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Partially Protective Vaccination Permits the Development of Latency in a Normally Virulent Strain of *Toxoplasma gondii*

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The virulent RH strain of *Toxoplasma gondii* is acutely lethal in mice and fails to establish chronic infection. Vaccination of BALB/c mice with a soluble tachyzoite antigen preparation, STAg, in combination with the immunostimulatory cytokine interleukin-12 results in partial protection against RH lethal challenge. Nevertheless, brain tissue obtained from surviving, vaccinated mice as late as 1 year after RH infection contained latent parasite forms as demonstrated by subinoculation into naive recipients. The tachyzoites arising in the subinoculated animals were genetically indistinguishable from the original RH inoculum. Microscopic examination revealed that the persistent parasite forms present in the brains of vaccinated and challenged mice have a tissue cyst-like morphology and express the bradyzoite antigen BAG-1 but not the tachyzoite-specific antigen SAG-2 but are different from the cysts formed by avirulent *T. gondii* strains in that the internal parasite stages display ultrastructural features intermediate between tachyzoites and bradyzoites. Moreover, the zoites within the RH tissue cysts are clearly distinct from conventional bradyzoites in their sensitivity to pepsin-HCl digestion. In contrast to the observations made with partially resistant STAg/interleukin-12-vaccinated animals, no latent forms could be detected in brain tissue after RH challenge of mice immunized with a live attenuated tachyzoite vaccine which confers total protection against this parasite isolate. The above findings demonstrate the potential of a virulent *T. gondii* strain to generate latent parasite stages, a process which may be promoted under conditions of incomplete vaccination.

The major goal of vaccination against microbial pathogens is to prevent disease. While desirable, this need not necessarily require the induction of a complete sterilizing immunity against the infecting agent. Indeed, by allowing the persistence of subclinical infection, suboptimal vaccination may promote the maintenance of a concomitant immune state, thereby prolonging the protection of the host against acute disease. On the other hand, because it permits low-level infection, partial immunization may have unforeseen detrimental effects related to the potential for recrudescence.

In the present report, we describe a situation in which incomplete immunization results in the appearance of dormant infectious stages not normally encountered when nonimmune or completely protected hosts are challenged with the same virulent isolate. These persistent forms display a previously unrecognized set of structural and biological features and represent a potential source of reactivated acute infection in immunocompromised hosts.

The vaccine-induced generation of latency we observed occurred in a setting of experimental immunization of mice against the intracellular protozoan parasite *Toxoplasma gondii*, an important opportunistic pathogen in AIDS patients and individuals taking immunosuppressive drugs (16). This organism exists as three major genetically defined subspecies which differ in both their virulence and host persistence (11). Avirulent strains of the parasite undergo transformation in the host from a rapidly replicating tachyzoite stage to dormant protease-resistant bradyzoites residing within tissue cysts (12). In contrast, it has been difficult to demonstrate dormant stages of virulent parasite isolates since they are usually lethal to susceptible experimental hosts.

While it is possible to induce solid protection against infection with virulent strains by immunization with live attenuated variants, vaccination with dead parasite preparations or defined *T. gondii* antigens has typically resulted in lower levels of immunity (14, 15). In an attempt to enhance the protection induced by nonliving immunization, we have tested the effect of the immunostimulatory cytokine interleukin-12 (IL-12) as a vaccine adjuvant when coadministered with a tachyzoite antigen preparation (soluble tachyzoite antigen [STAg]). This protocol resulted in the induction of significant levels of protection against challenge with the highly virulent RH parasite strain. Unexpectedly, however, the vaccinated mice surviving challenge infection were found to harbor latent parasite forms, which transferred acute infection upon subinoculation.

MATERIALS AND METHODS

Animals. Female BALB/c mice (7 to 8 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, Maine). Gamma interferon (IFN-γ)-deficient mice on a C57BL/6 background (GKO) (2) and wild-type C57BL/6 (B6) mice of either sex were obtained from the NIAID Taconic Contract Facility (Germantown, N.Y.) and used as recipients of brain homogenates.

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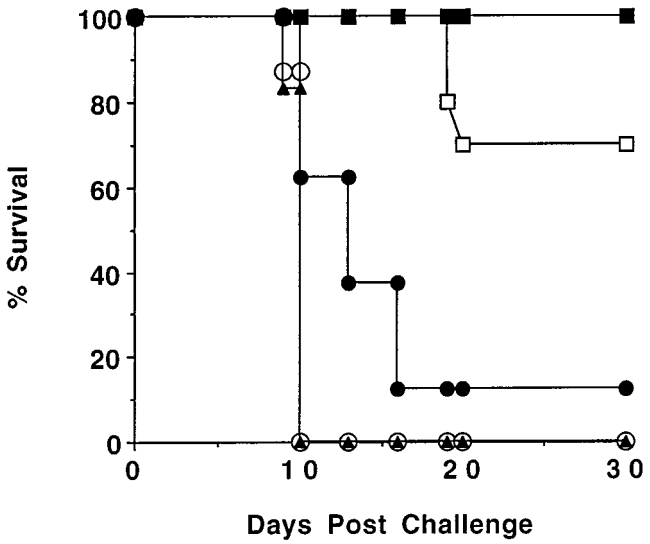

FIG. 1. Survival of mice after challenge with 2,000 strain RH tachyzoites. Groups of 10 mice were vaccinated with live ts-4 (filled squares), IL-12 alone (unfilled circles), STAg alone (filled circles), or STAg plus IL-12 (unfilled squares). A group of six nontreated mice were included as controls (triangles).

Cultivation of parasites. The ts-4 and RH strains of *T. gondii* were propagated at 34 and 37°C, respectively, by biweekly passage in human foreskin fibroblast cultures as previously described (20).

Parasite antigen preparation. STAg was prepared by sonication of RH parasites in the presence of protease inhibitors and centrifugation at 100,000 × *g* (4). The supernatant was subsequently dialyzed against 1× phosphate-buffered saline (PBS).

Vaccination. Female 7- to 8-week-old BALB/c mice were vaccinated with either the live ts-4 vaccine strain or STAg either alone or in combination with IL-12. For vaccination with live parasites, mice were injected twice intraperitoneally (i.p.) with 2×10^4 ts-4 tachyzoites 2 weeks apart (8). For vaccination experiments employing soluble parasite antigen, mice received two bimonthly subcutaneous injections of 20 μg of STAg or 0.1 μg of recombinant murine IL-12 (generously provided by Genetics Institute, Cambridge, Mass.) or both into the right footpad. Two weeks after the last vaccination, the mice were challenged subcutaneously with 2×10^3 RH parasites.

Subinoculation assay for the presence of dormant parasites. To detect the presence of dormant RH parasites in the brains of vaccinated and challenged mice, the organs were split along the midsagittal axis. Half the brain was homogenized in 1 ml of PBS by passage through a 19-gauge, and subsequently a 21-gauge, needle and injected i.p. into a single GKO or C57BL/6 mouse. Cumulative survival of the animals was then measured. To confirm the presence of parasites in the subinoculated recipients, several animals were sacrificed on day 5 or 6 postinjection and cytospin smears were prepared from their peritoneal exudate cells. Slides were stained with Diff-Quik as described in the manufacturer's instructions and examined microscopically for tachyzoites as described previously (20).

Genetic characterization of parasite isolates. To confirm the genotype of the latent parasite forms observed in vaccinated mice, peritoneal cells from mice subinoculated with brain tissue were pelleted by centrifugation, frozen, and stored at −70°C. DNA was extracted and the *SAG1* locus was analyzed by PCR-restriction fragment length polymorphism analysis as previously described (22).

Pepsin resistance assay. Resistance to peptic digestion was used as a criterion for the presence of conventional tissue cysts (12). To perform the assay, each brain was homogenized by syringe passage in 2 ml of PBS and split into two aliquots. One of the samples was then left at 4°C, while the second was enzyme digested. This step was carried out by adding 10 ml of digestion fluid and incubating the sample at 37°C for 60 min. The digestion fluid consisted of 5.2 g of pepsin (Sigma, St. Louis, Mo.) per liter in 0.17 M NaCl–0.084 M HCl. After incubation, the enzyme-treated and control brain suspensions were centrifuged at 400 × *g* for 20 min, and each of the pellets was resuspended in 1 ml of PBS and injected i.p. into a single mouse per sample.

Histopathology and immunohistology. For visualization of cysts, slices of brain tissue from strain ME49-infected mice or from STAg-plus-IL-12-vaccinated and RH-challenged mice were fixed in 4% phosphate-buffered formaldehyde, processed for paraffin embedding, and stained with periodic acid-Schiff stain (PAS) and hematoxylin-eosin. Four-micrometer-thick serial sections were also prepared and stained by the immunoperoxidase method (1, 24) with rabbit anti-SAG-2 (tachyzoite-specific marker) or anti-BAG-1 (bradyzoite-specific marker) antisera. The preparation and specificity of each antiserum were described previously (17, 18).

Electron microscopy. Brains from three mice sacrificed 28 days after RH challenge allowed the visualization of cyst-like structures by electron microscopy. Each brain was divided into three portions. One portion was passaged into GKO mice to confirm the presence of parasites. The second portion was fixed in 2% paraformaldehyde in 0.1 M phosphate buffer, and the third was chopped into small 1-mm cubes and fixed in 4% glutaraldehyde in 0.1 M phosphate buffer. A small block of the paraformaldehyde-fixed material was dehydrated and embedded in LR White for immunoelectron microscopy, and the remainder was embedded in wax for immuno-light microscopy. The glutaraldehyde-fixed tissue was postfixed in osmium tetroxide and stained en bloc with uranyl acetate prior to dehydration in ethanol, treatment with propylene oxide, and embedment in Spurr's epoxy resin. One-micrometer-thick sections were examined by light microscopy to identify tissue cysts. Suitable areas were thin sectioned and stained with uranyl acetate and lead citrate prior to examination in a JEOL 1200 EX electron microscope. For immunoelectron microscopy, cysts were identified in LR White-embedded material and thin sections were placed on Formvar-coated nickel grids. They were then stained with a rat monoclonal antibody, CC2, that reacts with the cyst wall as described previously (10).

RESULTS

Vaccination with STAg in combination with IL-12 induces partial protection against challenge with a virulent parasite strain. Based on previous studies (9, 21) demonstrating a role for IL-12 in host resistance to *T. gondii*, a vaccination protocol was developed in which BALB/c mice were vaccinated twice subcutaneously with a soluble tachyzoite extract (STAg) admixed with recombinant murine IL-12. Nonvaccinated animals or control mice immunized with IL-12 alone died within 10 days when challenged subcutaneously with 2,000 tachyzoites of the highly virulent RH strain (Fig. 1). In contrast, BALB/c mice immunized by inoculation with the temperature-sensitive RH mutant, ts-4, were completely protected against the same challenge. Vaccination with STAg alone did not confer significant protection against lethality, although half of the mice survived longer than mice vaccinated with IL-12 alone (median survival time, 13 days [STAg alone] versus 10 days [IL-12 alone]). Importantly, animals vaccinated with STAg in combination with IL-12 displayed significant resistance, with 70% of the mice surviving the challenge infection (Fig. 1). Nevertheless, the vaccinated survivors exhibited acute morbidity during weeks 1 to 3 following RH challenge, as evidenced by ruffled

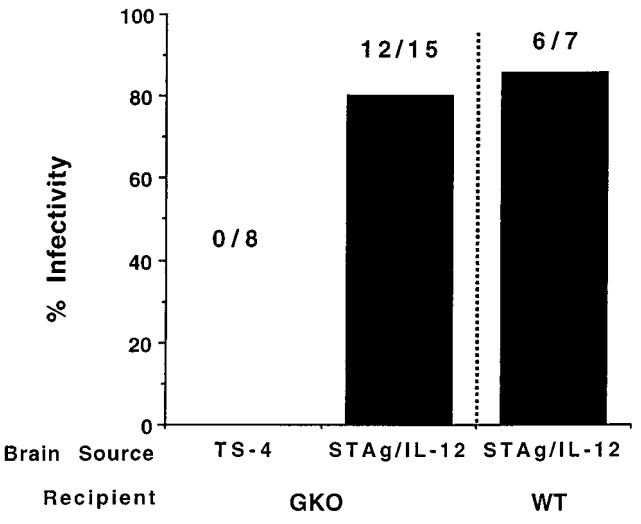

FIG. 2. Infectivity of brain homogenates from RH-challenged mice immunized with either STAg plus IL-12 or ts-4 in IFN-γ-deficient (GKO) and wild-type (WT) C57BL/6 mice. Each mouse was injected with a single half of a brain. Lethality was used as a measure of infectivity. The numbers of animals succumbing relative to the total number inoculated is indicated above each bar.

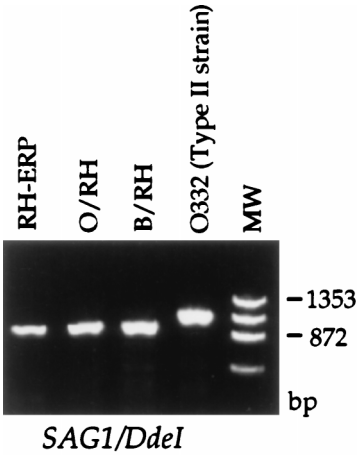

FIG. 3. Tachyzoites derived from persistent forms are genotypically identical to the original RH challenge parasites. *Dde*I digestion of SAG-1 amplification products show a band for RH parasites recovered from recipients (B/RH) identical with other type I strains (the original RH used for challenge [O/RH] and RH tachyzoites from E. Pfefferkorn [RH-ERP]) and distinct from a type II (O332) strain. Lane MW contains a ϕX174/*Hae*III size ladder. Approximately 50 to 100 ng of DNA was applied per lane.

fur and hunched stature. Thereafter, their appearance was normal. In contrast, the same acute symptoms were not observed after challenge of mice vaccinated with live ts-4.

Dormant RH parasites are present in STAg-plus-IL-12-vaccinated mice surviving RH challenge. To ascertain whether vaccination of mice with STAg plus IL-12 induces sterilizing immunity against RH tachyzoites, samples of brain tissue from mice surviving challenge (sacrificed between 30 and 365 days after RH exposure) were homogenized and subinoculated i.p. into IFN-γ-deficient (GKO) mice, which are exquisitely susceptible to *Toxoplasma* infection (20). While the brains from ts-4-vaccinated, RH-challenged mice were uniformly negative, the majority (12 of 15 or 80%) of brains from STAg-plus-IL-12-vaccinated mice induced mortality of recipient GKO mice within 9 days of subinoculation (Fig. 2). Microscopic examination of peritoneal cells from sample animals sacrificed at 5 to 7 days after brain passage revealed the presence of significant numbers of intracellular as well as extracellular tachyzoites. To ensure that the persistent parasites that transferred infection into GKO mice were indeed of the RH strain and not a contaminating avirulent strain, DNA was extracted from peritoneal exudate cell samples from mice subinoculated with brain tissue and the *SAG1* genotype of the parasites was determined by PCR-restriction fragment length polymorphism analysis. This analysis demonstrated that the recovered parasites had a type I allele, identical to tissue culture-derived RH and clearly distinct from the pattern associated with avirulent (type II) parasite isolates (Fig. 3).

Infections with the virulent RH strain are lethal in immunocompetent as well as IFN-γ-deficient hosts. To confirm that the persistent parasites in STAg-plus-IL-12-vaccinated mice retain the same virulent phenotype as do the RH organisms used for challenge, the brains from these animals were also transferred i.p. into wild-type C57BL/6 mice. All but one of seven subinoculated brains transferred lethality to the recipients within 9 days (Fig. 2). Thus, while vaccination with live attenuated ts-4 induces sterilizing immunity against RH tachyzoites, immunization with STAg in combination with IL-12 results in the persistence of latent parasites which retain the virulent phenotype of the challenge strain.

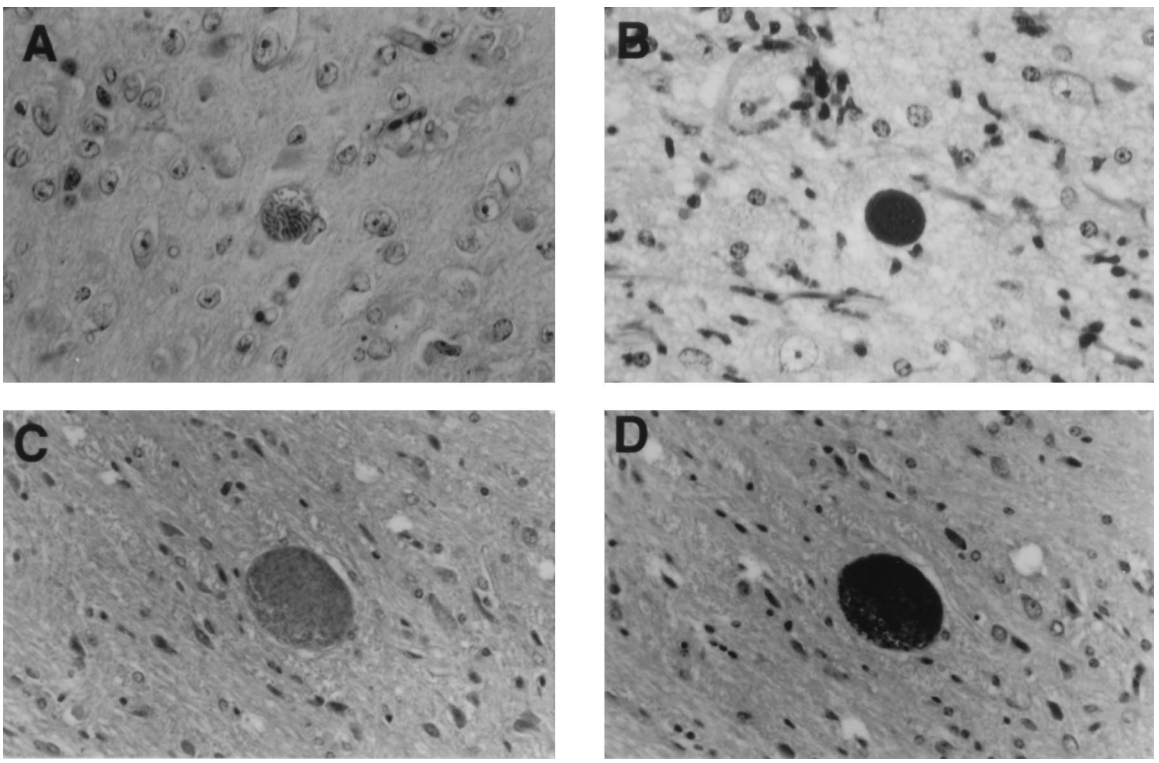

FIG. 4. Light microscope and immunohistochemical characterization of RH latent forms in brain tissue of BALB/c mice. An RH cyst-like structure is shown (A) which appears less intensely PAS positive than a brain cyst of the avirulent ME49 strain (B). RH cysts were negative for SAG-2 (C) but positive for BAG-1 (D).

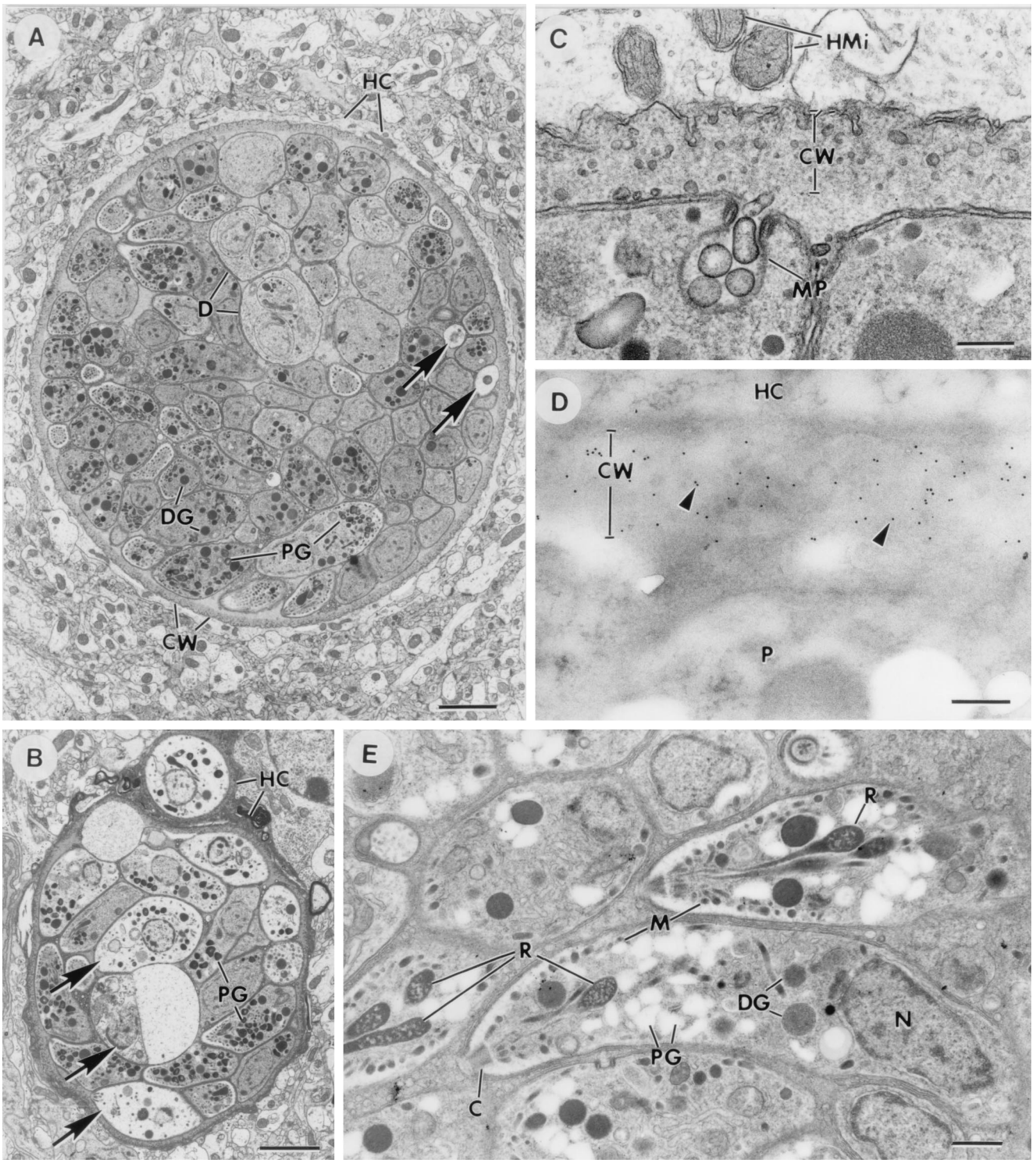

FIG. 5. Transmission electron micrographs of tissue cysts of the RH strain in the brains of STAg-plus-IL-12-vaccinated mice obtained 30 days after challenge. (A) Low-power photograph showing a tissue cyst enclosed by a cyst wall (CW) located within the host cell cytoplasm (HC). The cyst contains variable-appearing zoites, only a proportion of which contain numerous dense granules (DG) and polysaccharide granules (PG). Organisms undergoing division (D) and degeneration (arrows) are visible. Bar, 2 μm. (B) Small cyst within an electron-dense host cell (HC) containing more mature zoites with numerous polysaccharide granules (PG) and dense granules. A number of organisms have swollen and lucent cytoplasm consistent with degenerative changes (arrows). Bar, 2 μm. (C) Cross section through the periphery of a cyst showing the structure of a cyst wall (CW). Note the active micropore (MP) at the surface of the enclosed zoite. HMi, host cell mitochondrion. Bar, 200 nm. (D) Area similar to that shown in panel C from an LR White-embedded section immunostained with monoclonal antibody CC2. Note the numerous 5-nm gold particles (arrowheads) specifically labelling the cyst wall (CW). HC, host cell; P, parasite. Bar, 200 nm. (E) Detail of the interior of a cyst showing the crescent-shaped zoites with a basally located nucleus (N). The organisms contain the characteristic conoid (C), micronemes (M), dense granules (DG), and polysaccharide granules (PG). Note that the rhoptries are elongated, with a honeycombed interior. Bar, 0.5 μm.

Morphological characteristics of RH tissue cysts. Histologic sections of brains from long-term survivors were stained with PAS and counterstained with hematoxylin-eosin, a procedure normally used to identify parasite cysts in infected tissues. Structures resembling conventional cysts were identified at a low frequency (approximately one per five sections). These structures (Fig. 4A) were less intensely stained by PAS than tissue cysts of the ME49 strain of *T. gondii* (Fig. 4B) examined in comparable brain sections. To further characterize the differentiation state of the cyst-like structures, immunohistochemical staining with antisera to stage-specific tachyzoite (SAG-2) or bradyzoite (BAG-1) antigens was performed. Although morphologically distinguishable, the cyst-like structures were comparable to conventional ME49 cysts in their reactivity with the monoclonal antibodies, staining positively for BAG-1 (Fig. 4D) but negatively for SAG-2 (Fig. 4C). These findings suggest that the persistent RH parasite forms represent a developmental stage distinct from mature tissue cysts but in which transformation to bradyzoites has commenced.

Ultrastructural examination of the cyst-like structures in the brains of RH-challenged, vaccinated mice supported the conclusion of the light microscopic studies that these forms represent an intermediate parasite stage. Multiple sections through over 30 individual RH cysts were examined by electron microscopy. The cysts were of different sizes and contained a variable number of organisms, but all were located within intact host cells and were limited by a cyst wall (Fig. 5A and B). The cyst wall consisted of a unit membrane with numerous invaginations into an underlying granular layer (Fig. 5C). This homogeneous layer stained positively with the monoclonal antibody CC2, a cyst wall marker (Fig. 5D). These features are identical to those described for tissue cysts of avirulent strains, e.g., strains RRA and ME49 (5, 6, 10). The organisms within the cysts were heterogeneous in appearance (Fig. 5A). Many of the zoites appeared undifferentiated, lacking apical organelles and polysaccharide granules, consistent with the less-intense PAS staining. A number of these organisms were undergoing multiplication by endodyogeny (Fig. 5A). Among the more mature zoites, a proportion showed evidence of degenerative changes (Fig. 5B). It was possible to observe both proliferating and degenerating organisms within the same cyst (Fig. 5A). The more mature zoites were crescent shaped with a posteriorly located nucleus and numerous micronemes and polysaccharide granules (Fig. 5E), typical features of bradyzoites. However, the majority of rhoptries were elongate and their contents had a honeycomb appearance (Fig. 5E). This is a characteristic of tachyzoite rhoptries and differs from the typical appearance of bradyzoite rhoptries, which are more bulbous and uniformly electron dense, which were observed in a minority of organisms. In addition, the zoites appeared to be metabolically active as evidenced by active micropores (Fig. 5C) and the appearance of numerous vesicles around the Golgi body, characteristics not normally seen in bradyzoites. Therefore, although these organisms show a number of the bradyzoite-like characteristics, they retain some features of tachyzoites and may represent incompletely differentiated bradyzoites. On average, the more mature, crescent-shaped zoites represented 25 to 50%, while the degenerating parasites comprised less than 10%, of the internal parasites. The remaining fraction of zoites appeared undifferentiated, and none could be classified as typical tachyzoites or bradyzoites.

RH tissue cysts are distinct from conventional tissue cysts in that their bradyzoites are sensitive to pepsin digestion. A defining feature of bradyzoites within conventional tissue cysts of *T. gondii* is their ability to resist proteolytic digestion by pepsin-HCl. This characteristic presumably accounts for the successful transmission of the parasite following ingestion. To ascertain the functional maturation of the zoites within RH cyst-like structures, brain homogenates from STAg-plus-IL-12-vaccinated and RH-challenged mice were subjected to controlled digestion in pepsin-HCl at 37°C for 60 min and then subinoculated i.p. into GKO mice. Tissue cysts of the ME49 strain obtained from chronically infected mice and tissue culture-derived tachyzoites of the RH strain were included in each experiment as the positive and negative controls, respectively.

TABLE 1. RH cyst-like structures, in contrast to conventional tissue cysts, are sensitive to pepsin-HCl digestion

Type of test inoculum (size)	No. of mice infected/ no. inoculated (%)[a]	
	Undigested inoculum	Pepsin-HCl-digested inoculum[c]
RH tachyzoites (10^6)[b]	5/5 (100)	0/5 (0)
ME49 cysts (50)[b]	4/4 (100)	4/4 (100)
ME49 cysts (5)[b]	5/5 (100)	5/5 (100)
Half brain from STAg-plus-IL-12-vaccinated, RH-challenged mouse	6/8 (75)	0/8 (0)
Half brain from ts-4-vaccinated, RH-challenged mouse	0/4 (0)	0/4 (0)

[a] GKO mice were injected with the test inoculum, and 6 to 7 days later peritoneal cells were harvested and microscopically examined for the presence of tachyzoites.
[b] This test inoculum was incubated and injected together with the equivalent of a half brain from uninfected mice.
[c] Digestion with pepsin-HCl was performed for 1 h at 37°C as described in Materials and Methods.

As shown in Table 1, undigested inocula containing 5 or 50 cysts of strain ME49 or 10^6 RH tachyzoites resulted in positive infection of recipient mice. As expected, the infectivity of the ME49 cysts resisted peptic digestion whereas the tachyzoites were completely sensitive. As observed previously, brain homogenates of most STAg-plus-IL-12-vaccinated, RH-challenged mice induced infections upon subinoculation. However, in direct contrast to the samples containing ME49 tissue cysts, pepsin digestion destroyed the infectivity of the brains from the RH-challenged mice. Thus, although cyst-like in morphology, the dormant RH parasite forms are clearly distinct from the conventional tissue cysts of *T. gondii* strains in their sensitivity to proteolytic digestion. In agreement with this finding, three of three GKO mice inoculated perorally with brains from RH-challenged, STAg-plus-IL-12-vaccinated animals failed to develop infections whereas three control mice simultaneously inoculated with the same tissues i.p. all developed acute toxoplasmosis and rapidly succumbed (data not shown).

DISCUSSION

In this study, we have demonstrated that partial immunization against challenge with a highly virulent strain of *T. gondii* results in the appearance of latent parasite forms not normally encountered during infection with these organisms. The data thus argue that under certain conditions, vaccination while protecting the host against disease can allow the development of dormant but potentially recrudescent infection.

The generation of the latent *T. gondii* forms was observed in an experimental vaccine model involving immunization with a tachyzoite extract (STAg) plus the immunostimulatory cytokine IL-12. As will be described in detail in a future report (21a), this procedure results in highly significant levels of protection against virulent challenge while immunization with ei-

TABLE 2. Summary of features of RH cysts, RH tachyzoites, and conventional tissue cysts (e.g., strain ME49)

Type of cyst or tachyzoite	Characteristic					
	Cyst wall	BAG-1 Ag[a]	SAG-1 Ag	Nuclear localization	Rhoptry shape and content	Pepsin-HCl resistance of bradyzoites
RH cysts	Present	Positive	Negative	Posterior	Elongate, honeycombed	Sensitive
ME49 cysts	Present	Positive	Negative	Posterior	Bulbous, electron dense	Resistant
RH tachyzoites	Absent	Negative	Positive	Central	Elongate, honeycombed	Sensitive

[a] Ag, antigen.

ther STAg or IL-12 alone is ineffective (Fig. 1). Brain tissue from vaccinated animals surviving RH challenge was found to contain latent parasites as determined by subinoculation as late as 1 year following parasite exposure, indicating that these forms represent a stable rather than a transient stage. The immune response is clearly important for both the generation and persistence of these forms, since reversion to tachyzoites (with an indistinguishable genotype) occurs in both immunodeficient GKO as well as unimmunized immunocompetent recipients. Interestingly, however, in mice immunized with the live attenuated ts-4 vaccine, which display complete protection against RH lethality, no evidence of latent infection could be detected. Thus, it is likely that in these highly protected animals, complete elimination of the RH challenge occurs, while in mice vaccinated with STAg plus IL-12 the partial immunity established allows for the escape of some parasites and establishment of latency. Alternatively, the divergent outcomes of RH infection in the two types of vaccinated hosts may reflect qualitative differences in the immune responses induced by the immunization procedures employed.

The highly virulent RH strain of *T. gondii* was used for challenge infections in the present study. This isolate, established from a lethal case of encephalitis by Albert Sabin five decades ago (19), belongs to the type I genetic group of *T. gondii*. Mice inoculated with low numbers of tachyzoites belonging to this group of strains typically die within 10 days. Therefore, the existence of latent RH forms has been difficult to demonstrate during mouse infection. Previous reports of cyst formation by this virulent strain involved the use of naturally resistant host species (e.g., rats), chemotherapeutic treatment, or, as in the present case, prior immunization (3, 25, 26). In most of these investigations as well as in in vitro studies (23), the cyst-like forms arising were infrequent and minimally characterized, particularly in terms of infectivity.

The latent parasites described in the present report, although grossly resembling the cysts of avirulent strains, are nevertheless distinct in morphology and their expression of developmental markers and therefore likely represent an intermediate stage of differentiation (a comparison is summarized in Table 2). Clearly, the latent forms have undergone transformation into bradyzoites, as evidenced by the formation of a conventional cyst wall, a posteriorly located nucleus, and positive staining for a bradyzoite-specific antigen, BAG-1. Nonetheless, their rhoptries are elongated and contain a honeycombed matrix, an ultrastructural characteristic of rhoptries present in the tachyzoite stage (6). More importantly, they clearly lack the pepsin-HCl resistance phenotype that is a defining feature of the bradyzoite stage (12). Thus, it is likely that a developmental arrest resulted in the failure to remodel rhoptry contents and perhaps cell surface proteins responsible for the phenotype of pepsin-HCl resistance. Whether this partial arrest in bradyzoite development occurs with all type I strains is presently unclear. If so, the lack of pepsin resistance and, by extension, the lack of potential for oral infectivity raises an important question concerning how parasites of this genotypic group are transmitted in nature (7, 13). Nevertheless, the possibility that the maturational block may have arisen as a result of continuous long-term passage of the RH tachyzoites and, therefore, may be a peculiar feature of this laboratory strain cannot be ruled out.

Although not infective when inoculated perorally, the persistent RH cyst forms nevertheless represent a potential reservoir for recrudescence. Indeed, in preliminary experiments, neutralization of endogenous IFN-γ in six RH cyst-bearing animals resulted in pronounced morbidity and encephalitis 14 days after initiation of monoclonal antibody treatment (unpublished observations). Thus, individuals harboring these persistent forms as a consequence of partial vaccination are not protected and remain at risk of developing toxoplasmosis as a result of a breakdown of latency. The above observations, therefore, underscore the need for developing vaccination methods which induce sterilizing immunity and thereby eliminate the possibility of latent infection as exemplified in the situation described here where persistent forms are generated from a normally virulent parasite challenge.

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