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Neutralization of Maternal IL-4 Modulates Congenital Protozoal Transmission: Comparison of Innate Versus Acquired Immune Responses

Maureen T. Long and Timothy V. Baszler

IL-4 levels were modulated in mice to test the hypothesis that induction of a maternal type 1 response would decrease the frequency of congenital Neospora caninum transmission. This hypothesis tested the relationship between IL-4 and both innate and adaptive immunity utilizing two basic experimental designs. In the first, maternal IL-4 was neutralized with mAb during pregnancy in naive mice concomitant with initial, virulent infection. In the second, maternal IL-4 was neutralized before pregnancy concomitant with a priming inoculation consisting of live, avirulent N. caninum tachyzoites followed by virulent challenge during subsequent gestation. In mice that were naive before pregnancy, neutralization of IL-4 during gestational challenge did not result in decreased congenital transmission as measured by PCR performed on 1-day-old neonatal mice. In mice that were primed and modulated before pregnancy, congenital transmission from gestational challenge was significantly decreased compared with control mice. Reduction in transmission constituted a decrease in the numbers of mice transmitting N. caninum and a lower frequency of transmission by individual dams (p < 0.05). Decreased congenital transmission was associated with significantly lower levels of maternal splenocyte IL-4 secretion, lower IL-4 mRNA levels, and higher levels of IFN-γ secretion. Protected mice had significantly decreased Neospora-specific IgG1 compared with nonmodulated mice. These studies define a relationship between maternal Ag-specific immunity and the frequency of congenital transmission and demonstrate that modulation of type 2 cytokine responses can change the frequency of congenital protozoal transmission. The Journal of Immunology, 2000, 164: 4768–4774.

Neospora caninum is an emerging apicomplexan protozoan described worldwide. Maternal infection with N. caninum causes congenital infection resulting in abortion, neonatal neurological disease, or asymptomatic infection of offspring (1). Multiple species, including nonhuman primates, are susceptible to transplacental transmission (1, 2) with N. caninum abortion and congenital infection in cattle, causing significant industry losses (3).

Two salient features characterize the syndrome of neosporosis: 1) several epidemiological investigations indicate that congenital transmission, which results in normal but infected offspring, is a primary mode of N. caninum transmission in cattle (4–7); 2) vertically and horizontally acquired maternal infection results in repeated production of infected offspring in consecutive gestations (4, 8, 9). Because N. caninum perpetuates its life cycle primarily through vertical transmission and initial maternal infection results in minimal long term immunity, control of maternal infection is a primary focus of research for the prevention of clinical neosporosis.

Immunological hypotheses have been proposed to explain maternal susceptibility to Plasmodium falciparum during pregnancy, and these can also be extended to protozoan infection in general (10). One theory proposes that maternal immunity is a primary factor for enhanced susceptibility of the dam to malaria and toxoplasmosis (10, 11). An underlying assumption of this hypothesis is that pregnancy-induced change in maternal immunity affects factors such as maternal parasite load and, in the case of N. caninum, may result in an increase in the frequency of congenital transmission. Other experiments, also with P. falciparum, support a second hypothesis that specific parasite strains have a unique biological niche in the placenta, implying that transmission may occur independent of the state of maternal immunity (12). The current research investigates the relationship between maternal immunity and the susceptibility to Neospora congenital transmission.

Susceptibility of the pregnant host to protozoal infection (and congenital transmission) may be due to a type 2 cytokine bias maintained during gestation (11, 13, 14). The type 2 cytokine bias has been characterized in the murine placenta and is associated with successful implantation, maintenance of early pregnancy, and suppression of local inflammatory responses (15–17). Type 1 responses are down-regulated during pregnancy to induce maternal tolerance of the semiallogeneic fetus, and systemic and placental type 1 cytokines are associated with early embryonic death (16, 18–20). Paradoxically, decreased type 1 cytokines are conducive to pregnancy maintenance; however, the pregnant host may be unable to control parasitic challenge due to down-regulation of generally protective type 1 cytokines (21). Accordingly, enhanced susceptibility to protozoal infection during gestation increases the risk of placental infection and congenital transmission (11, 14).

Studies of toxoplasmosis and malaria have associated cytokine responses with susceptibility to congenital and placental infection in mice and humans, respectively (11, 14, 22–24). The role of IL-4 in the regulation of congenital protozoal disease is controversial.
Naive IL-4 knockout mice were shown to be susceptible to *Toxoplasma gondii* congenital transmission although more resistant to systemic disease (25). Others have shown that *T. gondii* congenital transmission was decreased by as much as 50% in IL-4 knockout mice (26). Both of these investigations focused on innate immune responses because only naïve mice were studied. The study presented here investigates the hypothesis that induction of maternal type 1 responses against *N. caninum* will prevent congenital transmission. Previous studies have identified a relationship between the presence of Ag-specific IL-4 responses in mice and susceptibility to systemic neosporosis (27). The purpose of the current study is to compare induction of innate and adaptive immune responses against *Neospora* congenital transmission through neutralization of IL-4 in an immunologically intact BALB/c model.

**Materials and Methods**

**Cell culture, Abs, and Ab reagents**

J. P. Dubey (Beltsville, MD) provided the *N. caninum* isolate (NC-1). DMEM, 2-ME, pyruvate, t-glutamine, and HEPES buffer were purchased from Sigma (St. LO). Vero cells (CCL 81), mAb against *N. caninum* (11B11; HB 188) and IFN-γ (R46A2, HB 170), and an IgG1 isotype control mAbs (Y13-259, CRL 1742) were acquired from American Type Culture Collection (Manassas, VA). mAbs 11B11 and Y13 were used for the neutralization of IL-4 and isotype control, respectively; mAbs 11B11 and R46A2 were used for capture Abs in the cytokine ELISA. Detection Abs, biotinylated RVD-24 and XM1G1.2 for IL-4 and IFN-γ were used for the cytokine ELISA and obtained from PharMingen (San Diego, CA). Caltag (South San Francisco, CA), manufactured the biotinylated anti-IgG1 and anti-IgG2a Abs, used for detection in the isotype ELISA. Avidin-biotin peroxidase and polyclonal goat anti-Neospora Abs were purchased from Cappel, Organon Teknika (West Chester, PA) and Veterinary Medical Research Development (Pullman, WA), respectively.

**Parasites and preparation of parasite Ag**

The NC-1 isolate was passaged at least once in vivo in mice since arrival in our laboratory. After isolation from mice, tachyzoites were passaged in vitro in Vero cell culture. Viability determined by fluorescein staining, and counted as previously described (25, 28–30). Parasites were passaged weekly either <10 or >40 times and used for virulent and avirulent challenge, respectively. Cell passage number was based on preliminary experiments wherein *N. caninum* was noted to lose virulence after several in vitro passages (data not shown). All infectious dosages were resuspended in a final volume of 200 μl/mouse in PBS. *N. caninum* Ag was used for stimulation of lymphocytes and prepared as previously described (31). The protein in the supernatant was quantitated by a commercial protein assay (BCA, Pierce, Rockford, IL) with BSA as a standard and stored at −80°C until use.

**Pregnancy studies**

For mating, a single male BALB/c mouse was placed with a single female BALB/c mouse, and the female was observed daily for the formation of a vaginal plug. The first day a vaginal plug was observed was designated day 0. Mice were infected i.p. between days 5 and 9 after vaginal plug formation depending on the particular experiment.

**Modulation of IL-4 during gestation.** To determine whether induction of a type 1 response would block congenital transmission in naive mice, IL-4 was neutralized during gestation. In these experiments, two groups of mice were utilized: 1) mice infected during gestation and treated with 5 mg mAb to IL-4; and 2) mice infected during gestation and treated with 2 mg mAb isotype control. In the first study, mice were treated with neutralizing Ab or the isotype control Ab and infected with 2 mg *N. caninum* tachyzoites during gestation. In the first study, mice were challenged at day 5 of gestation; in the second study, mice were challenged between days 7 and 9 of gestation. The mean congenital infection rate per dam was calculated, and differences between groups were detected by ANOVA. If significant differences between groups were found, pairwise comparisons were performed by the Student-Newman-Keuls method to identify differences (SigmaStat, Jandel Scientific, San Rafael, CA).

**PCR detection of *N. caninum***

Fetal tissues were lysed in a buffer containing 0.50 mM Tris-HCl (pH 8.0 at 25°C), 100 mM EDTA, 100 mM NaCl, 200 μg proteinase K, and 1% SDS overnight at 55°C. The DNA was extracted with phenol-chloroform-isomyl alcohol (25:24:1), precipitated with 3 M sodium acetate and 100% ethanol at −20°C, and washed with 70% ethanol; after drying, the DNA was resuspended in deionized water. A seminested PCR procedure was utilized to detect *N. caninum*. The target for the first reaction was a 321-bp sequence generated after priming reaction with NCP13 (5′-ACATTTACTCGTGTAAC-3′) and NP58 (5′-GGTGAACCGAGGGTGTGG-3′) that is specific and sensitive to one organism/10 mg of murine CNS tissue (36). The second target consisted of the same reverse primer and another primer sequence, NP7 (5′-CCCTCCCAATGTGCCAGGTTTG-3′), to generate a 220-bp target (36). Amplification for the first reaction consisted of a 50-μl reaction mix composed of 1 μg sample DNA, 2.5 mM MgCl2, 25 pmol each primer, reaction buffer (Promega, Madison, WI), 10 mM (NTPs), and 0.5 U Taq DNA polymerase. The same reaction mixture was utilized in the second round except that 2 μl of the first reaction and 3.5 mM MgCl2 were used. All reactions were performed in the same thermocycler (GeneAmp 2400, Perkin-Elmer, Norwalk, CT) with the following reaction parameters for the first round: denaturation, 94°C for 60 s; annealing, 55°C for 60 s; extension, 74°C for 60 s for a total of 25 cycles. Thirty cycles were performed in the second reaction with an annealing temperature of 56°C as the only variation. Reaction products were analyzed by electrophoresis through a 1.8% agarose gel stained with ethidium bromide and photographed under UV light (IA-200 Chemilmager, Imaging, a Innotech, San Leandro, CA). Controls consisted of the following: positive control composed of negative fetal tissue to which *N. caninum* tachyzoites were added; negative control tissues composed of fetal and neonatal tissues from a noninfected dam; and no DNA control. Targets were sequenced and confirmed to be the same as that published for this gene (data not shown). For confirmation of these findings, the entire process was repeated on the stored tissue samples.

**rt-PCR for IFN-γ and IL-4 mRNA***

Cytokine status was evaluated ex vivo in all three groups of mice modified before gestation and challenged with virulent *N. caninum* between days 7 and 9 of gestation (BLKBG, ISOBG, and SALBG) by measurement of relative amounts of murine IFN-γ and murine IL-4. Single-cell suspensions were obtained after spleens were removed from dams. Total
RNA from $6 \times 10^6$ splenocytes/mouse was extracted with acid guanidinium thiocyanate-phenol-chloroform (RNAsol, Cinna/Biotex Laborato-
ries, Houston, TX) without culturing or Ag stimulation according to man-
ufacturer’s recommendations. The purity and concentration of the samples
were determined spectrophotometrically. Relative amounts of mRNA for IFN-
$\gamma$ and IL-4 were determined by PCR amplification for each sample
using a mouse-specific PCR kit (Clontech, Palo Alto, CA) in a one-step
RT-PCR reaction (Titan TM, Boehringer-Mannheim, Indianapolis, IN).
Before amplification, 1 $\mu$g RNA was treated with DNase (DNase I, Life
Technologies, Gaithersburg, MD) following manufacturer’s recommenda-
tions. For PCR, manufacturer’s recommendations were utilized for ampli-
fication protocols and cycle parameters. Controls consisted of 1) mRNA
added in the place of cDNA to control for genomic DNA contamination, 2)
$\beta$-actin cDNA generated within each tube to control for tube to tube vari-
atation in efficiency and calculation of relative cytokine amounts, 3) positive
control tubes consisting of each target, and 4) negative control tubes with-
out cDNA. Amplicons were resolved on 1.8% agarose gels stained with
ethidium bromide and target bands for each mouse were quantitated by
densitometry. Values for densitometry were adjusted for background and
background and ratios of IL-4 to $\beta$-actin and IFN-$\gamma$ to $\beta$-actin were calculated for each dam.
The mean count and SD of the cytokine-modulated group, whereas 6 of 10
whelped in the cytokine-modulated group, whereas 6 of 10 whelped in the
neutralized group increased the number of whelping mice in the modulated
group. In a second experiment, 7 of 10 BLKDG mice whelped and 5 of
BLKDG mice whelped (Table I). Regardless of cytokine mod-
tification, there was no statistical difference in mean frequency of
transmission between the BLKDG and ISODG control group

### Immunohistochemistry

Four sections of maternal lung and two sections of liver were examined by an avidin-biotin-peroxidase complex immunoperoxidase method using polyclonal goat antiserum to *N. caninum* (41) except that anti-*N. caninum*
sperm was diluted at 1:1000 in Tris buffer with 5% normal rabbit serum.
Tachyzoites were counted in each section on each slide twice in a blinded
manner. The mean numbers of lesions and tachyzoites in each group were
calculated and compared by a Student *t* test.

### Results

**Neospora congenital transmission after neutralization of IL-4 in naive mice**

In the first set of experiments, IL-4 was neutralized during gesta-
tion in naive mice. Two experiments were performed, and in both
neutralization of IL-4 did not decrease *Neospora* congenital trans-
mission (Table I). In experiment 1, only 2 of 10 mice whelped in the
cytokine-modulated group, whereas 6 of 10 whelped in the
nonmodulated group, and all mice transmitted *N. caninum* to a
portion of their litters. This experiment was repeated with the modi-
fication that mice were neutralized and infected later in gestation
to increase the number of whelping mice in the modulated group.
In a second experiment, 7 of 10 BLKDG mice whelped and 5 of
10 ISODG mice whelped (Table I). Regardless of cytokine mod-
ulation, there was no statistical difference in mean frequency of
transmission between the BLKDG and ISODG control group of
mice.

### Table I. *N. caninum* transmission in naive mice with IL-4 neutralization during gestation

<table>
<thead>
<tr>
<th>Cytokine Modulation During Gestation</th>
<th>N. caninum-Positive Offspring/Mouse</th>
<th>Total Offspring/Mouse</th>
<th>Frequency of Transmission/Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Challenged with <em>N. caninum</em> at 5 days gestation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLKDG$^a$</td>
<td>3</td>
<td>7</td>
<td>0.43</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>ISODG$^a$</td>
<td>4</td>
<td>7</td>
<td>0.57</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.75</td>
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</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td><strong>Challenged with <em>N. caninum</em> at 7–9 days gestation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLKDG</td>
<td>3</td>
<td>6</td>
<td>0.50</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>ISODG</td>
<td>9</td>
<td>10</td>
<td>0.90</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Mice were treated with Ab to IL-4 during gestation.
$^b$ Mice were treated with isotype control Ab during gestation.

**Cytokine ELISA**

Secondary Ag-specific responses were evaluated only in the primed mice
that were rechallenged between 7 and 9 days of gestation (BLKBG and
ISOBG) as an in vivo measurement of cytokine bias. Serum was obtained from mice via tail vein bleeding at 4 and
6 wk after avirulent challenge and at 24 h after whelping (~10–12 days
after virulent challenge) via cardiac puncture for measuring Ab isotypes by
ELISA as previously described (39, 40). The samples were run as 2-fold
dilutions starting at 1:10 for the 4- and 6-wk bleedings and 4-fold dilutions
starting at 1:100 for the samples taken at 24 h after whelping (10–12 days
after virulent challenge). The OD of each well was determined by an elec-
tronic plate reader. The background cutoff was 0.05 OD unit. The OD value
of each sample was converted to titer, and the mean titer was compared
between the BLKBG and the ISOBG groups by Student’s *t* test.

**Isotype ELISA**

Ag-specific Ab isotypes were measured for each dam rechallenged at 7 and
9 days of gestation (BLKBG and ISOBG) as an in vivo measurement of
Neutralization of IL-4 in primed mice was undertaken with the intent that up-regulation of Ag-specific type 1 responses may result in protection (27). In the first experiment when mice were challenged during pregnancy at day 5 of gestation, both BLKBG and ISOBG mice had lower numbers of mice whelping than the SALBG mice (Table II). However, one of the mice in the BLKBG group did not transmit *N. caninum*. This experiment was repeated with the modification that mice were neutralized and infected later in gestation to increase the number of whelping mice in the modulated group. More mice whelped in the BLKBG group with significantly fewer mice transmitting *N. caninum* than in the other two groups (Table II). In addition, a significantly lower (p < 0.01) mean frequency of *N. caninum* transmission to offspring occurred in the BLKBG mice (0.10 ± 0.19) than in the ISOBG mice (0.60 ± 0.31) and SALBG mice (0.47 ± 0.15; p < 0.01). The naïve mice (SALBG) still had significantly more (p < 0.05) offspring per dam (7.57 ± 1.97) than the BLKBG mice (5.29 ± 1.29).

Cytokine mRNA levels

Relative cytokine responses were investigated in the experiment where mice were modulated before gestation to evaluate ex vivo, up-regulation of type 1 and down-regulation of type 2 responses in both primary (SALBG group) and secondary challenge (BLKBG and ISOBG) as a result of cytokine modulation. BLKBG mice did have significantly lower (p < 0.05) IL-4 responses compared with the ISOBG and SALBG mice indicating down-regulation of type 2 responses in the neutralized group (Fig. 1). IFN-γ mRNA levels comparing the BLKBG and ISOBG groups were not significantly different; however, the IFN-γ level was significantly lower (p < 0.05) in the SALBG group compared only to the ISOBG group.

Cytokine ELISA

Secretion of *N. caninum*-Ag-specific IFN-γ and IL-4 was measured in the BLKBG and ISOBG mice to confirm Ag specificity of the immune response and to demonstrate that secondary immune responses after rechallenge during gestation were biased by neutralizing mAb to IL-4 and avirulent *N. caninum* inoculation before gestation (Fig. 2a). BLKBG mice secreted less IL-4 in vitro (1.55 ± 0.13 ng/ml) than ISOBG mice (2.58 ± 0.61 ng/ml), although this difference was not significant. The BLKBG mice did secrete significantly more (p < 0.05) IFN-γ (92.17 ± 54.34 ng/ml) than the ISOBG mice (23.25 ± 13.19 ng/ml). When the ratio of IL-4 to IFN-γ was determined for each mouse and compared between groups (Fig. 2b), the mean of the BLKBG group was significantly different from the ISOBG (p < 0.05). Neither cytokine was detected in the negative control wells.

Maternal anti-*Neospora* IgG1 and IgG2a responses

Cytokines regulate isotype switching where IgG1 is induced by IL-4 and IgG2a by IFN-γ, providing an in vivo measurement of cytokine bias (39, 40). Minimal IgG1 was detected in BLKBG

Table II. *N. caninum* transmission in mice primed with avirulent *N. caninum* and IL-4 neutralized before gestation

<table>
<thead>
<tr>
<th>Cytokine Modulation Before Gestation</th>
<th><em>N. caninum</em>-Positive Offspring/Mouse</th>
<th>Total Offspring/Mouse</th>
<th>Frequency of Transmission/Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLKBGa</td>
<td>4</td>
<td>4</td>
<td>1.00</td>
</tr>
<tr>
<td>ISOBGb</td>
<td>1</td>
<td>5</td>
<td>0.83</td>
</tr>
<tr>
<td>SALBGc</td>
<td>4</td>
<td>4</td>
<td>1.00</td>
</tr>
<tr>
<td>Challenged with <em>N. caninum</em> at 5 days gestation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLKBG</td>
<td>2</td>
<td>9</td>
<td>0.22</td>
</tr>
<tr>
<td>ISOBG</td>
<td>2</td>
<td>8</td>
<td>0.25</td>
</tr>
<tr>
<td>SALBG</td>
<td>3</td>
<td>9</td>
<td>0.33</td>
</tr>
</tbody>
</table>

a Mice were treated with Ab to IL-4 before gestation.
b Mice were treated with isotype control Ab before gestation.
c Mice were treated with saline before gestation.
mice after primary challenge measured at 4 and 6 wk postinoculation, whereas the ISOBG mice had significantly higher (p < 0.05) IgG1 titers to *N. caninum* (Fig. 3a). This trend continued after the secondary virulent challenge, with BLKBG mice having lower IgG1 titers compared with the ISOBG mice (Fig. 3b). The IgG2a responses were lower than the IgG1 levels in mice at all measurement times, and no differences were detected between the groups (data not shown).

Dams of all fetuses were evaluated for parasite load by IHC performed on lung and liver. No *Neospora* tachyzoites were detected in either organ. Scattered accumulations of lymphocytes were present in maternal lung. The liver contained perportal accumulations of inflammatory cells.

**Discussion**

In naive mice, modulation of cytokine responses during gestation does little to change the frequency of congenital protozoal transmission whereas manipulation of Ag-specific maternal immune responses before gestation changes the susceptibility of the dam to congenital transmission. Specifically, down-regulation of type 2 Ag-specific immune responses before gestation results in a significant decrease in congenital transmission after challenge infection during gestation. Placental localization occurs rapidly within 6–8 days postinfection (28, 42), and innate immune responses may be unable to prevent congenital infection regardless of cytokine status. A primed immune response may be necessary to decrease maternal parasite burden, subsequent placental localization, and congenital transmission.

There is precedent in the literature for choosing the strategy of neutralizing IL-4 to reduce congenital *Neospora* transmission. During initial infection with *N. caninum*, nonpregnant mice, regardless of strain, produced high amounts of IL-4, IL-10, and IFN-γ, indicating a mixed cytokine response (43). However, when cytokine profiles were associated with parasite load and development of CNS disease, resistant mice produced little IL-4 and high amounts of IFN-γ whereas susceptible mice produced both IFN-γ and IL-4 (27). Thus, it was presumed that successful control of initial parasite burden might best be attained by attenuation or down-regulation of IL-4 and presumably type 2 cytokine responses.

Using *N. caninum*-primed mice, the IL-4 response was blocked after initial virulent challenge in treated mice and present but decreased in these mice during subsequent virulent challenge. The magnitude of the IL-4 response was variable depending on what method of measurement was used to characterize this response. When examining Ag-specific cytokine responses, in vitro secretion of IL-4 in the IL-4-neutralized mice was one-half that of the isotype control mice. By comparison, IgG1 responses were decreased in IL-4-neutralized mice 10- to 20-fold during neutralization and primary challenge with avirulent *N. caninum* and 3-fold during secondary challenge with virulent organism, indicating a partially blocked type 2 response. Ex vivo measurements followed the same trend for IL-4; the IL-4-neutralized mice had down-regulated IL-4 mRNA. All measurements of IL-4, which included regulation by measurement of mRNA, actual Ag-specific secretion as measured by protein, and induction of functional in vivo response indicated by isotype switching, demonstrated that down-regulated IL-4 was associated with the control of parasite congenital transmission.
The presence of IL-4 in some way enhances both systemic disease and congenital transmission. IL-4 is a pleotropic cytokine elaborated by type 2 Th cells, mast cells, and NK cells (44). When IL-4 is present during initial infection, its actions will predominate over type 1 cytokines in the differentiation of precursor lymphocytes (45). The predominance of IL-4 in mediating the phenotype of an immune response has been proposed to come from its effect on IL-12 and IFN-γ. IL-4 causes a termination of IL-12 signaling (46) through an initial IL-4 burst that occurs within 16 h after parasite challenge (47). Neutralization of IL-4 (and this burst) during initial challenge should modify subsequent development of T helper phenotypes. IL-4 also interacts with IFN-γ in a functional manner at the effector level because both cytokines compete for binding of IFN-γ activation sequence motifs in macrophages (48). In the presence of constant levels of IFN-γ, decreasing IL-4 levels may have the same effect on macrophage activation as increasing IFN-γ levels.

In vitro secretion of IFN-γ in response to N. caninum Ag was significantly increased in modulated mice compared with control mice, whereas the IFN-γ mRNA levels and IgG2a levels were not elevated in this group compared with both control groups. The IFN-γ mRNA response reflects regulation of this cytokine and, based on these data, there appears to be no additional up-regulation of this cytokine. If isotype measurements reflect an in vivo response, there is no evidence in this parameter that IFN-γ responses have resulted in an enhanced IgG2a response. However, IFN-γ secretion from splenocytes was actually increased in the IL-4-neutralized mice over that of the nonmodulated mice. Stimulation of splenocytes for 72 h may give results different from the in vivo isotype or ex vivo mRNA measurements because factors that regulate in vivo secretion of cytokines are not present in cell culture. Although it is not clear from this study how this datum translates to an enhanced in vivo response, the IL-4–neutralized mice appear capable of secreting more IFN-γ in response to N. caninum. This response may have provided significant local and systemic effects that resulted in protection against N. caninum transmission.

In vivo priming with avirulent N. caninum alone did not enhance type I responses as has been demonstrated for Leishmania sp. infection in mice (49). The ISOBG mice still transmitted N. caninum despite previous exposure, demonstrating that the mouse appears to develop little long term immunity to N. caninum after initial infection. This organism is unique compared with T. gondii (50, 51), and more similar to other protozoal agents, such as Plasmodium and Trypanosoma sp., in which long term infection is associated with recurrent problems for both mother and offspring during gestation (10, 23, 52). Because murine models have been unrewarding in terms of studying gestational effects in these diseases (53), murine neosporosis may serve as a model for investigating congenital disease where the organism induces little long term protection.

This study was unable to unequivocally correlate control of maternal parasite burden, as indicated by parasite load in maternal lung and liver, with the actual occurrence of protection against congenital transmission. Mice were evaluated at 10 days postinfection, and signifcant localization in peripheral organs was not detectable by microscopy. Because experimental N. caninum infection in nonpregnant mice typically results in microscopically detectable tissue parasites between 14 and 21 days postinoculation, other more sensitive methodology may be needed to evaluate early maternal parasite load. Alternatively, placental parasite load may correlate more closely with congenital transmission than systemic parasite load.

Modulation of maternal immune responses can induce immunity to N. caninum congenital transmission. Although in vivo priming and modulation block transmission, the exact effector mechanism has not been determined. Ultimate identification of the effector cell responsible for protection may require adoptive transfer of immune cells. Identification of protective cells will also provide an in vitro method of investigating Neospora-specific protective Ags.

References


