Toxoplasma* in NPM and NAM that included an initial resting phase followed by exponential growth phase in both groups. Despite these similarities, there was a significantly greater number of *Toxoplasma* per vacuole in NPM compared with NAM ($P < 0.001$) at 18 h after infection. The morphology and staining characteristics of intracellular *Toxoplasma* in NAM and NPM were similar. Beyond 18 h, continued intracellular multiplication and reinfection of other macrophages eventually resulted in the destruction of the monolayers in both groups.

Effect of macrophages from *Toxoplasma*-infected mice on *T. gondii*: relation of time after infection with *Toxoplasma*. The intracellular fate of *T. gondii* (RH strain) in TAM and TPM obtained at various intervals after chronic infection with *Toxoplasma* (C56 strain) is depicted in Table 4. TPM destroyed or in-

TABLE 2. *In vitro* infection of NAM, NPM, TAM, and TPM with *T. gondii* (RH strain)

Macrophages	No. of <i>Toxoplasma</i> in challenge inoculum ^a	Macrophages with intracellular <i>Toxoplasma</i> (%)	No. of <i>Toxoplasma</i> per infected macrophage
NAM	5×10^6	29 ± 3^b	1.4 ± 0.1^b
TAM ^c	5×10^6	60 ± 2	2.8 ± 0.3
TAM ^d	5×10^6	32 ± 1	1.3 ± 0.1
NPM	5×10^5	53 ± 3	1.8 ± 0.1
TPM	5×10^5	67 ± 5	2.2 ± 0.2

^a Sixty-minute incubation period.

^b Mean \pm 1 SEM of six experiments. Cells from 10 mice were pooled for each experiment. All experiments were run in duplicate.

^c TAM from mice infected with C56 *Toxoplasma* 40, 56, and 72 days earlier.

^d TAM from mice infected with C56 *Toxoplasma* 25, 90, and 168 days earlier.

hibited the multiplication of *Toxoplasma* at all time periods, whereas TAM were capable of destroying or inhibiting the multiplication of *Toxoplasma* for a limited time only. During the period TAM had the capacity to kill *Toxoplasma*, the mean number of organisms per vacuole in TAM and TPM at 18 h after infection did not differ significantly.

There were striking similarities in the intracellular fate of *Toxoplasma* in TAM and TPM when both groups were activated to kill this organism. (The following ranges and means of the percentages of infected macrophages represent data pooled for TAM and TPM when these cells were obtained from mice infected with *Toxoplasma* 42, 56, and 70 days earlier.) At zero time, 55 to 79% ($67 \pm 5\%$) of TPM and 56 to 64% ($60 \pm 2\%$) of TAM were infected with multiple organisms, each within an individual vacuole. By 8 h, the number of macrophages with intracellular organisms decreased to 10 to 14% ($12 \pm 2\%$) for TAM and 12 to 14% ($13 \pm 1\%$) for TPM. Thus, most intracellular *Toxoplasma* were eliminated during the first 8 h. This occurred without a significant decrease in densities of the infected monolayers compared with uninfected control monolayers. By 18 h, only 1 to 5% ($4 \pm 1\%$) of TAM and 3 to 9% ($7 \pm 2\%$) of TPM contained *Toxoplasma*. At this time most infected macrophages contained single trophozoites in individual vacuoles. Some trophozoites appeared normal but had not divided, and a few had undergone one division; others lacked dis-

tinct cellular features and appeared to be undergoing degeneration. At the time periods when TAM were activated to kill *Toxoplasma*, there was an increase in the total number of bronchoalveolar cells obtained by pulmonary lavage, and morphological changes in many of these cells, as described above, coincided with the acquisition of anti-*Toxoplasma* activity. When TAM supported the intracellular multiplication of *Toxoplasma* (days 25, 90, and 168), the total numbers of cells obtained by bronchoalveolar lavage, their morphology, and the mean number of *Toxoplasma* per vacuole at various time intervals after infection did not differ significantly from those of NAM.

Effect of macrophages on antibody-coated organisms. Coating of *Toxoplasma* with specific antibody had a striking effect on the rate of infection of NAM. After a 60-min incubation with 1×10^6 antibody-coated *Toxoplasma*, 37 to 62% ($47 \pm 10\%$) of macrophages were infected with one or more *Toxoplasma*. Of these cells, 35 to 45% ($40 \pm 4\%$) contained more than 10 *Toxoplasma*, whereas the remainder of the infected cells had 5.6 to 6.7 (6.2 ± 0.6) *Toxoplasma* per macrophage. Only 15 to 22% ($17 \pm 2\%$) of NAM contained 1.0 to 1.4 (1.2 ± 0.1) *Toxoplasma* per macrophage when challenged in parallel with 5×10^6 trophozoites not coated with antibody.

The intracellular fate of antibody-coated and untreated *Toxoplasma* in NAM is depicted in Fig. 1. Whereas NAM supported the intracellular multiplication of untreated *Toxoplasma*, these macrophages destroyed most antibody-coated organisms. Both the percentage of NAM with intracellular antibody-coated *Toxoplasma* (Fig. 1a) and the number of antibody-coated *Toxoplasma* per 100 NAM (Fig. 1c) were markedly reduced at 8 h after infection, with only 7 to 9% ($8 \pm 1\%$) of NAM containing single vacuoles with 1.2 to 1.4 (1.3 ± 0.1) *Toxoplasma* per vacuole. Thus, of the *Toxoplasma* exposed to immune sera, only a small fraction of the original number of intracellular organisms survived 8 h after infection, but most of these organisms went

TABLE 3. Multiplication of *T. gondii* in NAM and NPM

Macrophages	No. of <i>Toxoplasma</i> per vacuole at varying times after infection		
	0 h	8 h	18 h
NAM	1.0	1.2	3.55 ± 0.14^a
NPM	1.0	1.4	5.07 ± 0.08

^a Mean \pm 1 SEM of six experiments. Bronchoalveolar and peritoneal cells from 10 mice were pooled for each experiment. All experiments were run in duplicate.

TABLE 4. Intracellular fate of *T. gondii* (RH strain) in TAM and TPM obtained from mice at various intervals after infection with *toxoplasma* (C56 strain)

Macrophages	No. of <i>T. gondii</i> per vacuole at no. of days after infection ^a					
	25	42	56	70	90	168
TAM	3.6 ± 0.2^b	1.2 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	3.4 ± 0.3	3.5 ± 0.2
TPM	1.4 ± 0.3	1.1 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	1.5 ± 0.1	1.2 ± 0.1

^a C56 strain of *Toxoplasma*.

^b At 18 h after in vitro challenge. Each figure represents the mean of two experiments \pm SEM. All experiments were run in duplicate.

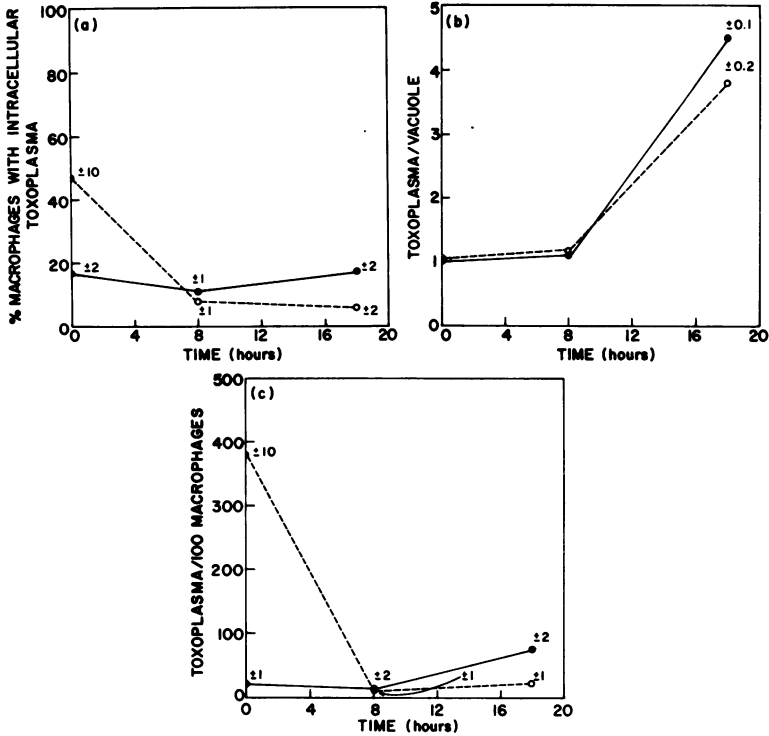


FIG. 1. Effect of NAM on antibody-coated (○) or untreated (●) *Toxoplasma* (RH strain). (a) Percentage of macrophages with intracellular *Toxoplasma*; (b) mean number of *Toxoplasma* per vacuole; (c) number of *Toxoplasma* per 100 macrophages. Numbers represent ± 1 SEM.

on to multiply, with 3.6 to 4.0 (3.8 ± 0.2) *Toxoplasma* per vacuole by 18 h after infection (Fig. 1b).

DISCUSSION

The results described above demonstrate that TAM are activated to inhibit or kill intracellular *T. gondii*, whereas NAM support multiplication of this organism. Thus, *T. gondii* can be added to the growing list of intracellular pathogens of humans that have been demonstrated to be able to survive and/or multiply within NAM, including *Histoplasma capsulatum* (9), *Cryptococcus neoformans* (4), *Listeria monocytogenes* (22), and *Mycobacterium tuberculosis* (8).

A marked difference was noted in the ability of *Toxoplasma* to infect NPM and NAM in vitro. For example, an inoculum that resulted in the infection of approximately 50 to 60% of cells in a monolayer of NPM resulted in the infection of less than 10% of cells in a comparable monolayer of NAM. Whether this difficulty in infecting NAM with *Toxoplasma* was due to the culture milieu rather than to an inherent resistance of these cells to infection with this parasite is unclear. A similar difficulty in infect-

ing NAM was encountered by Reynolds et al., who noted that a period of adjustment to in vitro conditions was necessary before a high percentage of NAM would ingest opsonized *Pseudomonas* (27). In another study, fresh (2 to 3 h postlavage), glass-adherent NAM formed rosettes with IgG-opsonized erythrocytes, but did not maximally phagocytize them until 24 to 30 h of culturing had occurred (26). Supporting the hypothesis that the duration of in vitro culturing of NAM influences their functional capabilities is the observation of Cohen and Cline of a progressive increase in the ability of NAM to phagocytize heat-killed *Candida albicans* with prolonged in vitro cultivation (7). The results of these authors suggest that the lower infection rate for NAM that we observed may well have been due to the brief time of their in vitro culture prior to challenge with *Toxoplasma*. We preferred to employ cells as early as feasible after their harvest, as we considered that results with such cells might more nearly reflect in vivo conditions.

As specific IgG receptors have been identified on NAM (26), one might predict that incubating these cells with organisms coated with this im-

munoglobulin would facilitate their phagocytosis. That this was true for our experimental model can be seen from the substantial increase in the rate of infection of NAM with *Toxoplasma* coated with IgG as compared with the rate achieved with noncoated organisms. Reynolds et al. demonstrated a similar enhancement of phagocytosis of *Pseudomonas* by NAM by opsonizing the bacteria with IgG; opsonized bacteria were ingested by 30 to 40% of NAM in contrast to nonopsonized bacteria, which were ingested by only 5 to 13% of macrophages (27).

Once *Toxoplasma* were intracellular within NAM, their survival and growth followed a course similar to that of *T. gondii* in NPM of the same mice. This course was similar to that described by others (19, 32). Initially there was a resting phase. This was followed by an exponential growth phase that continued throughout the period of observation. Before entering the growth phase, the organism obviously overcame the normal microbicidal mechanisms of the macrophage. It appears to accomplish this in NPM by preventing the fusion of lysosomes with the phagocytic vacuoles in which it resides (17). One can only speculate whether a similar mechanism of intracellular survival of *Toxoplasma* is operative in NAM.

That NAM can influence the growth of *Toxoplasma* under certain conditions was shown by their effect on the antibody-coated organisms. In contrast to the survival and multiplication of non-antibody-coated organisms within NAM, intracellular antibody-coated *Toxoplasma* were destroyed by these cells. The destruction of antibody-coated *Toxoplasma* in NAM was quite efficient, with a rapid dissolution of the intracellular parasites. One explanation to account for the altered intracellular course of antibody-coated *Toxoplasma* might be the inability of these parasites to prevent lysosomal fusion with phagocytic vacuoles as suggested by Jones et al. (18). A similar alteration of the intracellular fate of antibody-coated *Toxoplasma* has been observed in peritoneal macrophages of mice (1, 18) and in human monocyte-derived macrophages (2).

Of interest is the observation that, whereas the duration of demonstrable activation of TAM was transient, TPM of the same mice maintained their activation during the entire period of observation. (In other experiments in our laboratory, TPM have been shown to have the capacity to inhibit or kill this parasite for more than 1 year [J. Swartzberg, J. L. Krahenbuhl, and J. S. Remington, unpublished data].) In addition to this dichotomy in the persistence of microbicidal activity against *Toxoplasma* was

the difference in the time of appearance of activated macrophages at the two sites; activated macrophages appeared in the lungs later than they did in the peritoneal cavity. We have no data to define whether this delay was due to some factor associated with the method used to establish chronic infection (e.g., treatment with sulfonamides), the route of inoculation of the parasite, or some other mechanism, but we considered it most likely an artifact of the treatment schedule employed to establish chronic infection. Interestingly, Jones et al. observed that when mice were inoculated subcutaneously with cysts of the Pe strain of *Toxoplasma*, peritoneal macrophages inhibited the multiplication of *Toxoplasma* directly during the first 2 to 3 months after infection; thereafter, exposure of macrophages to immune lymphocytes and *Toxoplasma* antigen, or to the supernatants of such an interaction, was necessary to induce maximal growth inhibition by peritoneal macrophages (18).

Relevant to the duration of activated macrophages in the lungs is the question of the origin of these cells and the difference in the population of lymphocytes available to the lung and peritoneal cavity. The presence of activated macrophages in the lung occurred at a time when histological examination revealed a marked mononuclear cell infiltrate, including an increase in the number of intraalveolar cells resembling macrophages. One can only speculate as to the origin of the activated macrophage population that we studied in vitro. It might be resident alveolar macrophages, mononuclear phagocytes that infiltrate the lung from the circulation, or a combination of these. Pertinent to the question, but leaving it unresolved, are two recent observations. NAM were shown to respond to lymphokines (6) and to be activated in vitro (16) when exposed to sensitized respiratory tract lymphocytes and specific antigen. In addition, Truitt and Mackaness observed that the predominant effector of resistance against reinfection with aerosolized *L. monocytogenes* in mice previously immunized with this bacterium was the influx of mononuclear cells from the circulation into infected areas of the lung (30).

Of interest is the fact that, in the mouse model, the mononuclear cell infiltrate of the lung at the time when activated macrophages were present resembles the histological description of the lungs in human cases of disseminated toxoplasmosis (5, 13, 24, 31). A distinct difference, however, was the absence of proliferating organisms in the lungs of mice, whereas in patients parasitization of alveolar macrophages and alveolar lining cells has been demonstrated repeatedly

(5, 13, 24). It is interesting to speculate that the absence of *Toxoplasma* in the mouse lungs may have been largely due to the presence of activated macrophages.

The disappearance of activated macrophages from the lungs, in contrast to their persistence in the peritoneal cavity, might be explained by the removal of the stimulus for the continued activation of macrophages in the lung, such as the removal or destruction of *Toxoplasma*. Such an explanation would be predicated on the assumption of the persistence of these stimuli or their effect in the peritoneal cavity. Another alternative is that there may be an inherent difference in the process by which these cell populations undergo activation.

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