A Combination of Antigenic Regions of *Toxoplasma gondii* Microneme Proteins Induces Protective Immunity against Oral Infection with Parasite Cysts

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Infection with *Toxoplasma gondii* causes morbidity and mortality in congenitally infected and immunocompromised individuals. Both humoral and cell-mediated immunity are involved in host resistance to invasion of the parasite. Among putative vaccine candidates, the *T. gondii* microneme proteins appear to be promising, because they are responsible for the invasion process. The present work focused on studying the immunogenicity of microneme proteins in infected individuals and in a mouse model of chronic toxoplasmosis. We identified 5 distinct antigenic regions within MIC2, MIC4, MIC2-associated protein, and apical membrane antigen 1 gene products, which were recognized by (1) T cells from both adults with acquired infection and children with congenital infection and (2) antibodies from all patients. Finally, we demonstrated that DNA immunization with microneme fragments elicited effective protection in mice (84% reduction in brain-cyst burden), suggesting that a combination of these antigenic regions should be considered in the design of potential vaccines against toxoplasmosis.

*Toxoplasma gondii* is an intracellular protozoan parasite that infects all warm-blooded animals, including humans, and that causes significant morbidity and mortality in congenitally infected and immunocompromised individuals [1–4]. Congenital infection can lead to miscarriage, neonatal malformations, or other deficiencies occurring after birth, such as blindness and severe cognitive impairment [5, 6]. In immunocompromised patients, reactivation of chronic *T. gondii* infection can cause encephalitis and focal neurological lesions [7]. In animals, toxoplasmosis is of great economic importance worldwide, because it causes abortion and stillbirth in all types of livestock, particularly in sheep and goats [8]. Moreover, *T. gondii* tissue cysts in the meat of infected animals are the main source of infection in humans [9–11].

Infection of the host by *T. gondii* leads to an acute systemic dissemination of the rapidly multiplying parasite. The acute phase is followed by a chronic phase, in which *T. gondii*, enclosed in a dense intracellular cyst matrix, persists in the brain, the heart, and skeletal muscle [12]. Acute infection results in the activation of cells from the innate and adaptive compartments of the immune system, leading to life-long immunity [13]. The *T. gondii* tissue cysts effectively evade the immune system and remain alive during the lifetime of the host.

Cell-mediated immunity plays the predominant role in host resistance to *T. gondii* infection [14, 15], although recent reports have highlighted the importance of the humoral response [16, 17]. Most of the work on the antigens involved in protective immunity has focused on *T. gondii* molecules that belong to 3 major protein families: the surface antigens (SAGs), the dense granule excreted-secreted antigens (GRAs), and the rhoptry antigens (ROPs).
The microneme proteins look particularly promising because they are responsible for the adhesion and invasion process of all apicomplexan parasites [18, 19]. We recently demonstrated the involvement of the *T. gondii* MIC3 and MIC5 proteins in the human B cell response to parasite infection [20]. In the present work, we focused our attention on determining whether other microneme gene products are involved in the induction of B cell– and T cell–mediated immunity against the parasite in congenitally infected children and in healthy adults with acquired infection. To this end, the MIC1 [21], MIC2 [22], MIC2-associated protein (M2AP) [23], MIC4 [24], MIC6 [25], MIC7 [26], MIC8 [26], MIC9 [26], MIC10 [27], and MIC11 (GenBank accession number AF539702), and apical membrane antigen 1 (AMA1) [28] microneme genes were investigated by use of phage-display technology.

In the present study, we identified 5 distinct protein fragments within MIC2, MIC4, M2AP, and AMA1 gene products that are recognized by antibodies and T cells from infected individuals. Furthermore, we showed that DNA immunization with a mixture of the selected microneme fragments elicits effective protective immunity in mice after oral challenge with a cystogenic *T. gondii* strain.

**SUBJECTS, MATERIALS, AND METHODS**

**Construction and selection of a phage-display microneme library.** *T. gondii* tachyzoites (strain RH) were used for extraction of mRNA, as described elsewhere [29]. cDNA was used as polymerase chain reaction (PCR) template when the coding regions of the MIC1, MIC2, MIC4, MIC6, MIC7, MIC8, MIC9, MIC10, and MIC11, and AMA1 genes were amplified by use of specific oligonucleotides. PCR products were homogeneously mixed, and the resultant mixture (10 μg) was randomly fragmented by use of 1.5 ng of DnaseI (Sigma-Aldrich) for 20 min at 15°C. cDNA fragments that had a molecular size of 200–1200 bp were cloned into vector pGM (Stratagene); 5 × 10⁸ independent clones were yielded. Selection of the microneme library with serum samples from infected individuals and isolation of recombinant phage clones were performed as described elsewhere [20]. In brief, magnetic beads linked to Protein G (Dynal) were incubated with serum for 40 min at room temperature. The beads were then incubated with 5 × 10⁶ pfu of recombinant phages in 1 mL of blocking solution (5% nonfat dry milk, 0.25% Triton X-100, and 10 mmol/L NaCl) for 1 h at 37°C. After centrifugation at 10,000 g for 30 min at 4°C, the supernatant was incubated with glutathione-Sepharose (Amersham-Pharmacia), and recombinant GST fusion proteins were eluted in accordance with the manufacturer’s instructions. Finally, protein purity and content were assessed by SDS-PAGE analysis and Bradford assays, respectively.

For T cell experiments, recombinant proteins were affinity purified as described above and were then dialyzed against PBS. Dialyzed proteins were adsorbed overnight at 4°C with Polymyxin-B agarose (Sigma-Aldrich) before protein content was as assessed described above.

**ELISAs.** ELISAs for recombinant GST fusion products were performed by coating Maxisorb plates (Nunc) with the recombinant proteins at a concentration of 5 μg/mL in 50 mmol/L NaHCO₃ (pH 9.6). After incubation overnight at 4°C, the plates were left in blocking solution (5% nonfat dry milk and 0.05% Tween 20 in PBS) for 1 h at 37°C and were subsequently incubated with human or mouse serum (diluted 1:100 in blocking solution) for 1 h at 37°C. The plates were then washed, and anti-human IgG or anti-mouse horseradish peroxidase–conjugate antibodies (Sigma-Aldrich) were added to each well. Tetramethylbenzidine (Sigma-Aldrich) was used to reveal enzymatic activity. The results were recorded as the difference between the optical densities at 450 nm and 620 nm, as read by an automated ELISA reader (Labsystem Multiskan). The assay was conducted in triplicate for each sample, and mean values were calculated.

**Proliferation assays.** Peripheral-blood mononuclear cells (PBMCs; 1 × 10⁵ cells), isolated from heparinized blood, were suspended in 0.2 mL of RPMI 1640 medium (Gibco) containing 10% human serum. Cells were incubated in the presence or absence of recombinant antigens at 37°C. After 6 days, 1 μCi of [³H]-thymidine was added, and the cells were further incubated for 18 h before being harvested in a beta counter (LKB). Recombinant GST fusion proteins were used at 10 μg/mL and included the following: GST-MIC2a, GST-MIC2b, GST-MIC3, GST-MIC4, GST-M2AP, and GST. Pho-tohemagglutinin (0.5 μg/mL) was used as a positive control for PBMC proliferation. All cultures were prepared in triplicate, and SDs were ≤20% of the means of the triplicate cultures. Antigen-
specific PBMC proliferation was expressed as a stimulation index (SI = the mean of triplicate wells containing recombinant GST fusion proteins divided by the mean of triplicate wells containing GST). A positive response was defined as having an SI greater than the cutoff; for each recombinant GST fusion protein, cutoff values were determined as the mean reactivity of the negative population plus 2 SDs.

Patients. Sixty serum samples from 60 women who acquired T. gondii infection (i.e., who seroconverted) during gestation were included in the study. All serum samples were collected at the Referring Centre for Perinatal Infections of Campania Region (Italy). The serum samples, which were collected after delivery, were analyzed by use of IgG and IgM standard tests (ELFA IgG and IgM; bioMerieux). Thirty-seven serum samples from 37 children with congenital T. gondii infection, who had been enrolled at the center for postnatal management, were also included in the study. Postnatal diagnosis of congenital infection was performed by determining levels of T. gondii–specific IgM and IgA antibodies during the first 3 months of life and levels of T. gondii–specific IgG at birth and at 3, 6, 9, and 12 months of age. Congenital toxoplasmosis was proved on the basis of the persistence of T. gondii–specific IgG beyond 12 months of age. Serum samples from 30 T. gondii–specific IgG–negative subjects, who had been referred to the center for treatment of other infectious diseases (cytomegalovirus infection, hepatitis C, or rubella), were included as controls. The serum samples were analyzed in a blinded fashion. For obtainment of peripheral-blood samples, as part of a routine screening program for congenital toxoplasmosis in Italy, blood was drawn into Vacutainer tubes from 15 women who acquired T. gondii infection during gestation (the samples were collected after delivery), from 15 children with congenital infection (age range, 1–7 years), and from 10 T. gondii–specific IgG–negative women. All of the experiments performed with human samples were conducted at Kenton Laboratories (Rome, Italy) and at the University of Rome “La Sapienza,” in compliance with the current laws of Italy.

Mice and parasites. Female BALB/c mice, 7–8 weeks of age, were purchased from Harlan. Their microbiological status was conventional, and they were maintained in groups of 5 mice/cage, with food and water ad libitum. The avirulent T. gondii strain SSI119 [32] was maintained at Statens Serum Institut (Copenhagen, Denmark) by continual passage in CD1 mice through oral infection with 10–20 parasite cysts. Cysts were obtained by homogenizing the brains of infected mice. All animal experiments were performed at Statens Serum Institut under the inspection of the Animal Experiments Inspectorate, Danish Ministry of Justice.

DNA immunization and T. gondii challenge. To prepare plasmids, cDNA inserts of phage clones tx-2a, tx-1b, tx-11b, tx-13b, and tx-15b and cDNA encoding for the previously identified MIC3 fragment [20] were amplified by PCR with specific primers, to introduce (1) a translation start codon followed by a HindIII restriction site and (2) a translation stop codon followed by a XhoI site at the 5′ and 3′ DNA ends, respectively. The amplified DNA fragments were subsequently cloned into the HindIII and XhoI sites of the mammalian vector pcDNA3.1 (Invitrogen). The plasmids were purified from transformed E. coli by use of the EndoFree Plasmid Kit (Qiagen), in accordance with the manufacturer’s instructions. Purified plasmids were dissolved in endotoxin-free PBS and were stored at −20°C. After digestion with restriction enzymes, the integrity of the DNA plasmids was checked by agarose gel electrophoresis. DNA concentration was determined by measuring the optical density at 260 nm. For the purified plasmids, the ratios of the optical-density values measured at 260 nm and at 280 nm were 1.80–1.95, indicating that the preparations were free from protein contamination.

The vaccination protocol was as follows: Mice (10 mice/group) received injections of 50 μL of a homogeneous mixture of pcDNA3.1 microneme plasmids or empty pcDNA3.1 vector into each tibia anterior muscle at a concentration of 2 mg/mL. Three and 6 weeks after vaccination, the mice were given repeated injections in accordance with the same protocol. Tail bleeds were performed on vaccinated mice 3 weeks after the last DNA injection, to ascertain the levels of T. gondii–specific antibodies.

Mice immunized with either plasmids encoding for microneme fragments or empty pcDNA3.1 vector (the control mice) were infected orally with 30 cysts of avirulent T. gondii strain SSI119. One month after the challenge, the mice were killed, and their brains were removed and homogenized. The mean number of cysts per brain was determined microscopically by counting 4 samples (10 μL each) of each homogenate. The results are expressed as the mean ± SE for each group.

Statistical analysis. Levels of significance for the differences between the groups of mice were determined by use of Student’s t test (2-tailed); equal variance in the 2 groups was assumed.

RESULTS

Identification of antigenic regions within T. gondii microneme proteins. cDNA corresponding to the MIC1, MIC2, M2AP, MIC4, MIC6, MIC7, MIC8, MIC9, MIC10, MIC11, and AMA1 microneme genes was isolated by PCR, randomly digested by use of the E. coli DpnI enzyme, and used to construct a X-display library of cDNA fragments [33]. The resultant library contained 5 × 106 independent clones whose DNA inserts had a mean size of 200–300 nt. The phage-display microneme library was affinity selected by use of serum samples from infants with congenital infection and from women with acquired infection [20]. All of the identified phage clones matched the sequences of the MIC2, M2AP, MIC4, and AMA1 genes (table 1).

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Table 1. Affinity selection of the microneme cDNA display library with serum samples from children with *Toxoplasma gondii* congenital infection.

<table>
<thead>
<tr>
<th>Patient (age, months)</th>
<th>Titer of <em>T. gondii</em>-specific IgG, IU/mL</th>
<th>Clinical manifestation(s)</th>
<th>Microneme gene(s) identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1)</td>
<td>2549</td>
<td>RT and EC</td>
<td>MIC2</td>
</tr>
<tr>
<td>2 (16)</td>
<td>300</td>
<td>RT</td>
<td>MIC2 and M2AP</td>
</tr>
<tr>
<td>3 (48)</td>
<td>2125</td>
<td>RT and EC</td>
<td>MIC2, M2AP, AMA1, and MIC4</td>
</tr>
</tbody>
</table>

**NOTE.** AMA1, apical membrane antigen 1; EC, endocranial calcification; M2AP, MIC2-associated protein; RT, retinochoroiditis.

At the end of this procedure, 5 recombinant phage clones encoding for distinct antigenic regions of the microneme proteins (figure 1) were chosen for further examination. Clones tx-2a and tx-1b, matching the sequence of the MIC2 gene [22], have respective insert lengths of 78 and 144 residues. Clone tx-11b, matching with the sequence of the M2AP gene [23], has an insert length of 226 residues and approximately corresponds to the whole sequence of the *T. gondii* protein. Clone tx-13b, matching with the sequence of the AMA1 gene [28], has an insert length of 194 residues and corresponds to the carboxy-terminal region of the AMA1 gene, without the transmembrane sequence and the cytoplasmic tail. Finally, clone tx-15b, matching with the amino-terminal region of the MIC4 gene [24], has an insert length of 169 residues.

**Immunoreactivity of microneme proteins with antibodies from *T. gondii*-infected individuals.** To characterize the immunological properties of the selected antigen fragments, cDNA inserts of clones tx-2a, tx-1b, tx-11b, tx-13b, and tx-15b (here referred to as MIC2a, MIC2b, M2AP, AMA1, and MIC4, respectively) were subcloned into the bacterial vector pGEX-SN [20]. This resulted in the expression of antigen fragments as fusion proteins at the carboxy-terminal region of the GST protein. Recombinant GST fusion proteins were purified under native conditions from the cytoplasm of *E. coli* cells by 1-step affinity chromatography.

The immunoreactivity of GST-MIC2a, GST-MIC2b, GST-MIC3, GST-M2AP, GST-AMA1, and GST-MIC4 were determined for 97 *T. gondii*-specific IgG–positive serum samples, obtained from either children with congenital infection or women with acquired infection. Thirty *T. gondii*-specific IgG–negative serum samples were assayed as controls. The IgG ELISA performance of the 6 recombinant GST fusion proteins was assessed
by use of the 97 *T. gondii*-specific IgG–positive serum samples, and for each recombinant product the cutoff value was determined as the mean plus 2 SDs of the optical-density readings obtained from the 30 *T. gondii*-specific IgG–negative serum samples. The results of the ELISA analysis are reported in table 2. With the exception of GST-MIC4, all of the recombinant GST fusion proteins reacted with ≥60% of the *T. gondii*-specific IgG–positive serum samples from both groups of infected individuals (those with congenital and acquired infection). None of the *T. gondii*-specific IgG–negative control serum samples reacted with the recombinant GST fusion proteins.

**Table 2.** Comparison of immunoreactivity of recombinant glutathione S-transferase (GST) fusion proteins with IgG antibodies from *Toxoplasma gondii*-infected individuals.

<table>
<thead>
<tr>
<th>Recombinant fusion protein</th>
<th>Serum samples from healthy adults with acquired infection (n = 60)</th>
<th>Serum samples from children with congenital infection (n = 37)</th>
<th>Serum samples from <em>T. gondii</em>-uninfected individuals (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST-MIC2a</td>
<td>55 (92)</td>
<td>34 (92)</td>
<td>0</td>
</tr>
<tr>
<td>GST-MIC2b</td>
<td>37 (62)</td>
<td>25 (68)</td>
<td>0</td>
</tr>
<tr>
<td>GST-MIC3</td>
<td>54 (90)</td>
<td>29 (78)</td>
<td>0</td>
</tr>
<tr>
<td>GST-MIC4</td>
<td>26 (43)</td>
<td>17 (46)</td>
<td>0</td>
</tr>
<tr>
<td>GST-M2AP</td>
<td>44 (73)</td>
<td>33 (89)</td>
<td>0</td>
</tr>
<tr>
<td>GST-AMA1</td>
<td>47 (78)</td>
<td>22 (60)</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of serum samples that reacted with each recombinant GST fusion protein. The immunoreactivity of the recombinant antigen fragments were assayed by ELISA. For each fusion protein, the cutoff value was determined as the mean reactivity of the negative population plus 2 SDs. AMA1, apical membrane antigen 1; M2AP, MIC2-associated protein.

**Proliferative response of PBMCs from infected individuals to microneme antigens.** The recognition of MIC2a, MIC2b, MIC3, M2AP, and MIC4 antigen fragments by PBMCs from *T. gondii*-infected individuals is shown in figure 2. Of 15 PBMC samples from healthy adults with acquired *T. gondii* infection, MIC2a induced PBMC proliferation in 3 (20%), MIC2b induced PBMC proliferation in 3 (20%), MIC3 induced PBMC proliferation in 7 (47%), MIC4 induced PBMC proliferation in 2 (13%), and M2AP induced PBMC proliferation in 4 (27%). Of 15 PBMC samples from children with congenital infection, MIC2a induced PBMC proliferation in 1 (7%), MIC2b induced...
PBMC proliferation in 2 (13%), MIC3 induced PBMC proliferation in 5, MIC4 induced PBMC proliferation in 1 (7%), and M2AP induced PBMC proliferation in 2 (13%). Overall, PBMCs from 9 (60%) of 15 individuals with acquired infection responded to at least 1 of the tested antigens. Similarly, of 15 children with congenital infection, recombinant antigens induced PBMC proliferation in 8 (53%). None of the *T. gondii*-specific IgG–negative control serum samples displayed significant values of PBMC proliferation in the presence of the recombinant GST fusion proteins.

**Effective protection of DNA vaccination in mice.** We evaluated the ability of *T. gondii* microneme proteins to induce, in a DNA-vaccine formulation, an effective protective immunity in mice against chronic toxoplasmosis. DNA encoding for MIC2a, MIC2b, MIC3, MIC4, M2AP, and AMA1 antigen fragments were subcloned into a mammalian expression vector, and a homogeneous mixture of the corresponding plasmids was used to immunize the mice. A *T. gondii*-specific IgG response against the microneme fragments was generated 3 weeks after the last vaccination in all of the immunized mice, whereas no corresponding antibody production was observed in the control mice (which received empty pcDNA3.1 vector) (data not shown). Figure 3 shows the antibody response in immunized mice to the different recombinant proteins expressed in bacteria as GST fusion products. Interestingly, the mice immunized with plasmids encoding for microneme fragments developed *T. gondii*-specific IgG against MIC2a, MIC4, M2AP, and AMA1 protein products, whereas no IgG response was detected against MIC2b and MIC3.

The protective efficacy of plasmid immunization is shown in figure 4. Cyst burden was reduced by 84% in the mice vaccinated with the mixture of microneme plasmids, compared with that in the control mice (*P* = .0021). The mean numbers of cysts per brain in the microneme plasmid–immunized mice and the control mice were 187 and 1163, respectively.

**DISCUSSION**

Micronemes are secretory organelles and are widely conserved among parasites of the phylum Apicomplexa. These organelles are presumed to play a predominant role in the invasion process by discharging protein products capable of interacting with host cell receptors [18, 19]. Despite the variety and complexity of microneme proteins of *T. gondii*, several of these polypeptides exhibit a remarkable likeness to vertebrate adhesive proteins, as they possess integrin-like domains, thrombospondin-like domains, epidermal growth factor–like domains, and lectin-like domains [22, 25, 26, 28, 34].

To date, most of the work on *T. gondii* micronemes has been focused on cellular biological mechanisms, particularly on attempts to identify host cell receptors and to understand the mechanisms of microneme protein assembly and processing. The involvement of this protein family in the host immune response to *T. gondii* infection has been underinvestigated. We recently provided the first experimental evidence that the MIC3 and MIC5 proteins contain antigenic regions that are recognized by immunoglobulins of healthy adults with acquired *T. gondii* infection [20].

The present work allowed the identification of other immunodominant regions of microneme proteins within *T. gondii*.
MIC1, MIC2, MIC4, MIC6, MIC7, MIC8, MIC9, MIC10, MIC11, M2AP, and AMA1 gene products, which are recognized by antibodies from individuals with acquired and congenital T. gondii infection. Challenging a phage-display library of T. gondii microneme cDNA fragments with antibodies present in serum samples from children who were congenitally infected by the parasite enabled the identification of protein regions carrying B cell epitopes in MIC2, M2AP, MIC4, and AMA1 polypeptides.

The immunoreactivity of 5 distinct fragments of MIC2, M2AP, MIC4, and AMA1 proteins, expressed as recombinant GST fusion products, was assessed; overall, IgG in serum samples from 95% of both individuals with acquired and individuals with congenital infection (57/60 adults and 35/37 children, respectively) reacted with at least 1 of the identified antigen fragments (data not shown), which emphasizes the broad recognition of the microneme proteins by the human B cell response.

The microneme proteins MIC2, MIC3, MIC4, and M2AP contain T cell epitopes and elicit lymphoproliferative responses of PBMCs from T. gondii–positive individuals. In humans, the T cell response against the protozoan parasite has been detected during both acute and chronic phases of toxoplasmosis [35–39]. Recently, the use of recombinant proteins has allowed the characterization of T. gondii antigens that are involved in maintaining T helper–mediated immunity [40, 41]. It has been demonstrated in such studies that GRA1, GRA2, GRA6, GRA7, and SAG1 antigens activate CD4+ T cells, although none of the studies were able to induce PBMC proliferation in all positive subjects. Our data demonstrating that PBMCs from both healthy adults with acquired T. gondii infection and children with congenital T. gondii infection react with MIC2, MIC3, MIC4, and M2AP antigen fragments extend to microneme proteins the ability to induce a specific T helper response in humans. Moreover, the evidence that both patients with acquired and patients with congenital infection possess memory B and T cells specific for these proteins highlights the immunogenicity of microneme protein products in humans.

Long-term immunity induced by primary infection provided the incentive for the development of a vaccine against T. gondii. DNA immunization has been shown to provide protection against viral, bacterial, and parasitic infection [42]. DNA vaccination activates especially cytotoxic T cells, which have been difficult to induce with protein subunit vaccines. The ability of plasmid DNA-encoding specific antigens to activate both CD4+ and CD8+ T cells suggests that DNA vaccines will be of particular usefulness in protection against T. gondii infection, for which protective immunity requires a strong contribution of the Th1-type response [14, 43]. Several trials of DNA immunization with genes encoding for T. gondii antigens have been conducted, mainly with membrane-associated SAGs [44, 45], GRAs [46, 47], and ROPs [48, 49].

In the present study, we evaluated the protective effects induced by microneme DNA immunization by orally infecting vaccinated mice with 30 cysts of the avirulent T. gondii strain SSI119 [32]. To this end, a homogeneous mixture of plasmids directing the cytoplasmic expression of selected MIC2, MIC3, MIC4, M2AP, and AMA1 antigen fragments in mammalian cells was used. An effective and highly significant degree of protection was obtained in mice immunized with this plasmid mixture, as measured by the strong reduction of cyst burdens, compared with that in control mice (84% reduction in brain cyst burden [figure 4]).

It is remarkable that a recent study showed protective effects of DNA immunization using a plasmid encoding for full-length MIC3 protein [50]. However, to obtain a strong reduction in cyst burdens, the authors needed to enhance the efficacy of MIC3 immunization by coadministering a plasmid encoding for a cytokine, such as granulocyte-macrophage colony-stimulating factor [51]. Our data show that a combination of antigenic regions of parasite proteins is sufficient to achieve the same or even stronger protection, without the need to coadminister DNA encoding for enhancing factors or to express full-length genes.

Most of the antigens that have been evaluated for the development of recombinant vaccines against T. gondii infection are polypeptides that are prevalently expressed by the parasite during the acute phase of infection. Nonetheless, we recently provided the first experimental evidence that protein products expressed exclusively in tissue cysts are immunogenic in humans [33], thus demonstrating that cyst-specific antigens contribute to the stimulation of both humoral and cell-mediated immunity against T. gondii infection. Consequently, in the design of an effective polycomponent DNA vaccine, it will be useful to blend microneme gene fragments with the antigenic regions of cyst-specific genes, with the goal of being able to perform DNA vaccinations that provide complete protection against toxoplasmosis.

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