INHIBITION OF MULTIPLICATION OF *TOXOPLASMA GONDII* BY HUMAN MONOCYTES EXPOSED TO T-LYMPHOCYTE PRODUCTS*

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Acquired resistance to a variety of obligate or facultative intracellular pathogens is dependent on cell-mediated immunity (1–3). Mononuclear phagocytes play a major role in this defense mechanism (2, 3). Lymphocytes from immunized subjects produce in response to antigenic stimulation substances which induce morphologic, metabolic, and functional changes in human monocytes (4, 5). However, little is known about the effects of these lymphocyte mediators on the interaction of human monocytes with specific intracellular parasites.

The obligate intracellular organism, *Toxoplasma gondii*, is well suited for the experimental evaluation of cellular immunity. Studies in animals have indicated that development of cellular immunity is the major factor in slowing the progression of toxoplasma infection in vivo (6, 7). Intracellular multiplication of this organism in vitro can be reproducibly quantitated by phase-contrast microscopy in normal (8) and immune mouse cells (6).

The present studies describe an in vitro model for the quantitation of the multiplication of *T. gondii* in monocytes from normal and immune human subjects. The findings show that the capacity to inhibit growth of toxoplasmas is induced in monocytes by a product released after exposure of T lymphocytes from immune subjects to toxoplasma antigen.

Materials and Methods

Subjects and Serum Preparation. Serum and mononuclear cells were obtained from healthy laboratory personnel. All sera were passed through a 0.45 μm Millipore filter (Millipore Corp., Bedford, Mass.), heat inactivated at 56°C for 30 min, and stored at −20°C. Sabin-Feldman dye tests and toxoplasma complement (C) fixation tests were performed by Dr. Anne Kimball, Cornell University Medical College, New York (9).

Collection, Separation, and Culture of Mononuclear Cells. Human peripheral mononuclear cells were separated from heparinized venous blood by centrifugation over a Ficoll-Hypaque gradient as described by Gordon et al. (10). The mononuclear band was resuspended in phosphate-buffered saline containing 0.3 mM EDTA and centrifuged at 500 *g* for 10 min. The cells were resuspended in RPMI-1640 with penicillin and streptomycin (RPMI) (Associated Biomedic Systems, Inc., Buffalo,

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N. Y.) and 5% heat-inactivated human serum (HIHS).[1] 0.5 ml of the mononuclear cell-rich suspension containing 10^7 cells/ml was carefully placed on 22-mm^2 glass cover slips in plastic dishes (35 × 10 mm, Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). After 90 min of incubation (37°C) in 5% CO_2/95% air about 10% of the cells were adherent to glass. The nonadherent cells were removed by washing four times with RPMI and designated as lymphocytes. Giemsa-stained smears of these cells revealed 95% lymphocytes, 3–4% monocytes, and 1–2% polymorphonuclear leukocytes. Adherent cells were 95% monocytes and 5% lymphocytes by morphologic criteria (11). Monocytes were maintained in culture for 1–3 days before infection with *T. gondii*.

Separation of Thymus-Derived (T) Lymphocytes and Bone Marrow-Derived (B) Lymphocytes. Lymphocytes were rosetted with neuraminidase-treated sheep red blood cells (SRBC) as described by Weiner et al. (12). The rosetted and nonrosetted lymphocytes were centrifuged over a Ficoll-Hypaque gradient (200 *g* for 10 min). Rosetted lymphocytes (T cells) were pelleted, owing to their greater density, whereas the nonrosetted lymphocytes appeared as a sharp band in the Ficoll-Hypaque mixture. The SRBC in the rosetted lymphocyte preparation were lysed with ammonium chloride (0.83% in distilled H_2O) (J. T. Baker Chemical Co., Phillipsburg, N. J.). Samples from the two populations of lymphocytes (T-cell rich or B-cell rich) were again rosetted and also treated with antihuman IgG fluorescein-labeled antibody (13) to determine the efficiency of the separation procedure.

Preparation of Lymphocyte Products. Mononuclear cells obtained from the Ficoll-Hypaque gradient were suspended in RPMI-5% HIHS in plastic tubes (17 × 10 mm; Falcon Plastics, Div. Of BioQuest). Lymphocyte products were prepared by incubating (5% CO_2/95% air, 37°C) 2.5×10^6 of these cells for periods varying from 15 min to 96 h with either: (*a*) 20 μg/ml of toxoplasma antigen or (*b*) 50 μg/ml of Con A or (*c*) 10 μg/ml of purified protein derivative (PPD). Control tubes lacked either antigen or lymphocytes. After incubation, tubes were cooled to 4°C and centrifuged at 1,400 *g* for 20 min. The supernates were designated as lymphocyte products and stored at 4°C.

Incubation of Lymphocytes or Lymphocyte Products with Monocytes. Monocytes were cultured for 1.5–96 h before the addition of 2.5×10^6 intact lymphocytes (starting mixture, T-cell rich or B-cell rich) and toxoplasma antigen or mitogen. The cells were then incubated (5% CO_2/95% air, 37°C) in 2.5 ml RPMI—5% HIHS for 2–72 h before infection with *T. gondii*. The concentrations of antigen or mitogen were identical to those used in the preparation of lymphocyte products. Control monocyte cultures excluded either lymphocytes or antigen and mitogen. Lymphocyte products were similarly incubated with monocytes for 2–48 h before infection with *T. gondii*.

Lymphocyte Transformation. Lymphocyte transformation studies utilized mononuclear cells from the Ficoll-Hypaque mixture and employed the micro method and concentrations of phytohemagglutinin (PHA), pokeweed mitogen (PWM), and concanavalin A (Con A) described by Weksler and Hutteroth (13). 2×10^5 cells were also incubated with either toxoplasma antigen (10 μg) or 0.5 μg of preservative-free PPD (Parke, Davis & Co., Detroit, Mich.) in 0.2 ml of RPMI with 20% heat-inactivated heterologous human AB serum. All studies were performed in triplicate and all cultures were incubated (5% CO_2/95% air, 37°C) for 72 h except those with PPD (120 h). Control cells were incubated with culture media alone.

HeLa Cells. HeLa cells obtained from Dr. L. Senterfit (Cornell University Medical College, New York) were cultured in plastic flasks in minimum essential medium (MEM) (Microbiological Associates, Inc., Bethesda, Md.) with 10% heat-inactivated fetal calf serum (HIFCS) (Grand Island Biological Co., Grand Island, N. Y.) and monolayers were prepared as described by Jones et al. (8). The procedures for incubation of HeLa cells with lymphocytes or lymphocyte products and the infection and quantitation of toxoplasma intracellular multiplication were the same as described for monocytes.

Maintenance and Collection of T. gondii and Preparation of Toxoplasma Antigen. The RH strain of *T. gondii*, originally isolated by Sabin (14), was obtained from Dr. Thomas Jones (Cornell University Medical College, New York). The strain was maintained by intraperitoneal passage in CFW male mice, and living toxoplasmas, as well as soluble toxoplasma antigen(s), were prepared from parasite-rich mouse peritoneal exudates (15). The toxoplasma antigen was quantitated by the protein content as determined by the Lowry method and stored at −20°C (16).

Infection of Monocyte Monolayers with T. gondii and Quantitation of Parasite Multiplica-

[1] *Abbreviations used in this paper:* Con A, concanavalin A; GT, generation time; HIFCS, heat-inactivated fetal calf serum; HIHS, heat-inactivated human serum; MIF, migration inhibition factor; PHA, phytohemagglutinin; PPD, purified protein derivative; PWM, pokeweed mitogen.

tion. The glass adherent monocytes were washed four times with RPMI before exposure to 1×10^6 toxoplasmas in 1 ml of MEM-20% HIFCS for 30 min at 37°C in 5% CO_2/95% air. The cells were then washed twice with RPMI to remove extracellular toxoplasmas and incubated (5% CO_2/95% air, 37°C) in 2 ml of RPMI 5% HIHS. After incubation for 1–24 h, infected monocytes were fixed in 2.5% glutaraldehyde in 0.1 M Na cacodylate (pH 7.4) at 4°C. The cover slips were removed from the dishes, inverted on glass slides, rimmed with paraffin, coded, and examined by phase-contrast microscopy with a 63× or 100× objective. The mean number of toxoplasmas per vacuole was determined by counting the parasites in 50 vacuoles for each monocyte preparation. Toxoplasma growth curves were constructed and the mean toxoplasma generation time (GT) was calculated by standard methods (17).

Results

Profile of Study Subjects. A profile of the study subjects and the results of their lymphocyte transformation studies with toxoplasma antigen and PPD are shown in Table I. Subjects A, B, and C had positive Sabin-Feldman dye tests documented for 60, 24, and 8 mo, respectively. Subjects D and E repeatedly had negative tests (<1:2).

Lymphocytes from all subjects responded to PHA, Con A, and PWM with increased (30- to 250-fold) incorporation of tritiated thymidine as compared with controls. Subjects A, B, and C (dye test positive) had increased incorporation of thymidine (about 10-fold) with toxoplasma antigen whereas the response of subjects D and E was insignificant. The mitogenic response to toxoplasma antigen of lymphocytes from the dye test-positive, and presumably immune subjects, was comparable in magnitude to that of cells from a tuberculin-sensitive individual after exposure to PPD.

Multiplication of T. gondii in Human Monocytes. 30 min after infection of monocytes in vitro, parasites were present within cytoplasmic vacuoles of 15–20%

TABLE I

Profile of Study Subjects and Results of Lymphocyte Transformation Studies with Toxoplasma Antigen and PPD

Subject	Age/sex	Sabin-Feldman dye test	Tuberculin skin test	Lymphocyte transformation* (cpm thymidine incorporated × 10^{-3})		
				Control‡	Toxoplasma antigen	PPD
A	36M	1:256		0.3 ± 0.1	4.4 ± 2.3	
B	28M	1:16	Positive	0.3 ± 0.1	2.2 ± 1.5	2.0 ± 1.1
C	36F	1:256		0.3 ± 0.1	3.1 ± 1.2	
D	30M	Negative	Negative	0.1 ± 0.1	0.6 ± 0.2	0.2 ± 0.1
E	40M	Negative		0.4 ± 0.1	0.5 ± 0.2	

* Results are expressed as the mean with one standard deviation. Statistical analysis utilized the unpaired Student's *t* test. The combined results of lymphocytes from subjects A, B, and C and toxoplasma antigen are significantly different from both their control cultures and lymphocytes from subjects D and E with toxoplasma antigen ($P < 0.0005$). The response of lymphocytes from subject B to PPD is significantly different from both his control cultures and lymphocytes from subject D and PPD ($P < 0.01$).

‡ Control lymphocytes were incubated with culture media alone.

of the cells. The age of the monocyte culture did not influence the percent of cells parasitized. However, a greater percentage of cells was parasitized if either the number of infecting toxoplasmas or the duration of their exposure to the monocytes were increased. Multiple vacuoles containing toxoplasmas were uncommonly seen within a single cell.

The growth curve of *T. gondii* in monocytes from subject D (dye test negative) is shown in Fig. 1. The parasites entered an exponential phase of growth after a 10

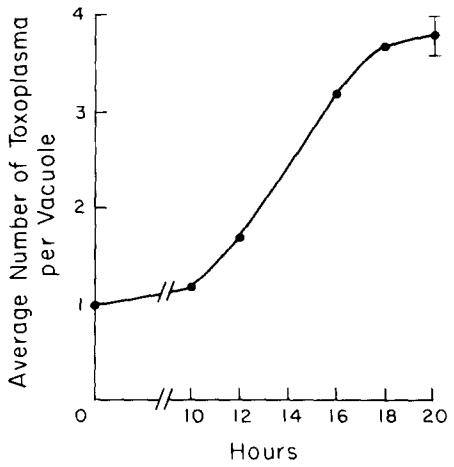

FIG. 1. Growth curve of *T. gondii* in monocytes from Sabin-Feldman dye-test-negative subject. The bar represents one standard deviation.

h lag period and the average number of toxoplasmas per vacuole increased from 1.0 to 3.8 by 20 h after infection. Further parasite multiplication during an additional 4–6 h of incubation destroyed the monocytes. The mean toxoplasma GT calculated from this curve was 5 h and 10 min. This is in agreement with the mean GT of this strain of *T. gondii* in other mammalian cells (8).

The multiplication of toxoplasmas in human monocytes was not influenced by the age of the monocyte culture (1.5–96 h) or the presence of toxoplasma immune HIHS either during the initial monocyte cultivation or during incubation of monocytes after infection. There was no difference in parasite multiplication in monocytes infected with toxoplasmas in media containing 20% dye test-positive (titer of 1:256) HIHS compared to dye test-negative HIHS or HIFCS. Observations were not made on possible influences of these various sera on the rate of uptake of toxoplasmas or on parasite survival during the early postphagocytic period.

Effect of Immune Lymphocytes and Toxoplasma Antigen on the Multiplication of T. gondii in Monocytes. Monocytes from the test subjects were cultured in vitro with various combinations of autologous lymphocytes and toxoplasma antigen for 24 h. After washing to remove lymphocytes and antigen, the monocytes were infected with toxoplasmas. As shown in Table II, multiplication of toxoplasmas was significantly inhibited in those monocytes which were previously exposed to both toxoplasma antigen and lymphocytes from dye test-positive subjects. Monocytes preincubated with only lymphocytes or antigen, but not both, did not acquire the capacity to suppress parasite multiplica-

TABLE II

Multiplication of T. gondii in Monocytes Cultured with Combinations of Autologous Lymphocytes and Toxoplasma Antigen

Subject	Sabin-Feldman dye test	Lymphocyte transformation with toxoplasma antigen	Average no. of toxoplasmas per vacuole 20 h after infection in monocytes preincubated with:*		
			Toxoplasma antigen	Lymphocytes	Lymphocytes and toxoplasma antigen
A	Positive	Yes	4.0 ± 0.2‡	3.9 ± 0.3	2.4 ± 0.2
B	Positive	Yes	4.0	3.7 ± 0.3	2.4 ± 0.2
C	Positive	Yes	3.8	3.8	2.3
D	Negative	No	4.1	3.7 ± 0.3	3.8 ± 0.2
E	Negative	No	3.8	3.7	3.7

* Monocytes were preincubated for 1 day before infection with either toxoplasma antigen, autologous lymphocytes, or both lymphocytes and toxoplasma antigen.

‡ One standard deviation is indicated when the mean is derived from four or more separate experiments.

tion. Lymphocytes from nonimmune subjects did not induce in monocytes the capacity to inhibit growth of toxoplasmas, even when toxoplasma antigen was present. The source of the monocytes (i.e., immune or nonimmune donors) was irrelevant; lymphocytes from immune subjects plus toxoplasma antigen induced in monocytes from nonimmune as well as immune persons the capacity to inhibit toxoplasma multiplication. Monocytes cultured with heterologous lymphocytes were indistinguishable from those cultured with autologous lymphocytes by morphologic criteria, i.e., spreading on glass and ruffled membrane activity.

The mean toxoplasma GT calculated from the parasite growth curves shown in Fig. 2 was prolonged from 5 h and 10 min to 8 h by exposure of immune lymphocytes and monocytes to toxoplasma antigen for 1 day before infection of the monocytes. There was no prolongation of the lag phase preceding toxoplasma multiplication but rather a slowing of the parasite division time after the lag period.

Lymphocyte Requirements and Kinetics of their Effect on Monocytes. The number of immune lymphocytes required with toxoplasma antigen to stimulate monocyte inhibition of toxoplasma multiplication was also studied.

Mononuclear cells (5×10^6) were cultured on glass for 1.5 h as described and then washed five times with RPMI to remove nonadherent lymphocytes. The lymphocytes were pooled, counted, and resuspended in fresh media with toxoplasma antigen. Different numbers of lymphocytes were then returned to the washed monocytes and incubated for 24 h before toxoplasma infection.

Maximal inhibition of toxoplasma multiplication resulted when monocytes were preincubated with $2\text{–}4 \times 10^6$ lymphocytes (approximately a 10:1 lymphocyte:monocyte ratio), whereas no suppression of parasite multiplication was seen with 1×10^6 lymphocytes. Increasing the number of lymphocytes to 1×10^7 did not enhance the inhibition of toxoplasma growth.

Observations were also made on the time required for immune lymphocytes

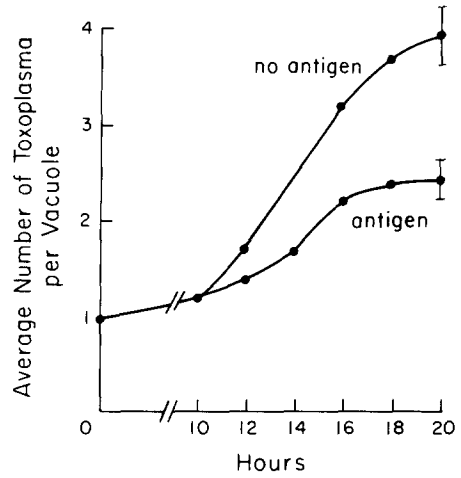

FIG. 2. Growth curve of *T. gondii* in monocytes from a dye-test-positive subject which have been preincubated with autologous lymphocytes in the presence or absence of toxoplasma antigen. The bars represent one standard deviation.

and toxoplasma antigen to induce in monocytes the capacity to inhibit toxoplasma multiplication. Monocyte cultures washed to remove the lymphocytes and antigen at times ranging from 2 h to 72 h all exhibited the same capacity to inhibit parasite multiplication (Table III). The addition of both toxoplasma antigen and lymphocytes from immune subjects after monocytes were infected resulted in a similar degree of inhibition of toxoplasma multiplication. Monocytes preincubated with immune lymphocytes and toxoplasma antigen for 24 h and then washed and maintained in fresh culture media for 48 h completely lost their capacity to inhibit multiplication of toxoplasmas.

Demonstration that T Lymphocytes are Responsible for the Induction in Monocytes of the Capacity to Inhibit Toxoplasmas. In order to determine the subpopulation of immune lymphocytes primarily responsible for the induction of inhibition of toxoplasma multiplication, rosetted T lymphocytes were separated from nonrosetted B lymphocytes on Ficoll-Hypaque. In four experiments, there was a mean of 68% rosetting T cells in the starting lymphocyte mixture from subject A (Table IV). After separation, the population enriched in T cells contained an average of 83% rosetting cells and the population enriched in B cells had an average of 15% rosetting lymphocytes. The percentage of immunoglobulin-bearing lymphocytes in the T- and B-cell-rich populations was 10% and 95%, respectively.

2.5×10^6 immune lymphocytes from either the starting lymphocyte mixture or the separated T- or B-cell-rich populations were preincubated for 24 h with monocytes and toxoplasma antigen. As shown in Table IV, there was inhibition of toxoplasma multiplication in the monocytes preincubated with either the starting lymphocyte mixture or with the T-cell-rich population. In contrast, monocytes preincubated with the B-cell-rich population acquired no demonstrable capacity to inhibit toxoplasma multiplication. The number of lymphocytes used here was just above the minimum required for full induction of monocytes,

TABLE III

Exposure Time of Monocytes to Toxoplasma Antigen and Immune Lymphocytes

Incubation period of immune lymphocytes and toxoplasma antigen with monocytes before toxoplasma infection	Average no. of toxoplasmas per vacuole in monocytes 20 h after infection
h	
2	2.3 ± 0.1*
24	2.4 ± 0.2
48	2.3 ± 0.1
72	2.5 ± 0.2
72 (without antigen)	4.0 ± 0.3

* One standard deviation.

TABLE IV

Multiplication of T. gondii in Monocytes Cultured with Toxoplasma Antigen and Lymphocyte Populations Enriched in T or B Cells

Lymphocyte population	Percentage of lymphocytes		Average no. of toxoplasmas per vacuole in monocytes 20 h after infection
	Rosette-forming cells	Immunoglobulin-bearing cells	
	%	%	
Starting mixture	68 (64–74)	34	2.4
T-cell rich	83 (72–89)	10	2.3
B-cell rich	15 (8–18)	95	4.0
None	—	—	4.2

thus a B-cell-rich population containing 15% T cells would be expected to be inactive.

Multiplication of T. gondii in Monocytes and HeLa cells Exposed to Lymphocyte Products. Supernates (lymphocyte products) were collected after incubation of toxoplasma antigen with lymphocytes from three dye test-positive subjects and a dye test-negative subject. These supernates were incubated with monocyte cultures for 24 h before toxoplasma challenge. As shown in Table V, supernates of lymphocytes from the dye test-positive subjects and antigen induced significant monocyte inhibition of toxoplasma multiplication, whereas no inhibition resulted from supernates of lymphocytes alone or supernates of lymphocytes from the dye test-negative subject and antigen. There was no difference in toxoplasma multiplication in monocytes exposed to these various lymphocyte products for short periods (2 h) compared to longer periods (48 h).

Supernates were also prepared from T- and B-cell-rich lymphocyte populations from a dye test-positive subject and toxoplasma antigen. T-cell-rich supernates induced monocytes to inhibit toxoplasma multiplication as effectively

as did the starting lymphocyte mixture supernates (Table VI). No inhibition was induced by the B-cell-rich supernates.

Supernates prepared by incubation of immune lymphocytes and toxoplasma at 37°C for as little as 15 min were active in inducing monocytes to inhibit toxoplasma multiplication. Supernates collected after 2, 24, or 72 h of incubation of lymphocytes and toxoplasma antigen could be diluted 1:4 without any loss of activity. Supernates prepared at 4°C were completely inactive. Active supernates could be stored at 4°C for 1 mo without loss of activity. Activity was lost when supernates were heated at 56°C for 30 min.

Supernates which induced human monocytes to inhibit toxoplasma multiplication did not influence parasite growth in HeLa cells. In two experiments, HeLa cells preincubated for 1 day with either active supernates or intact immune lymphocytes and toxoplasma antigen had an average of 8.2 toxoplasmas/vacuole 24 h after infection. HeLa cells preincubated with only lymphocytes or culture media had 8.7 toxoplasmas/vacuole.

TABLE V

Toxoplasma Multiplication in Monocytes Exposed to Lymphocyte Products

Composition of lymphocyte products (supernates) preincubated with monocytes before infection		Average no. of toxoplasmas per vacuole in monocytes 20 h after infection
Lymphocytes (dye test of donor)	Toxoplasma antigen	
Positive	Present	2.4 ± 0.1*, ‡
Positive	Absent	4.0 ± 0.1
Negative	Present	3.9 ± 0.1
Negative	Absent	3.9 ± 0.1
No lymphocytes	Present	4.1 ± 0.3

* One standard deviation.

‡ Combined results of experiments with lymphocytes from three dye test-positive subjects.

TABLE VI

Toxoplasma Multiplication in Monocytes Exposed to T- and B-Cell Lymphocyte Products

Lymphocyte population*	Average no. of toxoplasmas per vacuole in monocytes 20 h after infection
Starting mixture	2.4 ± 0.1‡
T-cell rich	2.4 ± 0.1
B-cell rich	3.9 ± 0.1

* Lymphocytes were cultured with toxoplasma antigen for 1 day and the resulting lymphocyte products were then incubated with monocytes for 24 h before toxoplasma challenge.

‡ One standard deviation.

Specificity of Lymphocyte-Antigen Effect on Monocytes. Monocytes from subjects B and D were incubated for 24 h with 3–4 $\times$ 10^6 autologous lymphocytes and either toxoplasma antigen (50 μg) or PPD (100 μg) before infection with *T. gondii*. Subject B had evidence of immunity to both *Mycobacterium tuberculosis* and *T. gondii*, as evidenced by a positive PPD and a positive Sabin-Feldman dye test, as well as a similar degree of lymphocyte blastogenesis in response to PPD or to toxoplasma antigen. Subject D had no evidence of immunity to either microbe (Table I). Monocytes cultured with lymphocytes from subject B and toxoplasma antigen inhibited toxoplasma multiplication, whereas monocytes preincubated with this subject's lymphocytes and PPD did not (Table VII). Toxoplasma multiplication in monocytes from subject D was uninhibited under all test conditions. No inhibition of toxoplasma multiplication occurred in monocytes incubated with Con A-stimulated lymphocytes, their supernates or supernates of lymphocytes from the tuberculin-positive subject, and PPD.

Discussion

The obligate intracellular parasite, *T. gondii*, is a common cause of human infection. Serologic surveys utilizing the Sabin-Feldman dye test indicate that about 40% of the United States population has had toxoplasmosis by age 50 (18). Although toxoplasmosis is commonly asymptomatic, it can produce a fulminant illness in the immunosuppressed patient or the fetus (19, 20). While host factors which control this infection are not completely understood, cell-mediated immunity appears to be a major determinant in halting the progression of this infection. There is abundant evidence that cell-mediated immunity plays a major role in the host defense against *T. gondii* in mice (6) and hamsters (7). In humans delayed-type skin hypersensitivity (21) and lymphocyte transformation to toxoplasma antigens have been demonstrated (22), and evidence also indicates that macrophages from immune subjects can inhibit toxoplasma growth (23, 24).

In the present report an in vitro system has been utilized to quantitate the intracellular multiplication of *T. gondii* in human monocytes, and to characterize aspects of the cellular immune response of man. *T. gondii* is well suited for

TABLE VII

Toxoplasma Multiplication in Monocytes Cultured with Autologous Lymphocytes and PPD or Toxoplasma Antigen

Monocyte culture conditions before infection with *T. gondii*		Average no. of toxoplasmas per vacuole in monocytes 20 h after infection
Lymphocyte source	Antigen	
Subject B (immune to toxoplasmosis and tuberculosis)	Toxoplasma	2.4 ± 0.2*
	PPD	3.8
Subject D (not immune to toxoplasmosis or tuberculosis)	Toxoplasma	3.8 ± 0.2
	PPD	3.8

* One standard deviation.

quantitative studies; it is sufficiently large to count directly, and determine viability under the phase-contrast microscope (8), and it is then possible to calculate the generation time of the intracellular organisms. Such a direct method circumvents many of the problems of studies employing bacterial models in which colony counts are employed; in these models extracellular multiplication of organisms often cannot be excluded, and intracellular localization cannot be determined with certainty in stained preparations.

Toxoplasma growth curves in cultures of human monocytes from immune and nonimmune subjects were identical when these cells were not exposed in vitro to toxoplasma antigen and immune lymphocytes. While this is in agreement with recent observations utilizing human macrophages (23), it conflicts with animal studies in which some peritoneal macrophages from toxoplasma-infected mice inhibit parasite multiplication without prior in vitro exposure to toxoplasma antigen (25). Anderson and Remington postulated that the contrasting results with human and mouse macrophages might relate to the prolonged in vitro culture period (5–8 days) of the human cells (23). This seems unlikely since in our studies the age of the monocyte cultures (90 min to 4 days) did not influence toxoplasma multiplication. It is more plausible that this variation in macrophage response is related to the generally longer interval between infection-immunity and study in humans. Jones et al. have now shown that mouse peritoneal macrophages inhibit toxoplasmas without additional antigen exposure in vitro during the period 16–75 days after infection, but after that time in vitro contact with antigen and immune lymphocytes is required (15). The source of cells studied in the human (blood) and mouse (peritoneal cavity) is also different and could contribute to the variation in macrophage response. Mouse peritoneal macrophages might be exposed in vivo to different numbers or types of lymphocytes, to localized antigen or intact parasites, or to different concentrations of serum factors. Furthermore, the peritoneal cavity might provide a greater opportunity for contact between the macrophage and these factors.

In the present study, toxoplasma multiplication was inhibited only in those monocytes preincubated with toxoplasma antigen and lymphocytes from a toxoplasma-immune subject or to soluble factors produced by these two agencies. Parasite growth curves indicated that this inhibition did not result from a prolongation of the "lag phase" preceding multiplication but from a slowing in the toxoplasma division time after the lag period.

The mechanism whereby the monocyte inhibits toxoplasma multiplication is unknown. Jones and Hirsch have demonstrated in electron microscopy studies that there is a lack of fusion of phagosomes containing viable toxoplasma and the lysosomal granules of normal mouse peritoneal macrophages (26). They have recently reported that toxoplasma-immune mouse macrophages which were capable of inhibiting parasite multiplication similarly demonstrate a failure of fusion between phagosomes and lysosomes (15).

Toxoplasma multiplication in human monocytes was not influenced by exposure of the parasites to heat-inactivated dye test-positive human serum before infection of the monocytes or by the addition of antibody to the medium after the toxoplasma were intracellular. Although these experiments do not exclude a role for cytophilic antibody, they indicate that conventional humoral

antibody does not play a role in the intracellular inhibition of toxoplasma multiplication (27).

Evaluation of the role of the various types of lymphocytes in human cellular immune reactions has been hampered by the unavailability, until recently, of suitable methods for separating T and B lymphocytes. The critical role of the T cell in experimental animal models is evident in studies in which the passive transfer of immunity to *Listeria monocytogenes* was abolished by pretreatment of the transferred lymphocytes with antitheta antiserum and C (28), and the demonstration that T cells are essential for resistance to mouse pox and murine listeriosis (29–31). In our studies we utilized the distinctive property of human T cells to form rosettes with SRBC (32) to obtain a T-cell-rich lymphocyte population. The T cell was then shown to be primarily responsible for stimulating monocytes to inhibit toxoplasma multiplication.

In the present study, supernates obtained from antigen-stimulated lymphocytes from immune subjects induced in monocytes the capacity to inhibit toxoplasma multiplication as effectively as did intact lymphocytes and antigen. In contrast, Fowles et al. reported intact lymphocytes and antigen were more effective in enhancing macrophage bacteriostasis than soluble lymphocyte products (33). These differences may reflect variations in the host species (human or guinea pig) or in the infectious agent (toxoplasma or listeria) studied. In the toxoplasma system the active material was released into the supernate quite rapidly (within 15 min), indicating that lymphocyte transformation and "standard" migration inhibition factor (MIF) release are not prerequisites for the inhibition of toxoplasma multiplication, since these processes require a much longer time. In previous reports a minimum of 18–24 h has been required to generate lymphocyte products which alter macrophage metabolism or function (33–35). The very brief contact period between lymphocyte and antigen in our system suggests the passive release of a preformed lymphokine. The absence of any monocyte-stimulating activity in supernates prepared at 4°C indicates that cellular metabolism is required either to produce or release the substance. The prompt release seen in our system is not too surprising, since in other studies on lymphocyte activation, membrane lipid turnover or synthesis, active pinocytosis, acetylation of histones, and phosphorylation of nucleoproteins were discernable within 15 min of the addition of a transforming agent (36).

The instruction of monocytes to inhibit toxoplasma multiplication by both intact lymphocytes and antigen, or their supernate, was also rapid, and completed in 90–120 min. In other in vitro systems, 1–10 days of contact between mononuclear phagocytes and lymphocyte mediators was required to induce metabolic or functional changes in the cells (37–42). This variability probably reflects the diversity of the in vitro systems employed in these studies. The addition of lymphocytes and antigen immediately after monocytes were infected with toxoplasmas also resulted in inhibition of parasite multiplication. When monocytes incubated with lymphocytes from immune subjects and antigen for 1 day were washed, and then cultured an additional 2 days in fresh media alone, they lost their ability to inhibit toxoplasma multiplication. A similar reversibility in this type of induced macrophage function has been reported by Nathan et al. (38).

Supernates of toxoplasma-immune lymphocytes and specific antigen which induced monocytes from normal or immune subjects to inhibit toxoplasma multiplication did not influence parasite growth in HeLa cells. This finding suggests that the active principal in the supernate is not interferon (43). Kinetic studies and the sensitivity of the supernate to heat suggest that the lymphocyte product is not MIF (44). Concentration and fractionation studies in progress are directed towards the further characterization of this lymphocyte product.

Monocytes cultured with either lymphocytes from a tuberculin-positive subject and PPD or supernates from Con A-stimulated lymphocytes did not inhibit toxoplasma multiplication. These preliminary observations suggest a specificity in the expression as well as in the induction of cellular immunity against *T. gondii* and are in agreement with the findings of Hoff and Frenkel (45) and Hirsch et al. (46).

Summary

The multiplication of *Toxoplasma gondii* was quantitated in human monocytes in vitro by phase-contrast microscopy. Toxoplasma multiplication was identical in monocytes from subjects with established toxoplasma immunity and nonimmune subjects but was significantly inhibited in cells from both sources if the monocytes were preincubated with immune lymphocytes and toxoplasma antigen. Supernates prepared from toxoplasma-immune lymphocytes incubated with toxoplasma antigen were also effective in inducing in monocytes the capacity to inhibit toxoplasma multiplication. Supernate activity was evident after lymphocytes and antigen were incubated for as little as 15 min. The instruction of monocytes was also rapid and reversible. Monocytes were fully induced to inhibit toxoplasma multiplication after a 2 h exposure to an active supernate, but they lost their inhibitory capacity on culture in vitro for 48 h in the absence of immune cells or their products. The lymphocytes participating in the monocyte induction were identified as T cells. The in vitro stimulation of monocytes appeared to exhibit some specificity, since no inhibition of toxoplasma multiplication occurred in monocytes incubated with either purified protein derivative and lymphocytes from tuberculin-positive subjects, concanavalin A-stimulated lymphocytes, or their supernates. Supernates which induced monocytes to inhibit toxoplasma multiplication did not influence parasite growth in HeLa cells.

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