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Effect of Mouse Lymphokines and Cloned Mouse Interferon-γ on the Interaction of *Rickettsia prowazekii* with Mouse Macrophage-Like RAW264.7 Cells

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We studied the effects of crude mouse lymphokines and cloned mouse inteferon-γ on the interaction of *Rickettsia prowazekii* with mouse macrophage-like RAW264.7 cells. Treatment of RAW264.7 cells with lymphokines before infection, after infection, or both before and after infection with *R. prowazekii* led to killing of a substantial proportion of the RAW264.7 cells. Such cytotoxicity required both lymphokines and viable *R. prowazekii* and did not occur in mouse fibroblastic L929 cells. Untreated cultures of RAW264.7 cells supported good growth of the Breinl strain of *R. prowazekii*, but in lymphokine-treated cultures, little or no rickettsial growth occurred in the cells that survived the cytotoxic reaction. In addition, treatment of RAW264.7 cells with lymphokines before rickettsial infection was associated with suppression of the initial infection. The effects of cloned mouse interferon-γ were similar to the effects of crude mouse lymphokines. Assessment of cytotoxicity, inhibition of the initial infection, and inhibition of rickettsial growth in RAW264.7 cells pretreated with various concentrations of interferon-γ indicated that the effects of the lymphokines could be explained by the interferon-γ that was present in these preparations. Treatment of RAW264.7 cells with interferon-γ makes them unsuitable host cells for *R. prowazekii*.

The bacterial etiological agent of epidemic typhus, *Rickettsia prowazekii*, has a predilection for multiplication within the vascular endothelial cells of its human host (31). Virulent *R. prowazekii* can also infect and multiply within human monocyte-derived macrophages, but rickettsiae treated with immune serum are killed by the macrophages (1, 5). An avirulent strain of *R. prowazekii*, strain E, fails to multiply in most human macrophages (5).

The mouse macrophage-like cell line RAW264.7 is strikingly similar to human monocyte-derived macrophages in its interactions with *R. prowazekii* (21). The virulent *R. prowazekii* strain Breinl proliferates well in RAW264.7 cells, but the avirulent strain E fails to grow in most of the cells. Furthermore, virulent *R. prowazekii* treated with specific antiserum is cleared from these macrophage-like cells (21). These data suggest that RAW264.7 cells constitute a good model system for the study of *R. prowazekii*-macrophage interactions. RAW264.7 cells are easily cultured and readily lend themselves to experiments that might be difficult or impossible to perform with human monocyte-derived macrophages.

The survival and growth of *R. prowazekii* within nonprofessional phagocytes (mouse fibroblasts) are inhibited when the fibroblasts are treated with lymphokines produced by antigen- or concanavalin A-stimulated mouse spleen cells (24). In addition, the growth of *R. prowazekii* within human fibroblasts is inhibited when the fibroblasts are treated with lymphokines produced by concanavalin A-stimulated human peripheral blood mononuclear cells (24). Supernatant fluids obtained from cultures of antigen- or phytohemagglutinin-stimulated human leukocytes inhibit the growth of *R. prowazekii* not only in human fibroblasts but also in human endothelial cells and macrophages (30). Studies with *Rickettsia tsutsugamushi* have shown that treatment of resident mouse peritoneal macrophage cultures with supernatant fluids collected from antigen-stimulated cultures of mouse spleen cells markedly suppresses the ability of the macrophages to be infected with this rickettsia (13, 14). In addition, untreated mouse macrophages support the growth of *R. tsutsugamushi*, whereas lymphokine-treated macrophages have rickettsicidal activity (13).

Most, if not all, of the antirickettsial activity that is observed in mouse lymphokine-treated mouse fibroblasts that are infected with *R. prowazekii* can be explained by the interferon-γ (IFN-γ) that is present in the lymphokine preparations (22–24). Furthermore, mouse IFN-γ has antirickettsial activity: cloned mouse IFN-γ produced by Chinese hamster ovary cells and cloned mouse IFN-γ produced by *Escherichia coli* inhibit the growth of *R. prowazekii* within mouse fibroblasts (22).

The present study examines the effects of crude mouse lymphokines and cloned mouse IFN-γ on the interaction of *R. prowazekii* with mouse macrophage-like RAW264.7 cells. We report that treatment of RAW264.7 cells with lymphokines or cloned IFN-γ and infection of the cells with *R. prowazekii* together result in the killing of a substantial proportion of the RAW264.7 cells. In addition, rickettsial growth is inhibited in the cells that survive the cytotoxic reaction.

MATERIALS AND METHODS

Cell cultures. The mouse macrophage-like cell line RAW264.7 was obtained from the Cell Distribution Center at the Salk Institute (San Diego, Calif.) and grown in Dulbecco modified Eagle medium supplemented with 10% calf bovine serum. Mouse L929 cells were purchased from Flow Laboratories (McLean, Va.) and grown in either Eagle minimum essential medium or Dulbecco modified Eagle medium supplemented with 10% newborn bovine serum. Cells were grown in a CO_2 incubator at 34°C. The bovine sera were heated at 56°C for 30 min before use.

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Rickettsiae. *R. prowazekii* E or Breinl was harvested and purified from infected yolk sacs as described previously (24). Viable rickettsiae were enumerated by the antibody hemolysis method of Walker and Winkler (26).

Lymphokines. Lymphokines were prepared from cultures of spleen cells from BALB/c mice or C57BL/6J mice according to previously described procedures (22, 24). BALB/c mouse lymphokines were the supernatant fluids collected from concanavalin A- or antigen-stimulated cultures of spleen cells obtained from normal or immunized mice, respectively (24). When BALB/c mice were immunized, the antigen was either *Corynebacterium parvum* CN6134 (Burroughs Wellcome Co., Research Triangle Park, N.C.) or *R. prowazekii* E. C57BL/6J mouse lymphokines were the supernatant fluids collected from concanavalin A-stimulated cultures of spleen cells obtained from normal or *Listeria monocytogenes*-challenged mice (22).

Cloned mouse IFN-γ and control medium for cloned IFN-γ. Cloned mouse IFN-γ produced by Chinese hamster ovary cells, control medium for this IFN-γ, and cloned mouse IFN-γ produced by *E. coli* were kindly provided by Genentech, Inc., South San Francisco, Calif.

Interferon assay. A cytopathic effect inhibition assay modified from that of Havell and Vilček (8) as described previously (23) was used for measuring IFN. Because an international reference preparation of mouse IFN-γ was not available, IFN activity was calibrated against a World Health Organization international reference preparation of mouse type I IFN (G-002-904-511). IFN activity is expressed as international units (IU).

Treatment of cells with lymphokines or IFN, infection of cells with rickettsiae, assessment of cytotoxicity, and measurement of rickettsial growth. RAW264.7 cells and L929 cells were X-irradiated with a General Electric Maximar 100 X-ray unit at a dosage adequate to prevent cell division (3,000 to 5,000 rads). RAW264.7 cells were adjusted to a density of 2.5×10^5 cells per ml, and L929 cells were adjusted to a density of 1.2×10^5 cells per ml in Dulbecco modified Eagle medium plus 10% serum. Cells were planted in eight-chambered slides (0.3 ml per chamber) (Miles Scientific, Div. of Miles Laboratories, Inc., Naperville, Ill.) and incubated overnight in a CO_2 incubator at 34°C. Cells were then treated for 24 h with tissue culture medium alone or with various dilutions of lymphokines or cloned IFN-γ in tissue culture medium. The cells were infected with *R. prowazekii* as described previously (24). RAW264.7 cells were incubated for 1 h with approximately 100 viable rickettsiae per cell, and L929 cells were incubated for 1 h with approximately 200 viable rickettsiae per cell. After infection, the cells were washed, the appropriate medium was added, and the slides were incubated for up to 48 h. All treatments were done in duplicate, and at least 100 cells were examined in each chamber. For assessment of cytotoxicity, the cells were stained with trypan blue. For assessment of the initial rickettsial infection and rickettsial growth, slides were dried, fixed, and stained as previously described (24). Slides were examined with an oil immersion objective, and the number of rickettsiae present in each of 100 cells was counted for each duplicate. When a cell contained over 100 rickettsiae, it was assigned a value of 100; this practice causes an underestimate of the number of rickettsiae at later times. The percentage of cells infected with rickettsiae (%R), the average number of rickettsiae per infected cell (RI), and the average number of rickettsiae per cell (NR) were determined.

Supernatant fluids of T lymphocyte clones. Supernatant fluids were collected from five Lyt-1^+,2^- murine T lymphocyte clones cultured in the presence of concanavalin A or the antigen bovine serum albumin. These clones were maintained in the presence of irradiated spleen cell feeder layers; hence, control supernatant fluids were collected from cultures that contained spleen cell feeder layers and concanavalin A or bovine serum albumin.

RESULTS

Cytotoxicity of lymphokine treatment and *R. prowazekii* for RAW264.7 cells but not for L929 cells. Treatment of irradiated RAW264.7 cells with mouse lymphokines and infection of these cells with viable *R. prowazekii* E led to killing (trypan blue positivity) of more than half of the cells within 4 to 6 h after infection (Table 1). Such cytotoxicity was not observed in uninfected control or uninfected lymphokine-treated RAW264.7 cells, in control cells that were incubated with live or dead *R. prowazekii*, or in lymphokine-treated cells that were incubated with dead *R. prowazekii*. At 0 and 4 h after infection, the total number of cells present in measured areas of lymphokine-treated RAW264.7 cell cultures that were infected with viable *R. prowazekii* was similar to the numbers observed in comparable areas of uninfected lymphokine-treated cultures, uninfected control cultures, and control cultures infected with viable *R. prowazekii* (Table 2). Hence, preferential loss of cells from RAW264.7 cell cultures subjected to a given treatment was not observed at these time points. The percentage of cells stained by trypan blue in the lymphokine-treated RAW264.7 cell cultures infected with viable *R. prowazekii* did not increase between 4 and 24 h after infection (Table 1). In some experiments, this percentage actually decreased (data not shown). In experiments in which a decrease was observed, many cells had been lost from the substratum of the lymphokine-treated infected cultures by 24 h after infection. The data in Table 2 indicate that extensive cell loss had occurred by 24 h after infection in the lymphokine-treated RAW264.7 cell cultures that were infected with rickettsiae. Extensive loss of RAW264.7 cells was not observed, however, in uninfected lymphokine-treated cultures, uninfected control cultures, or control cultures infected with viable rickettsiae (Table 2).

TABLE 1. Effect of lymphokines and infection with *R. prowazekii* E on the viability of irradiated RAW264.7 or L929 cells[a]

Cell line	Treatment of cells	Rickettsiae[b]	Cytotoxicity[c] at the following time after infection:		
			0–1 h	4–6 h	20–24 h
RAW264.7	Control	None	7 ± 2	14 ± 3	12 ± 3
	Control	Dead	8 ± 0	9 ± 0	17 ± 1
	Control	Live	8 ± 1	12 ± 1	23 ± 6
	LK	None	8 ± 2	14 ± 1	15 ± 3
	LK	Dead	10 ± 2	9 ± 2	16 ± 0
	LK	Live	17 ± 1	55 ± 6	59 ± 9
L929	Control	None	5 ± 4	2 ± 0	3 ± 1
	Control	Live	4 ± 1	4 ± 3	4 ± 1
	LK	None	2 ± 1	3 ± 1	7 ± 1
	LK	Live	3 ± 1	3 ± 1	11 ± 1

[a] Cells were treated for 24 h before infection and after infection with tissue culture medium alone (control) or medium that contained 20% crude mouse lymphokines (LK) collected from BALB/c mouse spleen cell cultures stimulated with concanavalin A or the antigen *C. parvum*.

[b] Rickettsiae were killed by heating at 56°C for 30 min or by heating at 45°C for 2.5 h, and dead rickettsiae were diluted in the same manner as the live rickettsiae.

[c] Expressed as percent trypan blue-positive cells. Each value represents the mean ± the standard error for two or more experiments.

Cytotoxicity was not observed under any conditions in L929 cells, even when these cells were treated with lymphokines and infected with viable *R. prowazekii* (Table 1). The total numbers of L929 cells present in measured areas of uninfected control cultures, control cultures infected with viable *R. prowazekii*, uninfected lymphokine-treated cultures, and lymphokine-treated cultures infected with viable *R. prowazekii* were comparable at 0, 4, and 24 h after infection (Table 2). Hence, L929 cells were not preferentially lost from any of these cultures.

Either the avirulent strain E or the virulent strain Breinl of *R. prowazekii* caused cytotoxicity in RAW264.7 cells that were treated with mouse lymphokines (Tables 1 and 3). Lymphokines were able to induce cytotoxicity when they were added to RAW264.7 cells either before rickettsial infection only or after rickettsial infection only (Table 3), as well as when they were added both before and after infection (Table 1).

Cytotoxicity was also observed in nonirradiated, lymphokine-treated RAW264.7 cells that were infected with *R. prowazekii* (Table 4). In addition, the extent of cytotoxicity was directly proportional to the initial rickettsial infection, and infection of cells with as few as three rickettsiae per cell was sufficient to cause cytotoxicity in lymphokine-treated cells (Table 4).

Effect of lymphokine treatment on the initial rickettsial infection and growth of *R. prowazekii* in RAW264.7 cells. The effect of treating RAW264.7 cells with lymphokines for 24 h (before infection) on the ability of these cells to be initially infected with *R. prowazekii* was variable, but the data suggested that lymphokine-treated cells were less readily infected than control cells. When irradiated RAW264.7 cells were treated with medium alone or with 10 to 20% lymphokines collected from BALB/c mouse spleen cell cultures, and then infected with *R. prowazekii* Breinl, the means ± standard errors for %R in control and lymphokine-treated cultures at 0 h after infection were 87 ± 4 and 63 ± 7, respectively. The means ± standard errors for RI in control and lymphokine-treated cultures were 3.7 ± 0.5 and 2.2 ± 0.3, respectively (n = 5 experiments). In three of the five experiments the NR in the lymphokine-treated cultures was less than 60% of the NR in the control cultures.

TABLE 2. Effect of lymphokines and *R. prowazekii* E on total cell number[a]

Cell line	Treatment of cells	Rickettsiae	Total no. of cells[b] at the following time after infection:		
			0–1 h	4 h	24 h
RAW264.7	Control	None	227 ± 19	203 ± 12	183 ± 8
	Control	Live	239 ± 36	205 ± 15	185 ± 11
	LK	None	231 ± 18	194 ± 15	215 ± 14
	LK	Live	242 ± 15	195 ± 21	NC[c]
L929	Control	None	197 ± 8	163 ± 7	196 ± 4
	Control	Live	187 ± 12	177 ± 7	204 ± 6
	LK	None	175 ± 16	161 ± 8	177 ± 4
	LK	Live	171 ± 17	170 ± 6	184 ± 10

[a] Irradiated cells were treated for 24 h before infection and after infection with tissue culture medium alone (control) or medium that contained 20% crude lymphokines (LK) collected from BALB/c mouse spleen cell cultures stimulated with concanavalin A. By 4 h after infection, lymphokine-treated RAW264.7 cell cultures that were infected with *R. prowazekii* contained 77% trypan blue-positive cells. In all other cultures less than 9% of the cells were trypan blue positive.

[b] The number of cells in an area defined by a Whipple disk (0.5 mm^2) was determined. Each value represents the mean ± the standard error for four determinations (duplicate slides).

[c] NC, Not counted because of extensive and uneven cell loss throughout the monolayers.

TABLE 3. Cytotoxicity of *R. prowazekii* Breinl for irradiated RAW264.7 cells treated with lymphokines either before or after infection[a]

Treatment of cells	Rickettsiae	% Trypan blue-positive cells at 4–6 h after infection[b]
Control	None	10 ± 4
LK, B	None	10 ± 2
LK, A	None	6 ± 1
Control	Live	12 ± 4
LK, B	Live	47 ± 5
LK, A	Live	39 ± 4

[a] Irradiated RAW264.7 cells were treated for 24 h before infection (B) or after infection (A) with tissue culture medium alone (control) or medium that contained crude mouse lymphokines (LK). Lymphokines collected from BALB/c mouse spleen cell cultures stimulated with concanavalin A or the antigen *C. parvum* were used at a concentration of 20%; lymphokines collected from concanavalin A-stimulated cultures of spleen cells obtained from *L. monocytogenes*-challenged C57BL/6J mice were used at a concentration of 0.7%.

[b] Each value represents the mean ± the standard error for three or more experiments.

In the experiments described in Table 5, the effect of lymphokines on rickettsial growth was examined in cultures of irradiated RAW264.7 cells treated with lymphokines before infection only, after infection only, or both before and after infection. In these experiments, which were complicated by the cytotoxicity described above, only cells which appeared to be intact microscopically were examined for rickettsiae. As expected, in control cultures of RAW264.7 cells, *R. prowazekii* Breinl grew well: %R increased slightly and RI increased approximately 18-fold during the 48-h incubation period. When RAW264.7 cells were treated with lymphokines, however, %R dropped dramatically during the 48-h incubation period and RI underwent little change. Thus, little or no rickettsial growth occurred in the lymphokine-treated cells that survived the cytotoxic reaction.

Effects and relative activities of various lymphokine preparations. Lymphokines collected from concanavalin A-stimulated cultures of spleen cells obtained from *L. monocytogenes*-challenged C57BL/6J mice had the same effects (cytotoxicity, inhibition of rickettsial growth, and inhibition of the initial rickettsial infection) as the other lymphokine preparations, but they induced these effects at much lower concentrations (data not shown). In contrast to the lymphokine preparations collected from stimulated cultures, supernatant fluids collected from unstimulated cultures of spleen cells obtained from normal C57BL/6J mice did not inhibit the initial rickettsial infection. Such fluids had cytotoxic activity at a 1/6 dilution but not at higher dilutions and caused only slight (~20%) inhibition of rickettsial growth at a 1/6 dilution, whereas a preparation collected from concanavalin A-stimulated cultures of spleen cells obtained from normal C57BL/6J mice had cytotoxic activity and markedly inhibited rickettsial growth at a dilution as high as 1/162. Addition of concanavalin A to an unstimulated culture fluid enhanced neither its cytotoxic activity nor its ability to inhibit rickettsial growth and did not render it able to inhibit the initial rickettsial infection (data not shown).

Presence of cytotoxic activity, antirickettsial activity, and IFN in supernatant fluids collected from murine T lymphocyte

TABLE 4. Cytotoxicity of various numbers of *R. prowazekii* for nonirradiated RAW264.7 cells treated with lymphokines before infection[a]

Treatment of cells	Rickettsial infection at 0 h		Cytotoxicity[b] at 5 h after infection
	%R	RI	
Control	0		4 ± 1
LK	0		12 ± 1
Control	49 ± 6	2.5 ± 0.4	9 ± 4
	72 ± 3	4.0 ± 0.8	10 ± 4
	82 ± 4	6.3 ± 2.1	14 ± 6
LK	52 ± 6	2.8 ± 0.4	55 ± 1
	67 ± 1	4.8 ± 1.1	75 ± 3
	73 ± 1	7.6 ± 1.8	89 ± 2

[a] Nonirradiated RAW264.7 cells were incubated for 24 h before infection in tissue culture medium alone (control) or in medium that contained 14 to 20% crude mouse lymphokines (LK) collected from BALB/c mouse spleen cell cultures stimulated with concanavalin A. Cell suspensions were prepared and incubated in polypropylene tubes with various dilutions of *R. prowazekii* E for 1 h at 34°C. After washing, samples of each cell suspension were cytocentrifuged onto microscope slides, fixed, and strained. The remaining cells were incubated at 34°C for 5 h and were then stained with trypan blue.

[b] Expressed as percent trypan blue-positive cells. Each value represents the mean ± the standard error for two experiments.

clones. Supernatant fluids collected from five Lyt-1$^+$,2$^-$ murine T lymphocyte clones cultured with concanavalin A or the antigen bovine serum albumin could substitute for crude mouse lymphokines in inducing cytotoxicity in RAW264.7 cells that were also infected with *R. prowazekii* (Table 6). These supernatant fluids also contained IFN and inhibited rickettsial growth in mouse L929 cells (29).

Comparison of the effects of mouse lymphokines and cloned mouse IFN-γ on the interaction of *R. prowazekii* Breinl with RAW264.7 cells. We examined the cytotoxic activity, inhibition of the initial rickettsial infection, and inhibition of rickettsial growth in RAW264.7 cells treated before infection with various concentrations of IFN-γ as crude mouse lymphokines or cloned mouse IFN-γ produced by *E. coli* (Fig. 1). The curve for each parameter in mouse lymphokine-treated cells was strikingly similar to the curve for the same parameter in cloned mouse IFN-γ-treated cells. Thus, the effects of crude mouse lymphokines on RAW264.7 cells infected with *R. prowazekii* might be accounted for by the IFN-γ that is present in these preparations. Both cytotoxicity and inhibition of rickettsial growth were observed in cells treated with IFN-γ at 0.8 IU/0.3 ml as crude lymphokines or cloned IFN-γ, and these two parameters reached a maximum in cells treated with IFN-γ at approximately 3 IU/0.3 ml. As the IFN-γ concentration was increased further, the percent inhibition of rickettsial growth remained at its maximal value (~88%). However, at very high concentrations of IFN-γ, cytotoxicity declined somewhat. Marked inhibition of the initial rickettsial infection was observed only at IFN-γ concentrations of ≥12 IU/0.3 ml, and maximal inhibition occurred at an IFN-γ concentration of approximately 200 IU/0.3 ml.

Experiments with cloned IFN-γ produced by Chinese hamster ovary cells showed that cytotoxicity occurred in RAW264.7 cells treated with cloned IFN-γ after rickettsial infection only, as well as in RAW264.7 cells treated with cloned IFN-γ before infection only. Cytotoxicity was not observed in cells treated with control medium for this IFN-γ and infected with *R. prowazekii* (data not shown).

DISCUSSION

Most of the antirickettsial activity observed in mouse lymphokine-treated mouse fibroblasts can be accounted for by the IFN-γ that is present in the crude lymphokine preparations (22, 23). Although macrophage-reactive factors other than IFN-γ may be present in such preparations, the effects of crude lymphokines that were observed in treated RAW264.7 cells in this study also can be explained in the simplest model by the IFN-γ that is present in the lymphokine preparations.

Treatment of mouse macrophage-like RAW264.7 cells with crude lymphokines, supernatant fluids from stimulated T lymphocyte clones, or cloned IFN-γ made the cells unable to support the growth of *R. prowazekii*. After treatment with high concentrations of IFN-γ, infection of RAW264.7 cells with *R. prowazekii* was suppressed. Treatment with a wide range of IFN-γ concentrations and infection with *R. prowazekii* killed a substantial proportion of the RAW264.7 cells and inhibited rickettsial growth in the surviving cells.

Inhibition of rickettsial growth also occurred in lymphokine- or IFN-γ-treated fibroblastic L929 cells (22, 24), but cytotoxicity did not. Cytotoxicity was observed in another mouse macrophage-like cell line, J774.1, after lymphokine treatment and infection with *R. prowazekii* (data not shown); hence, cytotoxicity may be dependent on some function that is present only in macrophage-like cells.

It is also possible that cytotoxicity occurs only in lymphokine-treated macrophages or macrophage-like cells that are infected with this *Rickettsia* species. In studies conducted by others with *R. tsutsugamushi*, lymphokine treatment induced rickettsicidal activity in cultures of resident mouse peritoneal macrophages, but killing of lymphokine-treated, infected macrophages was not reported (13, 14). This may mean that there was no cytotoxicity or that it was not investigated.

Wisseman and Waddell (30) recently showed that supernatant fluids collected from antigen- or mitogen-stimulated

TABLE 5. Effect of lymphokine treatment of RAW264.7 cells on the growth of *R. prowazekii* Breinl within the cells[a]

Treatment of cells	Time (h) after infection	%R	RI
Control	0	85 ± 3	3.5 ± 0.4
	24	81 ± 4	16.3 ± 1.4
	48	94 ± 2	61.7 ± 4.0
LK, B	0	59 ± 13	2.1 ± 0.4
	24	22 ± 8	2.2 ± 0.5
	48	9 ± 3	2.8 ± 0.9
LK, A	0	81 ± 3	3.2 ± 0.3
	24	34 ± 6	3.7 ± 0.6
	48	16 ± 3	8.2 ± 2.5
LK, BA	0	63 ± 6	2.1 ± 0.3
	24	17 ± 4	2.6 ± 0.8
	48	20 ± 13	2.5 ± 1.1

[a] Irradiated RAW264.7 cells were treated with lymphokines (LK) for 24 h before infection (B), for 24 h before infection and after infection (BA), or after infection (A). Lymphokines were collected from BALB/c mouse spleen cell cultures stimulated with concanavalin A, the antigen *C. parvum*, or the antigen *R. prowazekii* and were used at a concentration of 10 or 20%. At 24 and 48 h after infection, only cells that appeared to be intact microscopically were examined for rickettsiae. Each value for %R and RI represents the mean ± the standard error for three or more experiments.

cultures of human leukocytes inhibited the growth of *R. prowazekii* Breinl in cultured human macrophages, endothelial cells, and fibroblasts. These supernatant fluids also had a cytolytic action on rickettsia-infected cells but not on uninfected cells. This cytolytic action, in contrast to the cytotoxicity we have described, occurred in fibroblasts. In addition, it was not observed when the fibroblasts were treated with supernatant fluids and then washed and suspended using trypsin-EDTA before rickettsial infection (30). No data were given about the possible cytolytic action of the supernatant fluids on rickettsia-infected macrophages and endothelial cells (30).

In our experiments, viable rickettsiae were required for cytotoxicity, and either the virulent strain Breinl or the avirulent strain E of *R. prowazekii* was effective. The effectiveness of both strains is interesting because in untreated cultures of RAW264.7 cells, strain Breinl grows well but strain E does not (21). Infection of cells with small numbers of rickettsiae was sufficient to cause cytotoxicity in lymphokine-treated cells; however, the percentage of cells killed increased when cells were infected with larger numbers of rickettsiae. The decreased percentages of cells killed in cultures treated with very high concentrations of IFN-γ and then infected with *R. prowazekii* may have been related to the marked inhibition of the initial rickettsial infection that was observed in these cultures.

Many studies have dealt with the inhibitory effect of interferons on the multiplication of cultured cells, and in some reports interferon treatment has been associated with cell death (3, 6, 25). It may be that infection with *R. prowazekii* makes RAW264.7 cells more sensitive to the effects of IFN-γ. On the other hand, treatment of RAW264.7 cells with IFN-γ may make the cells more sensitive to *R. prowazekii*. A phospholipase A activity is involved in the interaction of *R. prowazekii* and host cells (28) and may be responsible for the cytotoxicity observed in the present study.

TABLE 6. Presence of cytotoxic activity in supernatant fluids collected from mitogen- or antigen-stimulated murine T lymphocyte clones[a]

T lymphocyte clone	Inducer	% Trypan blue-positive cells[b] at 4–6 h after infection or mock infection at the indicated supernatant fluid dilution		
		1/10, infected	1/160, infected	1/10, mock infected
A6	ConA	73	23	4
	BSA	46	31	3
B10	ConA	46	61	5
	BSA	44	13	4
D8	ConA	33	7	3
	BSA	40	27	2
H1	ConA	45	33	1
	BSA	46	44	3
H8	ConA	37	17	5
	BSA	51	42	3
Control	ConA	6	ND[c]	4
	BSA	5	ND	3

[a] Supernatant fluids were collected from murine T lymphocyte clones cultured in the presence of concanavalin A (ConA) or the antigen bovine serum albumin (BSA). Irradiated RAW264.7 cells were treated with dilutions of each supernatant fluid for 24 h before rickettsial or mock infection.

[b] Each value represents the average of two determinations.

[c] ND, Not determined.

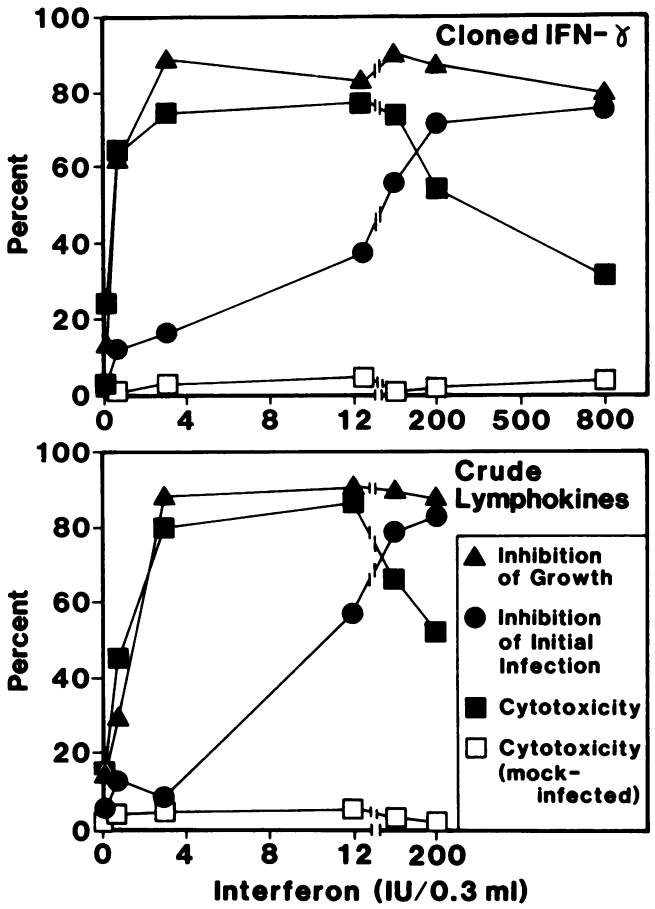

FIG. 1. Cytotoxicity, inhibition of the initial rickettsial infection, and inhibition of rickettsial growth in RAW264.7 cells treated with various concentrations of cloned mouse IFN-γ or crude mouse lymphokines. Irradiated RAW264.7 cells were treated for 24 h with various concentrations of IFN-γ as cloned mouse IFN-γ (produced by *E. coli*) or crude mouse lymphokines (produced by concanavalin A-stimulated spleen cells from *L. monocytogenes*-challenged mice). The cells were then infected with *R. prowazekii* Breinl. Percent inhibition of the initial rickettsial infection was calculated from the NRs at 0 h after infection. Cytotoxicity (percent trypan blue-positive cells) was assessed 4 to 6 h after rickettsial infection or mock infection. Percent inhibition of rickettsial growth was calculated from the NRs at 24 h after infection (expressed as percentages of the NRs at 0 h). Each value for cytotoxicity and inhibition of the initial rickettsial infection represents the mean of three experiments. Each value for inhibition of rickettsial growth represents the mean of two experiments. In untreated control cultures of RAW264.7 cells, %R at 0 h was 77 ± 7 (mean ± standard error) and RI at 0 h was 3.4 ± 0.4. NR increased 8.3- ± 2.4-fold in untreated control cultures during the 24-h period after infection.

The participation of IFN-γ in the activation of macrophages for killing of tumor cells, *Toxoplasma gondii*, and *Leishmania donovani* has been demonstrated (10, 12, 15–17). Our study shows that IFN-γ also alters the interaction of macrophage-like cells with *R. prowazekii*. Occurrence of cytotoxicity and inhibition of the initial rickettsial infection may reflect IFN-γ-induced alterations in the RAW264.7 cell membrane. Treatment of macrophages or macrophage-like

cells with lymphokines or IFN-γ results in cell surface changes (7, 9, 11, 18, 19, 32), and treatment of cells with IFN preparations can alter the cytoskeleton and the rate of endocytosis (2, 4, 20, 27). Such changes might perturb the process by which *R. prowazekii* enters macrophages.

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