Toxoplasma gondii: Fusion Competence of Parasitophorous Vacuoles in Fc Receptor–Transfected Fibroblasts

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After actively entering its host cells, the protozoan parasite *Toxoplasma gondii* resides in an intracellular vacuole that is completely unable to fuse with other endocytic or biosynthetic organelles. The fusion blocking requires entry of viable organisms but is irreversible: fusion competence of the vacuole is not restored if the parasite is killed after entry. The fusion block can be overcome, however, by altering the parasite's route of entry. Thus, phagocytosis of viable antibody-coated *T. gondii* by Chinese hamster ovary cells transfected with macrophage-lymphocyte Fc receptors results in the formation of vacuoles that are capable of both fusion and acidification. Phagocytosis and fusion appear to involve a domain of the Fc receptor cytoplasmic tail distinct from that required for localization at clathrin-coated pits. These results suggest that the mechanism of fusion inhibition is likely to reflect a modification of the vacuole membrane at the time of its formation, as opposed to the secretion of a soluble inhibitor by the parasite.

Toxoplasma gondii IS A PROTOZOAN PARASITE THAT IS PARTICularly adept at productively infecting mammalian cells. Although susceptible to oxidative killing (*1*), *T. gondii* efficiently enters macrophages without triggering the production of hydrogen peroxide (H_2O_2) or other toxic oxygen metabolites (*2, 3*). Moreover, after entering phagocytic and nonphagocytic cells, the parasite resides within a vacuole that fails to fuse with preexisting secondary lysosomes or to lower its internal pH (*4, 5*). Although the general features of *T. gondii* entry have been known for years, the mechanisms underlying these processes are not understood.

Tachyzoites of *T. gondii* attach to, invade, and replicate within all nucleated cells. During and after entry, tachyzoites discharge secretory organelles (*6*) (rhoptries and dense granules), enhancing cell entry (*7*) and resulting in the accumulation of one or more parasite-derived proteins in the newly formed parasitophorous vacuole space and, perhaps, in the parasitophorous vacuole membrane (*8*). Thus, the lack of fusion and acidification of vacuoles containing *T. gondii* vacuoles may occur either because the parasite produces an inhibitor of these processes, possibly by exocytosis, or because at the time of entry the parasite specifies the formation of a vacuole membrane that lacks signals needed for fusion with other intracellular compartments. Distinguishing between these two processes may not only suggest new therapeutic strategies for *T. gondii* infections, but also may provide new insights into the process of membrane recognition and fusion in general.

Absence of lysosomal membrane glycoproteins in vacuoles bearing live *T. gondii*. In previous studies of phagosome-lysosome fusion during *T. gondii* entry, light and electron microscopy were used to monitor the transfer of previously internalized endocytic tracers from lysosomes to the parasitophorous vacuole. These assays have been difficult to interpret and quantify, and may also systematically result in overestimation of the degree of fusion inhibition (*9*). We developed an approach with the use of endogenous markers to visualize directly the fusion of the lysosomal and vacuole membranes. The lysosomal membrane is enriched in a conserved family of heavily glycosylated membrane proteins designated lysosomal glycoproteins (lgp's) (*10*). Since lgp's are largely absent from the plasma membrane (*10, 11*), incoming phagocytic vacuoles would be expected to contain lgp's only after fusion with lysosomes.

To determine whether internalized *T. gondii* reside within vacuoles that were negative for lgp's, Chinese hamster ovary (CHO) cells were infected with viable tachyzoites (*12*), then analyzed at intervals by immunofluorescence with monoclonal and polyclonal antibodies to lgp120 or lgp96 or lgp58 (*10–13*). Only a small percentage (up to 16 percent in one experiment, but generally less than 5 percent) of vacuoles containing live parasites stained with antibody to lgp (anti-lgp) (Fig. 1, A and B, and Table 1). Vacuoles remained lgp-negative as parasites replicated intracellularly (Fig. 1, E and F); the low frequency of lgp staining was observed in cells infected for up to 38 hours, by which time individual vacuoles contained up to 40 tachyzoites. In contrast, vacuoles in CHO cells containing heat-killed tachyzoites were almost invariably lgp-positive even shortly after entry (Fig. 1, C and D, and Table 1). Similar results were obtained for internalization of tachyzoites by the J774 murine macrophage cell line. These results show that lgp staining permits a definitive assessment of fusion with lysosomes, and that only vacuoles containing live parasites fail to fuse with lgp-positive compartments.

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Complete fusion incompetence of vacuoles containing *T. gondii*. The fact that vacuoles failed to acquire lgp's even after long periods of infection (more than 24 hours) suggested that they were also unable to fuse with nonlysosomal organelles, such as late endosomes and Golgi-derived transport vesicles, that contain lower lgp concentrations (*10, 14*). Thus, it seemed likely that the parasite was able to effect a complete fusion block, preventing the vacuoles from fusing with any endocytic or biosynthetic organelle. To characterize the extent of the fusion block further, we next determined whether parasitophorous vacuoles were able to fuse with newly labeled endosomes. CHO cells were allowed to internalize various fluorescent tracers of fluid phase endocytosis [lucifer yellow (LY), fluorescein isothiocyanate dextran, Texas red ovalbumin], either before or after parasite entry. In cells infected with live tachyzoites and incubated for 10 minutes with LY (*15*), conditions that selectively label endosomes, very little tracer was delivered to parasitophorous vacuoles (Fig. 2, A and B, and Table 1). In contrast, most vacuoles containing dead parasites were LY-positive under the same conditions (Fig. 2, C and D). Similar results were obtained when infected cells were incubated for 30 or 60 minutes with LY, conditions sufficient to deliver LY to lysosomes (*15*). Thus, live *T. gondii* reside within vacuoles that are unable to fuse with either endosomes or lysosomes. As was suggested previously (*5*), the vacuoles were also unable to lower their internal pH, as indicated by their inability to accumulate the pH-sensitive fluorescent weak base acridine orange (*16*) (Table 1).

We next examined the question of whether the inability of newly formed vacuoles to fuse was irreversible or was dependent on the viability of the parasite after entry. If, for example, vacuole fusion was blocked as a result of the continuous secretion of one or more inhibitory substances, killing intracellular tachyzoites should result in eventual fusion of vacuoles with lysosomes. CHO cells were infected with live *T. gondii*, and 2 hours later the cells were treated with 10 μM pyrimethamine, an inhibitor of parasite dihydrofolate reductase, which effectively kills intracellular tachyzoites (*17*). Pyrimethamine treatment of infected CHO cells completely prevented the replication of intracellular organisms. Moreover, parasite-containing vacuoles, easily identified by light microscopy or by immunofluorescent staining with antiserum to *T. gondii* (anti–*T. gondii*), progressively disappeared over the next 36 to 48 hours. Vacuoles containing dead parasites remained negative for lgp staining (Fig. 1, G and H). Thus, although the fusion inhibition was dependent on the entry of live parasites, this requirement was manifested only at the time of entry (*18*). Since the viability of internalized organisms was not required, it is unlikely that *T. gondii* continuously releases a soluble inhibitor that controls vacuole fusion. This conclusion is further supported by the observation that a single host cell infected by a mixed population of nonviable and live organisms can contain both fusion-competent and fusion-incompetent vacuoles at the same time (Fig. 1B).

Route of parasite entry and subsequent fusion competence of the vacuole. In macrophages, the blocks in lysosome fusion and acidification can be overcome by coating live parasites with specific antibody prior to cell entry (*5, 19, 20*), implying that the route of

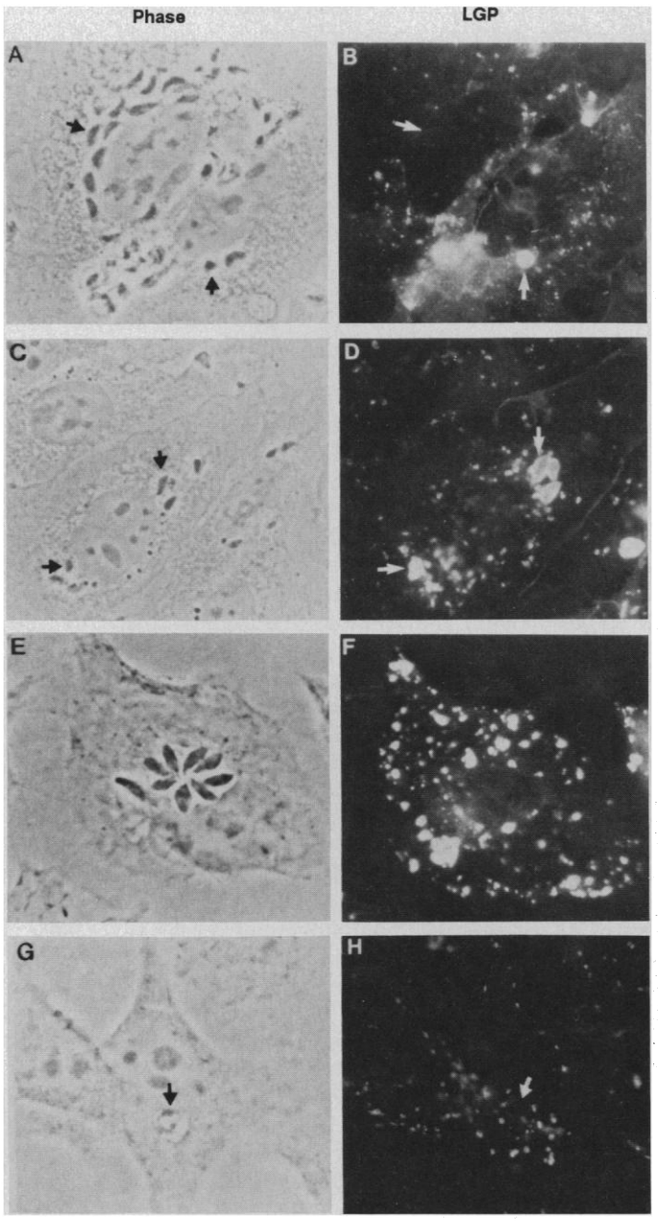

Fig. 1. Immunofluorescent staining of *T. gondii* vacuoles with antibodies to lgp. CHO-B1 cells (A, B, and E to H) and CHO-B2 cells (C and D) were infected with tachyzoites of *T. gondii*. The RH strain of *T. gondii* was used for all experiments. Tachyzoites were suspended in alpha minimal essential medium after being harvested from either the peritoneal cavities of 6- to 8-week-old BALB/c mice (Charles River) 3 days after the mice had been inoculated with 5×10^6 organisms, or from continuous culture in a human fibroblast monolayer, as described (*40*). Tachyzoites (25 μl) at 1×10^7/ml in alpha minimal essential medium were added to 300 μl of media overlaying cover slips containing CHO cells (ratio of parasite to cell, 10:1) in 24-well tissue culture plates. The plates were incubated (37°C in 5 percent CO_2), then washed with buffer at room temperature. Cover slips were fixed for 2 hours in 3 percent paraformaldehyde, then permeabilized in phosphate-buffered saline (PBS) containing 0.005 percent saponin and 10 percent normal goat serum. Cover slips were incubated sequentially with primary and secondary antibodies for 2 hours each in the same buffer. Murine monoclonal antibody E9A, recognizing an lgp of 96 kD in CHO cells (*13*), was used as the primary antibody. Equivalent results were obtained with monoclonal antibody 1C9, recognizing an lgp of 58 kD and with polyclonal antiserum to lgp 120. Cover slips were mounted in Mowiol (Calbiochem) and slides were examined by phase contrast (A, C, E, and G) and epifluorescence (B, D, F, and H) microscopy at ×1000 in a Zeiss Axiophot fluorescence microscope. Photographic film was TriX Pan 400, exposed and developed at 1600 ASA. Live parasites were allowed to infect cells for 2 hours (**A** and **B**) or 22 hours (**E** to **H**). Heat-killed tachyzoites were incubated with cells for 2 hours (**C** and **D**). In (**G**) and (**H**), cells infected for 2 hours were then incubated for an additional 20 hours in 10 μM pyrimethamine. Selected vacuoles (arrows) show either the presence or absence of lgp staining. Although the single vacuole in (G) is evident by phase-contrast microscopy, most vacuoles in pyrimethamine-treated cells were indistinct and were only detected by immunofluorescent staining with antibodies to *T. gondii*.

Table 1. Vacuole-lysosome fusion, vacuole-endosome fusion, and vacuole acidification for parasites entering transfected CHO-B1 cells. Numbers represent the percentage (range) of positive vacuoles in *N* experiments done on duplicate cover slips in which at least 50 vacuoles were counted for each cover slip. Results for a 1:50 dilution of polyclonal antibody to p30 are shown. Dilutions of 1:200 and 1:800 gave 59 and 18 percent lgp-positive vacuoles and 62 and 14 percent FcR-positive vacuoles, respectively. LY, lucifer yellow; AO, acridine orange.

T. gondii	lgp+		LY+		AO+		FcR+	
	%*	*N*	%	*N*	%	*N*	%	*N*
Live	3–16	14	3–6	4	4–11*	3	3–13	8
Heat-killed	92–98	11	58–87	4	63–84	2	17–24†	6
Live + anti p30	87–92	4	62–75	3	58–76	2	86–94	4

*Infected monolayers were incubated with acridine orange (2 μg/ml) for 10 minutes at 37°C. Monolayers were washed and maintained in cold buffer until live cells were examined by phase-contrast or epifluorescence microscopy. This acridine orange staining protocol differs from earlier studies with *T. gondii* (*1, 2, 5, 42*) in which cells were pre-loaded with acridine orange prior to infection, in order to assess phagolysosome fusion or parasite viability. †Positive vacuoles were faintly stained in comparison to vacuoles containing antibody-sensitized tachyzoites.

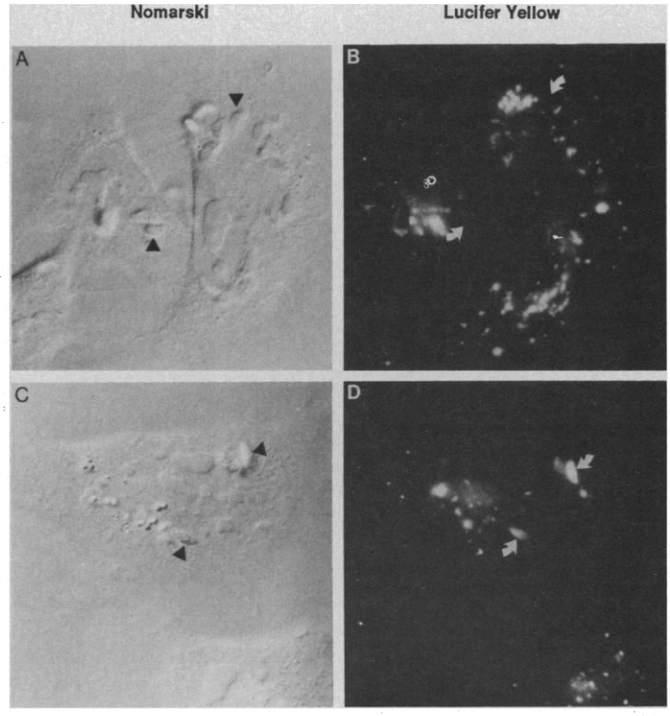

Fig. 2. Staining of vacuoles containing *T. gondii* with the fluorescent endocytic tracer lucifer yellow. CHO-B1 cells were incubated for 2 hours at 37°C with live (**A** and **B**) or heat-killed (**C** and **D**) tachyzoites. Monolayers were washed and incubated with lucifer yellow (1 mg/ml) for 10 minutes at 37°C. Monolayers were washed in cold PBS and maintained in cold buffer until live cells were examined by differential interference contrast (A and C) or epifluorescence microscopy (B and D). Selected vacuoles are illustrated by arrows.

entry determines the fusion potential of the vacuole. Interpretation is complicated by the fact that binding of antibody-coated parasites to receptors for the constant region of immunoglobulin G (IgG) [Fc receptors (FcR)] in macrophages also triggers generation of toxic oxygen metabolites capable of killing the parasite (*2*). The fact that fusion was observed under these conditions would simply reflect the internalization of nonviable parasites. To differentiate between these two possibilities, we analyzed the entry and fate of live *T. gondii* in CHO cells transfected with murine macrophage and lymphocyte IgG Fc receptors (FcRII-B2 or FcRII-B1, respectively, which are isoforms) (*21–23*). Unlike macrophages, the transfected CHO cells did not generate a respiratory burst after binding or entry of antibody-coated parasites (*24*).

Viable tachyzoites were opsonized with various dilutions of monoclonal or polyclonal antibodies to different *T. gondii* surface proteins (*25*), and then incubated with FcRII-expressing CHO cells at 37°C for 1 to 4 hours before fixation for immunofluorescence. IgG-coated *T. gondii* were efficiently internalized by the transfected cells. Intracellular parasite-containing vacuoles were enriched in FcR (Fig. 3B, FcR), as expected for FcR-mediated phagocytosis (*26*). In contrast, little or no FcR was detected in vacuoles after the entry of uncoated live or heat-killed parasites into the transfected cells (Table 1).

Virtually all of the vacuoles containing IgG-coated *T. gondii* rapidly fused with lysosomes, as indicated by the fact that each FcR-positive vacuole was also positive for lgp staining (Fig. 3A, lgp). The vacuoles were also capable of fusing with endosomes, as they were accessible to labeling with LY (Table 1). Finally, vacuoles containing antibody-coated parasites also underwent acidification, as indicated by their ability to accumulate acridine orange (Table 1).

Several considerations indicate that the inability of IgG-coated *T. gondii* to inhibit vacuole function was due only to their having been internalized via FcR. First, similar results were obtained with tachyzoites that were first sensitized with any of several antibodies to two different surface components of *T. gondii* (p22 and p30) (*25*) as well as with a polyclonal antibody to whole RH strain tachyzoites. Consequently, it is unlikely that the lack of fusion inhibition reflected antibody binding to a specific component on the parasite plasma membrane. There was also a correlation between the number of FcR-positive and lgp-positive vacuoles and the concentration of antibody used to coat the parasites (Table 1). Moreover, both binding and entry of opsonized parasites were decreased to that observed in the absence of antibody by prior incubation of transfected cells with the monoclonal antibody to FcRII (2.4G2) which blocks the ligand binding site of the receptor; resultant vacuoles were lgp-negative (*27*). Finally, antibody coating did not inhibit entry of viable tachyzoites into parental (that is, nontransfected) CHO cells, nor did it overcome the fusion block after entry into nontransfected cells (less than 10 percent of the intracellular vacuoles were lgp-positive).

Requirement for the FcR cytoplasmic tail in *T. gondii* phagocytosis. CHO cells transfected with either the FcRII-B1 or -B2 isoforms were equally capable of mediating the binding, internalization, and lysosomal fusion of antibody-coated *T. gondii*. This was surprising since only the FcRII-B2 isoform is characteristic of phagocytic cells such as macrophages; the FcRII-B1 isoform is the predominant species found in B lymphocytes—cells not normally associated with FcR-mediated phagocytosis (*28*). The FcRII-B1 and -B2 isoforms are identical in their extracellular and transmembrane domains; thus, antibody-coated particles bind these receptors with identical specificity and affinity. However, the two isoforms differ in their cytoplasmic tails. The FcRII-B1 tail contains an in-frame insertion of 47 amino acids that prevents this isoform from accumulating at clathrin-coated pits on the plasma membrane (*21*). Since both FcRII-B1 and -B2 transfectants were able to mediate the internalization and lysosomal fusion of IgG-coated parasites, it is apparent that neither process requires a functional coated pit localization domain.

To determine whether phagocytosis and fusion required any portion of the FcR cytoplasmic tail, we tested antibody-coated *T. gondii* for binding to CHO cells transfected with a mutant receptor containing only a single lysine residue in the cytoplasmic tail (tail-minus). We first compared attachment of live and heat-killed

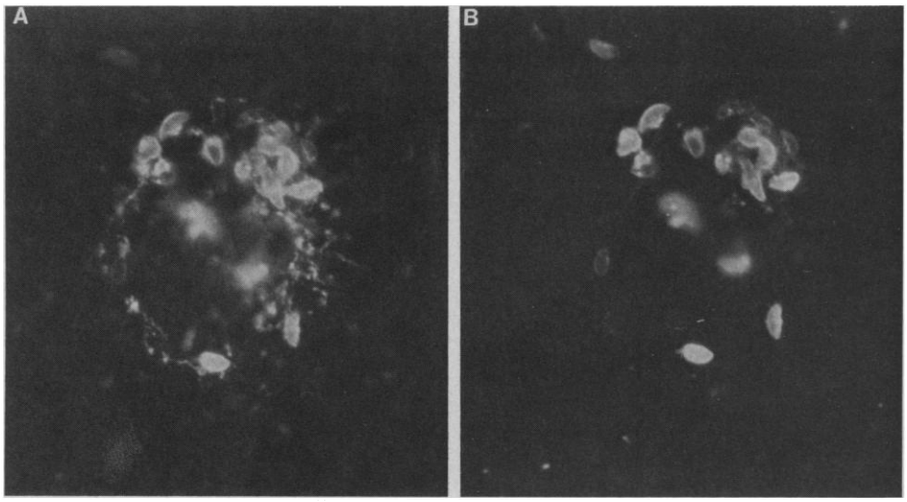

Fig. 3. Immunofluorescence staining of CHO-B1 cells infected with antibody-coated *T. gondii*. Live tachyzoites at 1×10^7/ml were first sensitized for 20 minutes at room temperature with a 1:50 dilution of polyclonal rabbit antiserum to p30. The sensitized parasites were added to CHO cells at a 10 to 1 ratio of parasites to cells and incubated for 2 hours at 37°C. The cells were fixed and stained with a monoclonal antibody, E9A, to lgp (**A**) or a monoclonal antibody, 1C14, to FcRII (*41*) (**B**)

tachyzoites in CHO-B1 and CHO tail-minus cells. The number of cell-associated, antibody-coated parasites was identical when CHO-B1 and CHO tail-minus cells were compared, regardless of whether parasites were alive or heat-killed (Fig. 4A). This finding is consistent with the two transfected cell lines having a similar number of cell surface FcR (1.2 to 1.5 $\times$ 10^6 per cell), and with the extracellular and membrane spanning domains of the wild-type and mutant receptors being identical.

While the presence or absence of a cytoplasmic tail had little effect on FcR binding of IgG-coated *T. gondii*, the efficiency of internalization was decreased in cells transfected with the tail-minus mutant relative to that observed in cells expressing FcRII-B1 (or -B2) (Fig. 4B). The fraction of heat-killed (and live) parasites internalized by CHO tail-minus cells was not augmented by coating parasites with IgG, in contrast to the results with CHO-B1 (or -B2) cells. Internalization of heat-killed (and live) IgG-coated parasites was also blocked by cytochalasin D (*27*), an agent that interferes with the normal function of actin-containing microfilaments. Thus, FcR-mediated entry into transfected CHO cells is analogous to phagocytosis in macrophages, which has been characterized as a cytochalasin-sensitive process.

We next examined the question of whether entry via a cytoplasmic tail–dependent phagocytic event was also needed for the fusion of vacuoles with lysosomes. Concentrations of antibody that led to nearly uniform lgp-positive vacuoles in CHO-B1 cells (87 to 92 percent, Table 1) resulted in only 14 to 19 percent lgp-positive vacuoles in CHO tail-minus cells. Furthermore, the percentage of FcR-positive vacuoles was decreased in cells expressing the tail-minus mutant (23 to 32 percent) relative to FcRII-B1 (86 to 94 percent, Table 1). Vacuoles containing antibody-coated parasites in CHO tail-minus cells were also unable to fuse with endosomes or to lower their internal pH. It is likely that parasites able to gain entry into these cells did so by an FcR-independent process presumably reflecting their normal physiological route.

While extracellular *T. gondii* is susceptible to acidic pH, it has thus far been impossible to directly determine the effects of lysosomal fusion on parasite viability. To address this issue, we next monitored the survival and infectivity of native and antibody-coated parasites in CHO-B1 compared to CHO tail-minus cells. The percentage of CHO cells harboring intracellular parasites after infection with native tachyzoites was similar at 2 and 24 hours in cells expressing either FcRII-B1 or the tail-minus mutant (Table 2). In the absence of antibody, parasites replicated with equivalent efficiency in both cell lines, since at 24 hours (compared to 2 hours) there was a six- to eightfold increase in the number of parasites per 100 cells. In contrast, most of the IgG-coated parasites were killed after entry into CHO-B1 cells. Little decrease in viability was observed for the fraction of IgG-coated parasites that entered CHO cells transfected with the tail-minus FcR mutant, or nontransfected cells. These findings demonstrate that, even in the absence of a respiratory burst, phagocytosis of the parasite under conditions that lead to fusion of the vacuole with endosomes and lysosomes results in parasite killing. Thus, inhibition of fusion and acidification is necessary for intracellular viability of *T. gondii*.

Mechanism of fusion inhibition. Although of critical importance to understanding many bacterial and protozoan infections, the mechanisms by which intracellular parasites inhibit phagosome-lysosome fusion remain largely unknown. The idea that the fusion block results from the secretion or release of inhibitory compounds from the infecting microorganism was first suggested by studies with *Mycobacterium tuberculosis*, in which inhibition of fusion was thought to reflect the release of complex sulfatides (*29, 30*). While the endocytosis of polyanions such as dextran sulfate by cultured macrophages may inhibit subsequent phagosome-lysosome fusion (*9, 30*), it is unclear whether such sulfatides play a physiological role during mycobacterial infections (*9, 31*).

Our results, obtained with a new assay for phagosome-lysosome fusion, strongly suggest that the fusion and acidification blocks

Table 2. Parasite survival and replication in transfected CHO cells. Experimental conditions were as described in the legend to Figs. 1 and 4, except that parallel cover slips were incubated for either 2 or 24 hours. The percentage of cells containing recognizable vacuoles at 2 and 24 hours is given. The average number of parasites per vacuole ranged between 4.8 and 6.9 for all cells at 24 hours. Results represent the range of values in three experiments, each done on triplicate cover slips. At least 100 cells were counted for each cover slip.

Parasites	Infected cells			
	CHO-B1		CHO tail-minus	
	2 hr	24 hr	2 hr	24 hr
	Live T. gondii			
Cells with vacuoles (%)	71–88	65–82	28–39	26–37
Tachyzoites/100 cells (No.)	74–105	643–930	40–57	280–392
	Live T. gondii *plus anti-p30*			
Cells with vacuoles (%)	93–97	18–26	73–81	72–79
Tachyzoites/100 cells (No.)	170–234	130–185	89–127	545–627

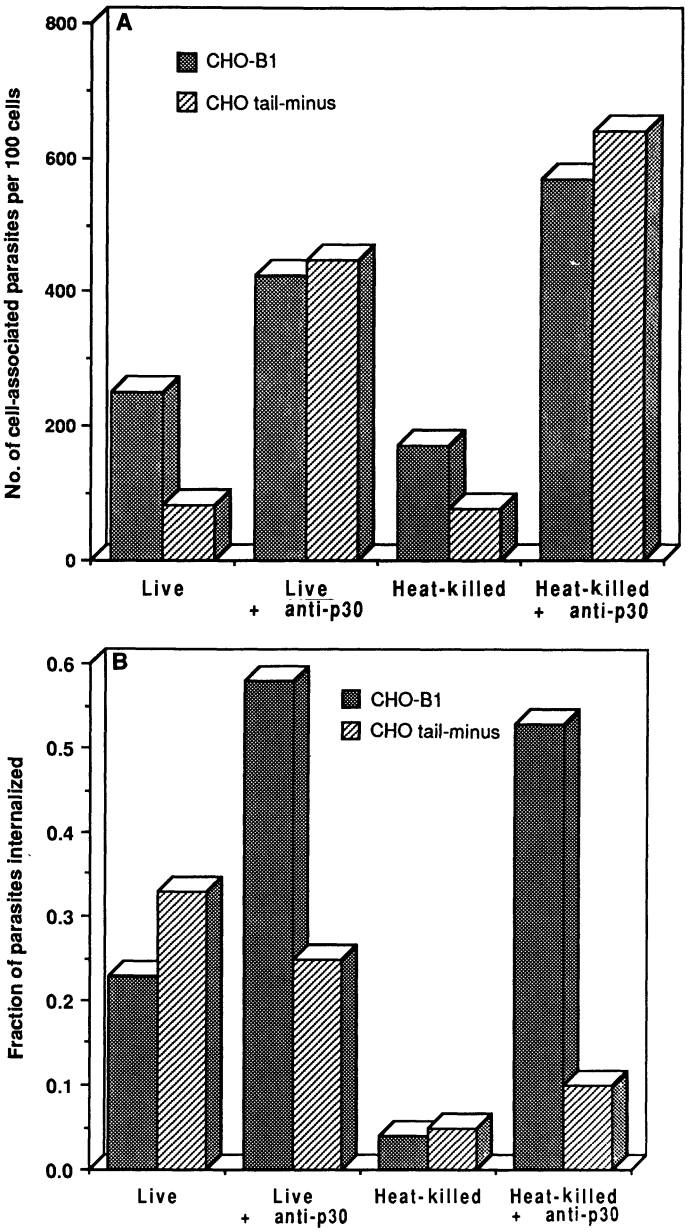

Fig. 4. Attachment and internalization of *T. gondii* by CHO-B1 and CHO tail-minus cells. Parasites were prepared and incubated with cells at a 10 to 1 ratio of parasite to cell as described in the legends to Figs. 1 to 3. After incubation for 2 hours at 37°C, cells were fixed and total cell-associated parasites (**A**) and fraction of total cell-associated parasites internalized (**B**) were measured. Parasites were scored as inside cells on the basis of a combination of phase contrast and differential interference contrast microscopy and immunofluorescence staining in the presence and absence of cell permeabilization with saponin. At least 100 cells were counted for each cover slip in each experiment. Results represent mean for three experiments, each done in triplicate. The standard deviations were all less than 17 percent of the sample means for results in (A) and less than 14 percent of the sample means for results in (B). The higher baseline association of live and heat-killed parasites with CHO-B1 than with either CHO tail-minus (or CHO-B2 cells) was a consistent, although unexplained, finding.

mediated by *T. gondii* are not dependent on the secretion of a soluble, parasite-derived inhibitor. Although the inhibition can only be mediated by viable tachyzoites, this requirement is manifested only at the entry stage. Since killing parasites after entry did not reverse either the fusion or acidification blocks, it is clear that there is no need for the continued secretion of an inhibitor by internalized organisms. The ability of even live parasites to inhibit vacuole function is not absolute, as demonstrated by the fact that viable, antibody-coated tachyzoites were unable to mediate the fusion or acidification blocks when allowed to enter FcR-expressing CHO cells. This effect was not due to the antibody interfering with some critical parasite surface component, since neither attachment and entry nor the fusion block were altered when opsonized *T. gondii* entered nontransfected cells. In addition, identical results were obtained with mono- and polyclonal antibodies to several different *T. gondii* surface proteins. Taken together, the results indicate that entry of live parasites is necessary but not sufficient to mediate fusion inhibition.

The ability to circumvent the fusion block by forcing live tachyzoites to enter via an alternative receptor indicates that the ability of *T. gondii* to inhibit fusion and acidification is linked to some irreversible event that occurs at the time of entry. Such an event seems likely to reflect the parasite's ability to modify the biochemical composition of the forming vacuole. Several lines of evidence already suggest that *T. gondii*, as well as other members of the apicomplexa group (for example, *Plasmodia* sp.), actively participate (*32*) in the energy-dependent entry process, which may selectively exclude, or include, specific plasma membrane components needed for (or inhibitory to) subsequent fusion events. Nascent vacuoles appear to be depleted in selected plasma membrane enzymes (*33*) as well as in intramembranous particles as determined by freeze-fracture analysis (*34*). Thus, it is conceivable that, among the excluded components, are those that are needed for subsequent vacuole fusion or acidification (or both). It is also possible that lipid components released as a result of rhoptry or dense granule discharge are incorporated into the forming vacuole membrane, as suggested with *P. falciparum* (*35*), further altering its properties. In any event, it is apparent that the vacuole lacks one or more molecules needed for normal function, or contains membrane components that are dominant inhibitors.

The fusion incompetence of *T. gondii* vacuoles is not likely to be the result of a primary block of acidification, since phagosome-lysosome fusion after uptake of inert particles is independent of acid pH in either compartment (*36*). In contrast, the inability of parasitophorous vacuoles to lower their internal pH may be a consequence of the fusion block. If active components of the vacuolar H^+-ATPase (proton–adenosine triphosphatase) must be delivered to the vacuole by fusion with endosomes, normal acidification would not occur (*37*). The lack of acidification might also reflect the active exclusion or inactivation of plasma membrane proton pumps or other ion channels during formation of the vacuole membrane.

Finally, the fact that IgG-coated *T. gondii* were efficiently bound and internalized by FcR-transfected CHO cells not only demonstrates the role of entry in mediating vacuole function, but also provides some initial insight into the requirements for phagocytosis in general. Clearly, even fibroblasts have the requisite cytoplasmic "machinery" to mediate phagocytosis once given an appropriate phagocytic receptor. Moreover, the fact that both the FcRII-B1 and -B2 isoforms mediate parasite entry demonstrates that FcR-mediated phagocytosis does not require a functional coated pit localization domain (*21*). The fact that the tail-minus FcR mutant failed to mediate efficient FcR-dependent entry, however, shows that some determinant (or determinants) on the receptor's cytoplasmic tail is required. IgG-coated erythrocytes are not efficiently internalized by the transfected cells, in spite of efficient binding (*23, 27*). By binding to laminin or other extracellular matrix receptors (*38*), *T. gondii* may provide a second signal to the CHO cells to trigger phagocytosis of bound particles via FcR (*39*). It should now be possible to identify this missing receptor and provide further insight into the requirements for phagocytosis—in addition to vacuole fusion.

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13. Molecules of 96 kD and 58 kD in CHO cells are recognized by monoclonal antibodies E9A and 1C9, respectively. These monoclonal antibodies identify constituents within intracellular vacuoles which precisely co-localize with vacuoles labeled by immunofluorescence and by immuno-gold frozen thin-section electron microscopy with polyclonal antiserum to lgp 120 (S. Schmidt, C. L. Howe, I. Mellman, unpublished results).
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