Mic1-3 Knockout of *Toxoplasma gondii* Is a Successful Vaccine against Chronic and Congenital Toxoplasmosis in Mice

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**Background.** We evaluated a new vaccine, Mic1-3KO, against both chronic and congenital toxoplasmosis in mice. Mic1-3KO is a mutant strain of *Toxoplasma gondii* RH that lacks the *mic1* and *mic3* genes.

**Methods.** OF1 mice were vaccinated with Mic1-3KO tachyzoites and challenged orally with *T. gondii* (strain 76K). Immune responses and protection against chronic infection (cyst load in brain tissue) and congenital infection (maternofetal transmission, survival, body weight, and chronic infection in pups) were evaluated.

**Results.** Mic1-3KO induced a strong humoral and cellular T helper (Th) 1 response and conferred highly significant protection against chronic infection (>96% reduction in cysts in brain tissue). Fewer infected fetuses were observed in vaccinated dams that were infected during pregnancy than in nonvaccinated infected dams (4.6% vs. 33.3%). All pups born to vaccinated infected dams survived and had the same weight as those born to nonvaccinated uninfected dams. Furthermore, they had significantly fewer cysts in brain tissue (>91%) than pups from nonvaccinated infected dams. During pregnancy, protection against congenital disease was associated with a cellular Th1 response regulated by interleukin-10. One month after delivery, vaccinated infected dams had >96% fewer cysts in their brain tissue than nonvaccinated infected dams.

**Conclusion.** Mic1-3KO is an effective vaccine against chronic and congenital toxoplasmosis.

*Toxoplasma gondii* is a significant human and animal pathogen that causes toxoplasmosis and may infect as much as one-third of the world’s population. This disease is often fatal in immunocompromised patients, such as those with AIDS or neoplastic disease and bone-marrow or heart transplant recipients. Primary infection during human pregnancy can lead to spontaneous abortion, neonatal death, and severe congenital defects, such as hydrocephalus, chorioretinitis, blindness, and mental retardation [1–3]. Congenital toxoplasmosis is also of considerable economic importance in the livestock industry, because it is one of the principal causes of abortion, fetal death, and stillbirths in sheep and goats.

Primary *Toxoplasma* infection leads to an immune response that confers lifelong protection against reinfection and against the congenital transmission of toxoplasmosis. Therefore, it should be possible to develop a safe and effective vaccine against acquired and congenital toxoplasmosis. A vaccine based on live attenuated S48 *Toxoplasma* tachyzoites is available, and it can protect pregnant sheep against toxoplasmosis [4]. However, with a naturally attenuated vaccine such as this, the possibility exists that it might at some point revert to a pathogenic form, which makes it a poor vaccine candidate for humans. Furthermore, if the challenge is of sufficient severity, it will not provide total protection. Furthermore, the attenuated S48 vaccine is difficult to manufacture and has a short shelf life.
Figure 1. Cytokine production by splenocytes from female Swiss OF1 mice vaccinated with Mic1-3KO. Splenocytes were recovered 2 months after vaccination and were stimulated in vitro with Toxoplasma gondii antigen (5 μg/mL). Cell-free supernatants were harvested and assayed for interleukin (IL)–2 and IL-4 activity after 24 h, for IL-10 activity after 72 h, and for interferon (IFN)–γ activity after 96 h. For IFN-γ, IL-2, and IL-10; P < .001 for IL-4. P < .14 for IL-4.

A mutant strain of T. gondii RH that lacks carbamoyl phosphate synthetase II (uracil auxotroph) has been constructed and is completely avirulent not only in immunocompetent mice but also in those that lack interferon (IFN)–γ [5]. A single injection of this mutant strain into BALB/c mice induced long-term protective immunity against intraperitoneal (ip) challenge with RH tachyzoites. One strategy for developing safer vaccines against toxoplasmosis is, thus, to create specific gene-deficient strains of T. gondii. For example, a new mutant strain of T. gondii RH that lacks the mic1 and mic3 genes (the Mic1-3 knockout [KO] parasite) was created. During early invasion, T. gondii secretes proteins from micronemes—specialized organelles located at the apical end of the parasite that play a central role in the recognition of and adhesion to host cells [6, 7]. Microneme proteins (MICs) contain adhesive motifs and, in some cases, bind to putative receptors on host cells. Twelve MICs have been identified and the host–cell surface binding properties of 2 (MIC1 and MIC3) have been demonstrated [8, 9]. MIC1 contains a tandemly duplicated domain that is distantly related to the thrombospondin–1–like domain of thrombospondin–related anonymous protein and that specifically binds lactose [10]. MIC3 contains several epidermal growth factor–like domains and a lectin–like domain that is required for binding [11]. Both MIC1 and MIC3 assemble with other MICs into independent complexes, MIC1/4/6 and MIC3/8. MIC1 is absolutely required for the MIC1/4/6 complex to leave the early compartments of the secretory pathway [12]; thus, disruption of the mic1 gene alone corresponds to a functionally triple-KO parasite. Disruption of either the mic1 gene or the mic3 gene causes slightly reduced virulence in mice, and the double-KO (Mic1-3KO) form is markedly impaired in virulence [13]. Furthermore, Mic1-3KO parasites have been shown to protect male OF1 mice against oral cyst challenge, which is the natural route of infection for acquired toxoplasmosis.

We vaccinated female OF1 mice with Mic1-3KO tachyzoites and evaluated the vaccine’s efficacy against chronic and congenital toxoplasmosis. Our data show that OF1 mice (both virgins and dams) vaccinated with Mic1-3KO were highly protected against oral challenge with T. gondii cysts and that this vaccine conferred highly significant protection against congenital toxoplasmosis. This protection was associated with a strong humoral and a potent Th1 cellular immune response.

MATERIALS AND METHODS

Mice. Eight-week-old male and female outbred Swiss OF1 mice and 8-week-old female inbred CBA/J mice were obtained from Janvier. All experiments were performed in accordance with the Tours University guidelines for animal experimentation.

Parasites. RH strain tachyzoites were maintained by serial ip passage in Swiss OF1 mice, and these were used to prepare T. gondii antigen (TAG) as described elsewhere [14]. Type II strain 76K cysts were obtained from the brains of orally infected CBA/J mice, were maintained by monthly passage, and were used for oral challenge. Type II isolates are predominant in human and sheep congenital toxoplasmosis [15, 16]. Mic1-3KO
Table 1. Effect of vaccination with Mic1-3KO tachyzoites on cyst formation in female Swiss OF1 mice.

<table>
<thead>
<tr>
<th>Experiment, group (no. of mice)</th>
<th>Cysts in brain tissue</th>
<th>Reduction, % (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Counted under the microscope&lt;40</td>
<td>No. positive/no. bioassayed in recipient mice1/7</td>
</tr>
<tr>
<td>1 Vaccinated infected (7)</td>
<td>96–99 (1)</td>
<td></td>
</tr>
<tr>
<td>Nonvaccinated infected (5)</td>
<td>1000 ± 857</td>
<td>ND</td>
</tr>
<tr>
<td>2 Vaccinated infected (7)</td>
<td>95.4–99 (5)</td>
<td></td>
</tr>
<tr>
<td>Nonvaccinated infected (3)</td>
<td>860 ± 173</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Mice were vaccinated by intraperitoneal injection on day 0 with 20 Mic1-3KO tachyzoites and were orally challenged with 45 strain 76K cysts on day 71. The cyst load in brain tissue was analyzed 1 month after challenge. Nonvaccinated mice infected with 45 strain 76K cysts were used as controls. Results are the mean ± SD of the no. of cysts in brain tissue from each mouse. We could not detect any cysts in brain tissue from vaccinated infected mice; therefore, these brain homogenates were bioassayed in recipient mice.

* Significant differences between control and vaccinated mice (in experiment 1; in experiment 2, Mann-Whitney exact test; ordinal scale: 1, cysts not detected; 2, 0–40 cysts; 3, >40 cysts).

Mouse vaccination. Female OF1 mice were vaccinated once ip with 20 Mic1-3KO tachyzoites that had been freshly harvested from cell cultures, using syringes with 26-gauge needles (Microlance; Becton Dickinson). Outbred mice were used to circumvent any genetic restriction of protection.

Humoral response. Titters of antigen-specific IgG antibodies in serum samples obtained from vaccinated mice were determined by an ELISA using TAg, as described elsewhere [14]. The antigen-specific antibody titer is given as the reciprocal of the highest dilution whose absorbance was 2.5-fold greater than the absorbance of the serum of untreated mice at the same dilution. Results are expressed as the mean ± SD of log titers.

Cytokine production. Spleen cells were prepared as described elsewhere [14]. Cells were stimulated with 5 μg/mL of TAg or medium alone. Cell-free supernatants were harvested and assayed for interleukin (IL)-2 and IL-4 activity after 24 h, for IL-10 activity after 72 h, and for IFN-γ activity after 96 h. Concentrations of IL-2, IL-4, IL-10, and IFN-γ were determined by ELISA (OptEIA Set; Pharmingen), in accordance with the manufacturer’s instructions.

evaluation of protection against acquired chronic toxoplasmosis in virgins and dams. Female Swiss OF1 mice, either vaccinated with Mic1-3KO tachyzoites or untreated, were orally challenged on day 71 (which corresponded to day 11 of gestation for pregnant mice) with 45 cysts of *T. gondii* strain 76K. Protection was evaluated 1 month after challenge by analyzing the cyst load in brain tissue. Mouse brains were homogenized in 5 mL of PBS, and the number of tissue cysts per brain was analyzed by counting 12 samples (10 μL each) of each homogenate under the microscope. When cysts were not found in brain tissue by counting, a bioassay for viable *T. gondii* cysts was performed. After centrifugation at 2000 g for 5 min, brain tissue was resuspended in 1 mL of RPMI 1640 and administered orally (2 doses of 0.5 mL each) to uninfected recipient Swiss OF1 mice (an entire brain-tissue sample to a single recipient mouse). Six weeks later, blood samples were obtained from the retro-orbital plexus, and serum was assayed for the presence of anti-Toxoplasma IgG by ELISA.

evaluation of protection against congenital toxoplasmosis. Female OF1 mice vaccinated with Mic1-3KO were mated with males on day 60. Females were placed in a male’s bedding for 48 h, to synchronize estrus, and were then caged with males (ratio, 1:3) for 72 h. Pregnant females were infected orally on day 11 of gestation with 45 cysts of strain 76K. The 2 control groups were nonvaccinated infected mice (oral infection on day 11 of gestation) and nonvaccinated uninfected mice. On day 17 of gestation, 4 vaccinated infected pregnant females and
Table 2. Maternofetal transmission of Toxoplasma gondii.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of litters</th>
<th>No. of fetuses</th>
<th>Positive fetuses, % Total Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated infected</td>
<td>4</td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>Nonvaccinated infected</td>
<td>3</td>
<td>33</td>
<td>11</td>
</tr>
</tbody>
</table>

NOTE. The presence or absence of T. gondii infection was assessed in fetuses before delivery, on day 17 of gestation (day 6 of infection). Fetuses from nonvaccinated infected mice (3 litters, 33 fetuses) and from vaccinated infected mice (4 litters, 43 fetuses) were homogenized separately in PBS, then inoculated intraperitoneally into recipient OF1 mice, to ascertain whether the fetuses were infected. Six weeks later, blood samples were obtained from the retro-orbital plexus, and serum was assayed for the presence of Toxoplasma antibody using ELISA. The no. of positive fetuses corresponds to the no. of mice positive according to ELISA for the presence of Toxoplasma antibody.

3 nonvaccinated infected pregnant mice were killed and tested for congenital infection in utero. Fetuses were delivered via transabdominal incision under sterile conditions. Fetal tissues were homogenized in 3 mL of PBS that contained penicillin (100 U/mL) and streptomycin (100 μg/mL; PBS + P/S) and then filtered through nylon mesh. After centrifugation at 2000 g for 5 min, fetal tissues were resuspended in 0.5 mL of PBS + P/S and inoculated ip into uninfected recipient mice (1 fetus/1 recipient mouse). Six weeks later, blood samples were obtained from the retro-orbital plexus. Serum samples were tested for Toxoplasma antibody IgG by ELISA. Naturally delivered pups were evaluated for protection against congenital infection by analyzing survival from day 1 to day 35 of age, weight on day 11 of age, and cyst load in brain tissue on day 35 of age.

Statistical analysis. Levels of significance of the differences between groups of mice were determined using the Mann-Whitney U test, the Mann-Whitney exact test (when applicable), or Fisher’s exact test (for maternofetal transmission and complete protection).

RESULTS

Immune Responses after Vaccination

By 23 days after vaccination with 20 Mic1-3KO tachyzoites, vaccinated female mice had seroconverted (experiment 1, n = 14; experiment 2, n = 15). Antibody titers were measured by ELISA using TAg: the anti–T. gondii IgG titers were 125/H11506 in experiment 1 and in experiment 2. These results indicated that Mic1-3KO induced a strong humoral response.

Cytokine production by TAg-stimulated splenocytes demonstrated that vaccination potentiated a strong Th1 immune response as measured by IFN-γ production. Supernatants from restimulated splenocyte cultures from 8 vaccinated GF1 mice contained more IFN-γ (285 ± 125 ng/mL) than supernatants

Figure 2. Survival and body weight of neonates born to Swiss GF1 mice vaccinated with 20 Mic1-3KO tachyzoites, then infected orally with 45 strain 76K cysts on day 11 of gestation (vaccinated infected). Neonates born to nonvaccinated uninfected mice and to nonvaccinated mice infected orally with 45 strain 76K cysts were used as controls. Neonate survival was monitored for 35 days (A), and pups were weighed when they were 11 days old (B).
from 6 untreated mice (3.9 ± 2.9 ng/mL) (fig 1). Restimulated splenocyte cultures from vaccinated mice also synthesized more IL-2 (467 ± 252 pg/mL) than cultures from untreated mice (20 ± 28 pg/mL) (fig 1). By contrast, no specific release of IL-4 was detected in any culture supernatant. IL-10 secretion, which controls the Th1 immune response, was greater in vaccinated mice (ng/mL) than in untreated mice (0.6 ± 0.5 ng/mL).

Protection against Chronic Infection
Vaccinated female mice were infected orally with 45 cysts of strain 76K on day 71 (2 months after vaccination). The number of cysts in brain tissue was counted microscopically 1 month after challenge (table 1). Nonvaccinated mice served as controls. In 2 independent experiments, all vaccinated infected mice (n = 7 in each experiment) had <40 cysts in brain tissue (we did not detect any cysts; the reduction in the number of cysts was >96% in experiment 1 and >95.4% in experiment 2), which is much fewer than in control nonvaccinated infected mice (experiment 1, 1000 ± 857 cysts; experiment 2, 860 ± 173 cysts). Brain homogenates from the experimental mice (vaccinated and infected but <40 brain cysts detected) were subsequently administered orally to uninfected recipient mice, to test whether the experimental mice had developed any cysts capable of causing reinfection. Anti-T. gondii IgG was produced by 1 of 7 (experiment 1) and 5 of 7 (experiment 2) mice, which indicates that some cysts had developed in vaccinated infected mice. Complete protection efficacy was 85.7% (6/7 mice; significant at P = .015) in experiment 1 and 28.5% (2/7 mice; not significant in experiment 2). The difference in the number of cysts in brain tissue was statistically significant in both experiments (experiment 1, P = .0012; experiment 2, P = .0025).

Protection against Congenital Toxoplasmosis
No abortions were observed in vaccinated mice. Vaccination with Mic1-3KO also did not modify the number of fetuses per litter in either experiment 1 (vaccinated, 11.2 ± 2.5; control, 11.8 ± 5.6) or experiment 2 (vaccinated, 12.07 ± 2.6; control, 11.4 ± 1.3).

Maternofetal transmission. Pregnant mice were infected on day 11 of gestation. Maternofetal transmission was investigated on day 17 of gestation by ip subinoculation of fetal tissues into naive mice (table 2). Transmission of the parasite to fetuses in experimental mice (vaccinated and infected) was significantly lower (P = .001; 2/43 fetuses positive [4.6%]) than in control mice (nonvaccinated infected; 11/33 fetuses positive [33.3%]).

Survival of neonates. Neonate survival was 100% for mice vaccinated with Mic1-3KO and then infected orally with strain 76K on day 11 of gestation in both experiments (fig 2). All neonates born to vaccinated infected mice in experiment 1 (n = 14) and experiment 2 (n = 15) survived until the end of the experiments (day 35 of age). In the control groups, litters born to nonvaccinated infected mice had lower survival (60% in experiment 1 and 64% in experiment 2).

Mean weight of pups. Live pups were weighed when they were 11 days old (fig 2). Pups born to experimental mice (vaccinated and then infected orally with strain 76K cysts on day 11 of gestation) weighed more (experiment 1, 5.8 ± 0.25 g; experiment 2, 5 ± 0.5 g) than pups born to control mice (nonvaccinated infected; experiment 1, 3.2 ± 0.95 g; experiment 2, 2.7 ± 1.1 g). The difference in weight was statistically significant in both experiments (P < .001). The mean weight of pups born to vaccinated infected mice was similar to that of pups born to nonvaccinated uninfected mice (experiment 1, 5.32 ± 0.58 g; experiment 2, 5 ± 0.9 g).

Cyst load in brain tissue. On day 35 after birth, pups were killed, and the number of cysts in brain tissue was analyzed (table 3). Each brain was homogenized in 3 mL of PBS. Brain cysts were counted under the microscope. Tissue cysts were detected in all pups (n = 53) born to nonvaccinated infected mice (n = 7; 618 ± 557 per brain). Forty-five percent of pups (n = 71) born to vaccinated infected mice (n = 14) had 56 ± 34 cysts per brain (91% reduction). The difference in the number of cysts in brain tissue was statistically significant (P < .0001). No cysts were detected in 55% of other pups (n = 86), which indicates that these pups had <25 cysts per brain (>96% reduction).

Cytokine Production after Infection during Pregnancy
We investigated cytokine production by maternal spleen cells on day 17 of gestation (day 6 of infection) (fig 3). IFN-γ and IL-2 production was much higher in TAg-stimulated splenocytes from vaccinated infected mice than in those from nonvaccinated uninfected mice. Stimulated splenocytes from nonvaccinated infected mice did not produce significant levels of IFN-γ and IL-2, compared with those from nonvaccinated un-

Table 3. Effect of vaccination on cyst load in brain tissue from neonates.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of litters</th>
<th>Cysts in brain tissue, mean ± SD (g)</th>
<th>Reduction in cysts, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated infected</td>
<td>14</td>
<td>56 ± 34* (45) [71]</td>
<td>91</td>
</tr>
<tr>
<td>Nonvaccinated infected</td>
<td>6</td>
<td>618 ± 557 (100) [53]</td>
<td>&gt;96</td>
</tr>
</tbody>
</table>

NOTE. Pups were killed, and the no. of cysts in brain tissue was analyzed on day 35 after birth. Brains of pups from nonvaccinated infected mice (6 litters, 53 pups) and from vaccinated infected mice (14 litters, 157 pups) were homogenized separately in 3 mL of PBS and counted under the microscope. * P < .0001.
infected mice. We found no specific release of IL-4 in any culture supernatant. However, as demonstrated in virgin mice 2 months after vaccination (fige 1), some IL-10 was secreted into the supernatants of restimulated splenocyte cultures from vaccinated and infected mice. No specific release of IL-10 was observed in cells from nonvaccinated mice.

Cyst Load in Brain Tissue of Mice 1 Month after Delivery

The cyst load in brain tissue was evaluated in dams ~1 month after delivery (table 4). Vaccinated female mice were orally challenged with 45 strain 76K cysts on day 71 (day 11 of gestation). In experiment 1, 10 vaccinated dams had <40 tissue cysts, and 4 dams had 40 tissue cysts; this corresponds to a 97.7% reduction in the number of cysts with reference to nonvaccinated infected dams (1798 ± 1139 cysts, n = 7). Brain homogenates were then bioassayed in nonvaccinated uninfected recipient mice; 5 brain homogenates contained cysts. In experiment 2, all vaccinated dams had <40 cysts in tissue (n = 15), a 96.7% reduction in the number of cysts with reference to nonvaccinated infected dams (1205 ± 846 cysts, n = 14). When bioassayed, 14 brain homogenates contained cysts. The efficacy of complete protection was 35.7% (5/14 mice) in experiment 1 and 6.6% (1/15 mice) in experiment 2 (not significant in both experiments (P < .0001).

DISCUSSION

At present, only 1 vaccine against toxoplasmosis has been licensed, and this vaccine uses a strain that has no ability to induce chronic infection. The possibility that live vaccines may revert to more-virulent forms gives impetus to the development of safer strains by the disruption of virulent genes.

Congenital infection occurs only when mothers first encounter T. gondii during pregnancy. Resistance to T. gondii is mainly mediated by Th1 cytokines, particularly IFN-γ and IL-2. Susceptibility of the pregnant host to toxoplasmosis may be due to a Th2 cytokine bias that is maintained during gestation [17, 18]. Pregnant mice are more susceptible to infection with T. gondii and have higher mortality than similarly infected nonpregnant female mice [17, 19]. This increased susceptibility of pregnant mice to T. gondii infection is associated with reduced Th1 function, as demonstrated by a decrease in the capacity to produce IFN-γ [20]. Furthermore, the survival of pregnant mice infected with T. gondii has been shown to be improved by the administration of Th1 cytokines, such as IFN-γ and IL-2 [20]. The immune response of mice to T. gondii during pregnancy has also been studied using transgenic IL-4–deficient BALB/c mice. Pregnant wild-type mice are more susceptible than IL-4–deficient mice to toxoplasmosis, and they have higher parasite loads. Pregnant IL-4–deficient mice have also demonstrated a lower transmission rate to fetuses than wild-type mice [18]. However, although pregnant C57BL/6 × 129SV IL-4–deficient mice were
Table 4. Effect of vaccination on cyst load in brain tissue from dams, 1 month after delivery.

<table>
<thead>
<tr>
<th>Experiment, group</th>
<th>Total no.</th>
<th>Microscopically counted&lt;sup&gt;a&lt;/sup&gt; (no.)</th>
<th>No. positive/ no. bioassayed in recipient mice&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Reduction, % (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Vaccinated infected</td>
<td>14</td>
<td>&lt;40 (10)</td>
<td>5/10</td>
<td>100 (5)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 (4)</td>
<td>ND</td>
<td>97.7–99 (9)</td>
</tr>
<tr>
<td>Nonvaccinated infected</td>
<td>7</td>
<td>1798 ± 1139</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>2 Vaccinated infected</td>
<td>15</td>
<td>&lt;40 (15)</td>
<td>14/15</td>
<td>100 (1)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96.7–99 (14)</td>
</tr>
<tr>
<td>Nonvaccinated infected</td>
<td>11</td>
<td>1205 ± 846</td>
<td>ND</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mice were injected intraperitoneally on day 0 with 20 Mic1-3KO tachyzoites. Vaccinated mice were orally challenged with 45 cysts of *Toxoplasma gondii* strain 76K on day 71 (day 11 of gestation), and the cyst load in brain tissue was analyzed 1 month after delivery. Nonvaccinated mice infected with the same no. of 76K cysts were used as controls. Results are the mean ± SD of the no. of cysts in brain tissue from each mouse. We could not detect any cysts in brain tissues from vaccinated mice, so their brain homogenates were bioassayed in recipient mice.

<sup>b</sup> Brain homogenates were administered orally to uninfected recipient OF1 mice, to ascertain whether the donor mice were infected. Six weeks later, blood samples were obtained from the retro-orbital plexus, and serum was assayed for the presence of *Toxoplasma* antibody by using ELISA. ND, not done.

<sup>c</sup> Significant differences between control and vaccinated mice (P<0.0001 in both experiments, Mann-Whitney exact test; ordinal scale: 1, cysts not detected; 2, 0–39 cysts; 3, >40 cysts).

more resistant to *T. gondii* infection than were wild-type controls, no reduction in fetal transmission was seen in these mice [21]. Effects from the genetic background of the mice may account for these apparently contradictory results.

The immune response generated by Mic1-3KO vaccination was clearly a Th1 response. Measurements of the cyst burden in the brain showed that the response effectively protected against adult acquired *T. gondii* infection after oral challenge with cysts of strain 76K type II.

Mice were mated 2 months after vaccination with Mic1-3KO, because reproductive failure was observed when mice were mated earlier (e.g., 1 month after vaccination; data not shown). It is possible that an ongoing anti-parasite Th1 response adversely affects both implantation and maintenance of the placenta, as reported by Krishnan et al. [22] in C57BL/6 mice infected with *Leishmania major*. Infection of C57BL/6 mice infected with *L. major* normally results only in a minor self-healing lesion at the site of inoculation and full recovery of the mouse mediated by a strong Th1 response. When C57BL/6 mice were infected with *L. major* at intervals before and during pregnancy, the frequency of implantation failure and fetal resorption (abortions) clearly increased. Unlike C57BL/6 mice, BALB/c mice infected with *L. major* showed a Th2 cytokine pattern with a resultant progressive infection and, ultimately, death; however, and again unlike C57BL/6 mice, BALB/c mice did not have lower implantation rates.

The Mic1-3KO vaccination reduced the rate of materno-fetal transmission of toxoplasmosis. Pregnant vaccinated mice showed a Th1 cytokine pattern after challenge, 6 days after infection. This is similar to the cytokine pattern seen 2 months after vaccination, before challenge, in virgin mice. In both pregnant vaccinated mice that were then challenged and virgin vaccinated mice, IL-10 was produced without a significant production of IL-4. This suggests that the protective Th1 immune response induced by vaccination is balanced during pregnancy. Indeed, a Th1-induced immune response before gestation is important for the prevention of congenital transmission. Long and Baszler [23] showed that modulation of the cytokine response in naive mice during gestation does little to change the frequency of congenital protozoal transmission (*Neospora caninum*), whereas the manipulation of antigen-specific maternal immune responses before gestation changes the susceptibility of dams to congenital transmission. In particular, the down-regulation of the Th2 antigen-specific immune response before gestation results in a significant decrease in congenital transmission after challenge infection during gestation. However, as described above, the Th1 immune response may be a cause of immune-mediated failure of pregnancy, and modulation of the Th1 response may underlie the delicate balance between the maintenance of pregnancy and resistance to the parasite.

During pregnancy, on day 17 of gestation, only 33% of the fetuses in control nonvaccinated mice showed signs of infection; however, by 35 days after birth, all pups in this group were infected. This may have been due to in utero transmission
at the end of gestation or during delivery or, possibly, to transmission via lactation [24–26]. However, we did not test this by placing the pups with foster mothers to prevent possible infection via lactation, and we showed that OF1 dams developed enough immunity to protect their pups. Indeed, all pups born to vaccinated dams survived (only 60.05% of pups born to control nonvaccinated dams survived); they did not weigh less than pups born to naive mice, and there were significant fewer cysts in their brains than in those from pups born to nonvaccinated mice (brain cysts were not detected in 55% of pups born to vaccinated dams). Further analyses are needed to ascertain whether these pups are infected. Serum samples were collected from 5 litters of pups born to vaccinated dams (n = 57 pups) before the pups were killed. Anti- T. gondii antibodies were not detected by ELISA in 11 pups (19%), which indicates that these pups were not infected.

A significantly smaller number of cysts in brain tissue was seen in vaccinated dams and in virgin mice than in nonvaccinated dams ~1 month after birth (>96% cyst reduction). Pregnant OF1 mice were not more susceptible to chronic infection than nonpregnant mice. No significant difference was seen between the 2 groups in their cyst load in brain tissue. Increased susceptibility to chronic infection has been reported in BALB/c mice [18] and in CBA/J mice (C. Beauvillain, INSERM U564, Angers, France, personal communication).

In conclusion, the present results demonstrate, to our knowledge for the first time, that Mic1-3KO is a potent, effective, and successful vaccine against toxoplasmosis because it provides highly significant protection against acquired and congenital T. gondii infection in female OF1 mice. This protection was associated with strong and specific humoral and cellular immune responses in adult mice. In addition, our model constitutes an attractive experimental model for the development of vaccines that may overcome the drawbacks of the existing 548 vaccine. This vaccine has to be evaluated in relevant animals, such as sheep.

Acknowledgments

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References