Toxoplasma gondii-Infected Cells Are Resistant to Multiple Inducers of Apoptosis

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Infection with certain intracellular pathogens, including viruses and bacteria, may induce host cell apoptosis. On the other hand, infection with some viruses inhibits apoptosis. Complex protozoan parasites, including Toxoplasma gondii and members of Plasmodium, Leishmania, and Microsporidia, are also obligate intracellular pathogens, yet relatively little is known regarding their subversion of host cell functions. We now report that cells infected with T. gondii are resistant to multiple inducers of apoptosis, including Fas-dependent and Fas-independent CTL-mediated cytotoxicity, IL-2 deprivation, gamma irradiation, UV irradiation, and the calcium ionophore beauvericin. Inhibition of such a broad array of apoptosis inducers suggests that a mechanism common to many, or perhaps all, apoptotic pathways is involved. The inhibitory activity requires live intracellular parasite and ongoing protein synthesis. Despite T. gondii-mediated inhibition of DNA fragmentation, infected cells can still be lysed by CTL. The Journal of Immunology, 1998, 160: 1824–1830.

Toxoplasma gondii is a protozoan, obligate intracellular parasite of the order Coccidia, for which felines are the definitive host (1). Human infection with T. gondii is an important cause of morbidity and mortality. In hosts with intact immunity, infection is usually benign, owing to a rapid, effective immune response that forces encystation of the tachyzoites (2). However, persons with defective cell-mediated immunity, including recipients of organ allografts (3), persons undergoing cytotoxic chemotherapy (4), neonates with perinatally acquired disease (5), and persons infected with HIV (6–11), are susceptible to severe, potentially fatal infection. Viable organisms may remain encysted and persons infected with HIV (6–11), are susceptible to severe, chemotherapy (4), neonates with perinatally acquired disease (5), and persons undergoing cytotoxic chemotherapy (4), neonates with perinatally acquired disease (5), and persons infected with HIV (6–11), are susceptible to severe, potentially fatal infection. Viable organisms may remain encysted within the host for extended periods of time, possibly for life (12). Encysted organisms may serve as reservoirs for recrudescence disease in the setting of compromised cellular immunity.

It has long been appreciated that viruses subvert normal host cell functions to replicate or establish latency, and infection with certain intracellular pathogens, including viruses and bacteria, may induce host cell apoptosis (13–20). Despite the medical importance of protozoans such as T. gondii, little is known regarding how these intracellular parasites subvert host cell processes to their own advantage (21). In this regard, T. gondii infects all nucleated cells, yet causes no obvious disturbance until the dividing parasites rupture the host cell (22). This is surprising in that the stress of infection could induce apoptotic cell death of the host cell (23). We wondered whether infected cells appeared unperturbed before rupture due to inhibition of apoptosis as has been reported for certain viral infections (24–29). The following studies were performed to investigate this hypothesis.

Materials and Methods

T. gondii and related cell lines

Tachyzoites of T. gondii were maintained in human foreskin fibroblasts (CRL 1634, American Type Culture Collection, Rockville, MD) as previously described (22). The T. gondii strain RH (from Lloyd Pfeffercorn) is a well-characterized laboratory isolate that has been used by us previously (22, 30).

Other cell lines

Murine MLR T lymphoblasts were derived by culturing 5 × 10^6 spleen cells from C57Bl/6 (B6) or B6smn.C, H-2^d (B6-gld) mice (The Jackson Laboratory, Bar Harbor, ME; both H-2^d) with 1 × 10^6 irradiated spleen cells from BALB/c mice (H-2^d) in individual wells of a 24-well plate in medium R10, which consists of RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% heat-inactivated FBS (HyClone, Logan, UT), 4 nM glutamine (Sigma Chemical Co., St. Louis, MO), MEM nonessential amino acids (Sigma), MEM vitamins (Sigma), 1 mM pyruvate (Sigma), 50 nM 2-ME (Sigma), and penicillin/streptomycin (Life Technologies). After 5 days of culture, fresh medium containing 10 U/ml recombinant human IL-2 (Hoffmann La Roche, Basel, Switzerland) was added. The resulting lymphoblasts were isolated by Lympholyte M density gradient (Cedarlane, Hornby, Canada) on day 7 and used within 3 days of separation.

The H-2^d-specific CD8-positive CTL line L3 was derived by Glasebrook and Fitch (31) from C57Bl/6 mouse spleen cells stimulated with DBA/2 spleen cells. L3 was propagated by periodic stimulation with irradiated BALB/c spleen cells. L1210-Fas, a lymphocytic cell derived from a DBA/2 mouse, which expresses H-2^d, was obtained from Pierre Golstein (Centre d’Immunologie INSERM-CNRS, Marseille, France). The Fas (CD95) gene has been transfected into this cell line and is constitutively expressed (32). A20 (American Type Culture Collection, TIB 208) is a B cell lymphoma cell line derived from a BALB/c mouse that expresses H-2^d. WEHI 231 (American Type Culture Collection, CRL 1702) is a pre-B leukemia cell that can be induced to undergo apoptosis by gamma irradiation (33). CTL-2 (American Type Culture Collection, TIB 214) is a T cell line that requires IL-2 for survival and undergoes apoptosis when IL-2 is removed (34). The mastocytoma cell line P815 (American Type Culture Collection, TIB-64) is a DBA/2 (H-2^d)-derived tumor obtained from the American Type Culture Collection. Cells were all maintained in R10 at 37°C and 5% CO2 in a humidified environment. Recombinant human IL-2 (10 U/ml) was added to cultures of normal T cells, L3, and CTL-2 cells for routine growth.
T. gondii infection

Tachyzoites were recovered from infected fibroblasts by forced passage through a 27-gauge needle. Recovered tachyzoites and cells were then incubated together in medium R10 overnight. The multiplicity of infection (moi)\(^1\) ranged from 1 to 10, as indicated.

The number of infected cells was determined by UV microscopy of samples stained with acridine orange. Ten microliters of cell suspension was placed on a slide, and a coverslip was carefully placed on top. After the preparation had dried, 100 cells were examined for the presence of Toxoplasma.

**Determination of apoptotic morphology**

Cell nuclei were examined for apoptotic morphology following the method of Duke and Cohen (35). Briefly, cells were suspended in a saline solution containing 20 \(\mu\)g/ml of the nucleic acid-binding dyes acridine orange (Sigma) and ethidium bromide (Sigma) and examined by fluorescence microscopy. The differential uptake of these dyes by cells allows the identification of viable and nonviable cells (36). Acridine orange enters into live and dead cells and stains the chromatin green. Ethidium bromide, in contrast, enters only into cells that have lost membrane integrity, staining the chromatin red. Apoptotic vs necrotic cell death may also be quantified using this combination of dyes. In normal cells or cells that have died by necrosis, the euchromatin and heterochromatin are visualized as heterogeneous nuclear structures. In vivid contrast, apoptotic nuclei show condensed chromatin that appears as bright-staining, featureless regions, often resulting in distinct beads. One hundred host cell nuclei per condition were counted, and the percentage of nuclei with apoptotic morphology was calculated.

**DNA ladder gel electrophoresis**

DNA in apoptotic cells is no longer intact and appears as a ladder of DNA bands at an interval of about 200 bp on electrophoretic gels. Uninfected A20 cells, A20 cells infected with *T. gondii* at a moi of 10, and freshly obtained *T. gondii* tachyzoites were incubated overnight at 37°C. Half of each culture was then treated with 20 \(\mu\)M beauvericin (Sigma) for 1 h. The cells were harvested by centrifugation and resuspended in 15 \(\mu\)l of a solution containing 2 vol of sample buffer (glycerol containing 0.01 M Tris-HCl, pH 8.0, and 7.5% bromophenol blue) and 1 vol of 10 mg/ml RNase A. The samples were loaded onto a 2% agarose gel from which the section above the wells had been replaced with 1% agarose, 2% SDS, and 53 \(\mu\)g/ml proteinase K. After electrophoresis, the gel was stained with ethidium bromide and visualized with UV light.

**JAM assay**

As first described by Matzinger, the JAM assay is a quantitative measure of DNA fragmentation (37). In brief, cells were labeled overnight with tritiated thymidine (Amersham, Arlington Heights, IL). The next day the cells were washed to remove the unincorporated radiolabel and were infected with *T. gondii*. Experiments were performed following overnight infection. After experimental treatment and incubations were complete, the cells were harvested onto a unifilter plate using a 96-well harvester (Packard, Meridian, CT), and the resulting counts were determined on a beta counter (Packard). When DNA was fragmented, the small pieces passed through the filter, resulting in lower counts. The percent DNA fragmentation was calculated using the formula: (spontaneous counts − experimental counts)/spontaneous counts \(\times 100\).

**Lysis assay**

Lysis of target cells by CTL was measured using a method similar to the JAM assay, as proposed by Eric Martz (38). A20 target cells were prepared in the same manner as that described for the JAM assay by labeling overnight with tritiated thymidine at between 0.5 and 2 \(\mu\)Ci/ml in tissue culture medium. After overnight culture, the cells were washed once. Further washing was not necessary because unincorporated thymidine was not retained on the harvesting filter for any of the conditions tested. The target cells were diluted to 5 \(\times\) 10\(^5\) cells/ml in tissue culture medium. H-2\(^d\) allospecific CTL L3 effector cells were washed and diluted to 2.5 \(\times\) 10\(^5\) cells/ml. One hundred microliters of target cells and 100 \(\mu\)l of effector cells were combined in 96-well microtiter plates with 50 \(\mu\)l of 125 \(\mu\)g/ml DNase I (Sigma). Target cells with medium alone were used as negative controls. The plates were allowed to settle to the bottom of the plate for 1 to 2 h before irradiation, to provide even exposure. Control cells were protected by aluminum foil.

**IL-2 withdrawal**

CTLL-2 were washed three times to remove IL-2 and resuspended in fresh R10 without IL-2. *T. gondii* was added at an moi of 1, 5, or 10 at the end of the three washes. The cells were then incubated overnight, and apoptosis was determined by nuclear morphology.

**Beauvericin treatment**

Beauvericin stock was prepared by dissolving beauvericin in absolute ethanol at 2 mM. Cells were treated by adding cell suspensions to beauvericin solution at 10 or 20 \(\mu\)M (39). The cells were then incubated for 1 h and assayed for apoptosis by nuclear morphology.

**Pyrimethamine treatment**

Pyrimethamine stock was made by dissolving 5 mg/ml of pyrimethamine (Sigma) in DMSO and was used at a 5 \(\mu\)g/ml final concentration. Overnight treatment with 5 \(\mu\)g/ml pyrimethamine kills *T. gondii* under these conditions (our unpublished observation).

**Cycloheximide and actinomycin D treatment**

Cycloheximide (Sigma) stock was prepared by dissolving 250 \(\mu\)g/ml in ethanol. A final concentration of 2.5 \(\mu\)g/ml was used in the assays. Actinomycin D (Sigma) stock was made by dissolving 500 \(\mu\)g/ml in water. A final concentration of 500 ng/ml was used.

**Statistical analysis**

Comparisons were performed using unpaired Student’s \(t\) test, assuming unequal variances. The \(p\) values reported are for the greatest differences observed in the dose-response analyses, considering a two-tailed value of \(p < 0.05\) significant.

**Results**

**Inhibition of CTL-induced apoptosis**

CTL are major effector cells for killing cells infected with viruses and other intracellular pathogens. CTL induce apoptosis in their targets by two distinct mechanisms (32). The first mechanism uses the pore-forming protein perforin and a group of proteases collectively referred to as granymes. The second mechanism is mediated by Fas ligand expressed on the CTL and Fas on the target. To investigate whether *T. gondii* infection might inhibit CTL-mediated apoptosis, we used established experimental conditions in

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\(^1\) Abbreviations used in this paper: moi, multiplicity of infection; hsp, heat shock protein.
which both or only one mechanism of killing could function (32, 40, 41). In the experiment depicted in Figure 1, alloreactive (H-2b anti-H-2b) MLR lymphoblasts were used as effector cells, and H-2d-bearing L1210-Fas tumor cells were used as target cells. To examine both mechanisms simultaneously, MLR lymphoblasts derived from B6 mice were employed (Fig. 1a). These lymphoblasts rapidly induced high levels of apoptosis in A20 tumor cells, which were significantly inhibited by T. gondii infection of the target cells (p < 0.001). However, it could not be determined whether Fas-dependent or independent cytotoxic pathways were inhibited using this experimental protocol.

To investigate killing in the absence of Fas ligation, MLR lymphoblasts derived from B6-gld, which are unable to express functional Fas ligand, were employed (Fig. 1b). T cells from these mice kill only by perforin/granzyme-mediated mechanisms; the Fas-mediated mechanism is not used (41). Apoptosis was induced in uninfected A20 cells by these effector cells, but was significantly inhibited by infection with T. gondii (p < 0.001).

The ability of T. gondii to inhibit Fas-dependent apoptosis mediated by CTL was examined using a modification of a protocol first described by Rouvier and colleagues (32). In brief, B6 MLR lymphoblasts were preincubated for 1 h with anti-CD3 Ab to induce Fas ligand expression, and EGTA was then added before incubation with target cells to prevent granule exocytosis (40). As in the case of perforin/granzyme-mediated cytotoxicity, Fas-dependent induction of DNA fragmentation by CTL was inhibited by T. gondii infection of target cells (Fig. 1c; p < 0.0007).

### CTL-mediated lysis in the presence of T. gondii infection

The abilities of T. gondii to inhibit DNA fragmentation and lysis were also compared (Table I). The allospecific cytotoxic T cell clone L3, which is able to use both granules and Fas ligand, was used to test for cytotoxicity against A20 tumor cells. Significant inhibition of CTL-induced DNA fragmentation by T. gondii infection was seen, but with no significant concomitant inhibition of target cell lysis.

The magnitude of inhibition of CTL-mediated apoptosis by T. gondii was directly related to the moi, demonstrating a dose-response effect. Because killing by both CTL cytotoxic pathways was affected, we speculated that T. gondii must inhibit an apoptotic induction pathway downstream of where the two mechanisms converge, suggesting that apoptosis induced by other means would also be inhibited. We therefore assessed the effect of T. gondii infection on four additional inducers of apoptosis: gamma irradiation, UV irradiation, growth factor (IL-2) withdrawal, and the fungal toxin beauvericin.

### Inhibition of gamma irradiation-induced apoptosis

To assess protection against radiation-induced apoptosis, we irradiated WEHI 231 cells, which are sensitive to gamma irradiation and undergo apoptosis when exposed to it (33). At 24 and 48 h following irradiation, the percentage of apoptotic cells in uninfected WEHI-231 was always higher than that in the infected population (Table II).

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**Table I. T. gondii infection results in decreased DNA fragmentation but does not alter susceptibility to CTL-mediated lysis**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% DNA Fragmentation</th>
<th>% Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>55 ± 11</td>
<td>59 ± 15</td>
</tr>
<tr>
<td>Infected</td>
<td>11 ± 23</td>
<td>51 ± 15</td>
</tr>
</tbody>
</table>

* A20 target cells were infected with T. gondii overnight at a moi of 10. L3 effector CTL were used at an E:T of 5:1 for 4 h. DNA fragmentation was measured by the JAM assay protocol and lysis was measured by DNase accessibility. Spontaneous counts for this experiment in the JAM assay portion averaged 130 cpm for uninfected cells and 200 cpm for infected cells; and 67 or 88 cpm for uninfected or infected cells, respectively. The experiment is representative of four experiments.

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**Table II. Inhibition of radiation-induced apoptosis following T. gondii infection**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Apoptotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated</td>
<td>Infected 24 h</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* WEHI 231 cells were irradiated with 1000 R. Data represent the percentage of cells with apoptotic nuclear morphology determined by visual microscopic examination (35). Results of the same cell population 24 and 48 h after irradiation are shown.

−, No treatment; +, treatment was performed. A moi of 10 was used for these experiments. At each time point, 100 cells were counted for each condition. These data are representative of three experiments.
Inhibition of UV irradiation-induced apoptosis

We also tested the effect of T. gondii infection on UV irradiation-induced apoptosis. In this assay, P815 mastocytoma cells infected overnight with T. gondii were protected from DNA fragmentation as measured by JAM assay. The fragmentation resulting from UV irradiation was inhibited in a dose-dependent manner by T. gondii infection (Fig. 2).

Inhibition of growth factor withdrawal-induced apoptosis

We next tested the effect of T. gondii infection on apoptosis resulting from IL-2 withdrawal using the IL-2-dependent cell line CTLL-2 (34). At 24 h after removal of IL-2, uninfected CTLL-2 were mostly apoptotic (Fig. 3). Infection with T. gondii protected against apoptosis, and as observed with CTL-mediated apoptosis, greater inhibition was observed at higher moi.

Inhibition of beauvericin-induced apoptosis

Finally, we examined the effect of T. gondii infection on induction of apoptosis by beauvericin (39). Beauvericin is a calcium ionophore that activates the apoptotic process in many cell types in as little as 5 min after addition, with nearly 100% of cells having apoptotic nuclei by 1 h (our unpublished data). Following overnight infection with T. gondii, cells were incubated for 1 h with beauvericin. Two doses of beauvericin and three moi were tested (Fig. 4). Increasing proportions of cells were protected at higher moi for both doses of beauvericin (p < 0.03). Similar results were obtained using A20 or WEHI-231 cells (data not shown).

Evidence that T. gondii infection protected cells against DNA fragmentation induced by beauvericin is also shown by gel electrophoresis (Fig. 5) and by JAM assay (Table III and data not shown).

FIGURE 2. T. gondii infection inhibits apoptosis induced by UV irradiation. P815 tumor cells were irradiated for 15 (filled circles), 30 (filled triangles), or 45 (open squares) min on a UV transilluminator. Target cells were labeled with 2 μCi/ml tritiated thymidine overnight, then washed and infected with T. gondii overnight before the assay. Data are the percent DNA fragmentation, with error bars representing the SDs of triplicate samples. Spontaneous counts of 1303, 1318, 1031, and 871 cpm for target cells infected with T. gondii at ratios of 0, 1, 5, and 10, respectively, were obtained. These data are representative of three experiments.

FIGURE 3. T. gondii infection inhibits apoptosis following IL-2 deprivation of CTLL-2 cells. The percentage of cells with apoptotic nuclear morphology 24 h after IL-2 withdrawal (circles) or in the continued presence of IL-2 (squares) is shown for uninfected cells and for cells infected with T. gondii at the indicated moi. Error bars represent the SDs of triplicate samples, with 100 nuclei per condition counted. Seventy-nine percent of CTLL-2 were infected. These data are representative of eight experiments.

FIGURE 4. Inhibition of beauvericin-induced apoptosis by T. gondii infection, as determined by apoptotic nuclear morphology. L1210-Fas cells were treated with no (squares), 10 μM (circles), or 20 μM (triangles) beauvericin for 1 h. One hundred cells per condition were counted. Fifty-six percent of cells were infected. Error bars represent the SDs of triplicate samples. These data are representative of three experiments.

FIGURE 5. Inhibition of DNA fragmentation following beauvericin-induced apoptosis in cells infected with T. gondii. T. gondii strain RH (TgRH)-infected and uninfected A20 cells and TgRH tachyzoites were either untreated or treated with 20 μM beauvericin for 1 h. The numbers of cells (× 10⁶) and tachyzoites (× 10⁷) are shown. DNA from these cells was then examined following electrophoresis through a 2% agarose gel.
Table III. Treatment with anti-T. gondii agents reduces protection against apoptosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Apoptotic</th>
<th>% DNA Fragmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Infected + pyrimethamine</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

Experiment 1: IL-2 Withdrawal

Uninfected 76
Infected 26
2-h pyrimethamine 47
Overnight pyrimethamine 60

Experiment 2: Beauvericin

Uninfected 73
Infected 18
Infected + ciprofloxacin 59

Experiment 3: Beauvericin

Uninfected 100
Infected 67
Infected + ciprofloxacin 59

Experiment 4: Beauvericin

Uninfected 100
Infected 67
Infected + ciprofloxacin 59

*A20 cells were infected with T. gondii overnight. Ciprofloxacin (2.5 μg/ml) or actinomycin D (500 μg/ml) was added 5 h prior to 1-h beauvericin treatment (20 μM). Data indicate the percent of cells with apoptotic nuclei as determined by UV microscopy. For each condition, 100 cells were counted. These data are representative of three experiments.

Table IV. Treatment with cycloheximide or actinomycin D reduces protection against beauvericin-induced apoptosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Apoptotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Infected</td>
<td>67 ± 4</td>
</tr>
<tr>
<td>Cycloheximide (5 h)</td>
<td>91 ± 3</td>
</tr>
<tr>
<td>Actinomycin D (5 h)</td>
<td>92 ± 7</td>
</tr>
</tbody>
</table>

A variety of viruses have been shown to encode proteins capable of inhibiting apoptosis. The majority of these proteins prevent apoptosis triggered by specific inducers working in a limited range of induction pathways. Only two viruses are known to be broadly active in inhibiting apoptosis: the cowpox virus, which encodes the crmA gene product, and baculovirus, which encodes the p35 protein. Both the crmA gene product and the baculovirus p35 protein act by blocking protease activity. Cells expressing crmA are rendered resistant to Fas or TNF receptor ligation (28, 43–46). Baculovirus p35 protein blocks Fas and TNF receptor activities, nerve growth factor withdrawal, and expression of the Drosophila reaper gene (24, 47–50).

Similar to cells overexpressing p35 or crmA, cells infected with T. gondii are resistant to multiple inducers of apoptosis. However, neither of these two viral gene products is known to inhibit apoptosis as broadly as a range of inducers as that in the case of T. gondii we now report. The mechanism by which T. gondii infection protects cells from induction of apoptosis has not yet been elucidated. However, according to current models of apoptotic induction, T. gondii probably blocks either the activation or the function of the three proteases Cpp-32 (51–53), ICE-LAP3 (54, 55), and Mch-2 (56). Our observation that T. gondii infection blocks apoptosis induced by granzyme B (CTL), which probably activates all three of these proteases directly (57), supports this concept.

Although the mechanism by which T. gondii infection inhibits the induction of apoptosis is unknown, it is an active process, requiring the presence of live parasites, as confirmed by the time-dependent abrogation of apoptosis protection upon killing of intracellular parasites with antibiotics. Furthermore, inhibition of apoptosis was directly dependent on the moi of T. gondii in a dose-dependent fashion, and inhibition of protein synthesis or mRNA transcription also abrogated this protection.

Although T. gondii-infected cells were resistant to induction of CTL-mediated apoptosis, target cell lysis induced by CTL was only minimally reduced (Table I). We interpret the 59% DNase accessibility results in the uninfected cells as due to a combination of both host cell DNA fragmentation and lysis, but that in this case, the additional contribution to loss of the radiolabel by direct lysis is negligible, since target cells engaged by CTL will fragment DNA anyway. This is evident from the observation that the level of DNase accessibility and the level of DNA fragmentation are essentially the same. On the other hand, in the infected cells, since fragmentation (JAM assay) is inhibited to 11%, we interpret the 51% DNase accessibility results to be due primarily to host cell lysis, with only a small component due to DNA fragmentation.

The observations that T. gondii infection renders host cells resistant to CTL-mediated DNA fragmentation, while not protecting against host cell lysis are not contradictory. Perforin, which can
form lytic lesions independent of apoptotic induction, is probably responsible for the lysis of infected cells. It is thus possible that Fas-mediated bystander activity is inhibited by T. gondii infection, reducing early destruction of infected hemopoietic cells, thereby enhancing the spread of the parasite throughout the host. Lysis, in the absence of apoptosis, mediated by direct killing may also not be as detrimental to the parasite as the normal apoptotic effects. Alternatively, T. gondii may not specifically evade immune destruction through its ability to inhibit apoptosis, but, rather, may prolong the life of the host cell, which might otherwise undergo apoptosis due to the stress of infection.

One other intracellular parasite, Leishmania, has been shown to inhibit apoptosis in infected cells (21). The system used to examine the effect of infection by Leishmania was the induction of apoptosis in bone marrow macrophages deprived of TNF-α. When macrophages were infected with Leishmania, the cells were activated to produce their own supply of TNF-α, thus protecting themselves from apoptosis. Not only the infected cells but also the bystander cells were protected in this manner. This demonstration of protection contrasts with our description of T. gondii protection in that the Leishmania system involved an external source of rescue induced by the parasite, limiting the rescue to a single system, whereas T. gondii infection rescued cells from a variety of apoptotic inducers, implying inhibition of the internal apoptotic signaling pathway.

Himeno and Hiseada recently described the role of heat shock protein 65 (hsp65) in protection against T. gondii infection (58). They suggested that hsp65 expression protects T. gondii-infected macrophages from apoptosis. Their description of the induction of apoptosis and its inhibition are not yet published, so direct comparison of their work and ours is not yet possible. However, it is unlikely that we are observing an hsp65-mediated event, as hsp65 expression, according to Himeno and Hiseada, was not induced by the RH strain of T. gondii, which is what we used, and hsp65 expression was dependent of the presence of y8 T cells, which were not present in our system.

Several important observations regarding our experimental system must be emphasized. First, our experimental model of CTL-mediated target cell lysis involved CTL not specific for T. gondii. Nonetheless, this model is valid for assessment of the consequences of CTL-mediated target cell lysis or DNA fragmentation, as these phenomena are end results of common CTL killing mechanisms and are Ag independent. In this regard, another group recently used a similar strategy to assess the fate of intracellular Mycobacteria following CTL-mediated host cell destruction by using influenza-specific CTL and influenza Ag-expressing, Mycobacteria-infected target cells (59). Further, if we had used T. gondii-specific CTL, we would not have been able to compare apoptosis inhibition in infected and uninfected cells, as the latter would not express the appropriate Ags and thus would not be recognized and lysed by T. gondii-specific CTL. We reason, however, that as mechanisms of CTL killing are Ag independent, cells infected with T. gondii would be relatively more resistant to CTL-mediated apoptosis than if they were not infected. How this inhibition of apoptosis influences the course of acute or chronic T. gondii infection will require other strategies for study.

Second, the level of apoptosis inhibition varied substantially between experiments, as did the degree of T. gondii infection. Nonetheless, the general observations we describe were highly reproducible. Furthermore, the variability in T. gondii infection between experiments was used to underscore the role of active T. gondii infection in mediating apoptosis protection. Thus, in the four experiments shown here in which the extent of T. gondii infection was quantitated, we observed that from 33 to 79% of cells were infected, yielding apoptosis inhibition of from 33 to 84%, with the level of apoptosis inhibition being directly proportional to the level of infection. In experiments in which different moi were used, the level of inhibition of apoptosis varied in direct relation to the number of parasites.

In summary, these experiments demonstrate that T. gondii infection inhibits apoptosis induced in numerous cell types by six different agents: CTL (Fas-dependent or independent), gamma irradiation, UV irradiation, growth factor deprivation, and a toxin. Inhibition of apoptosis at a common point in the apoptotic pathway is thus likely. Elucidation of the mechanism(s) and gene(s) involved probably will enhance our understanding of the apoptotic process. The ability to extend the life of infected host cells would be a substantial advantage to T. gondii or other obligate intracellular pathogens for enhancing their own survival and may be crucial for allowing T. gondii to survive long enough to produce cysts and provide for the spread of the organism. The consequences of apoptosis inhibition for the immunopathogenesis of T. gondii infection remain to be defined.

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References


