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VACCINATION OF MICE WITH *NEOSPORA CANINUM*: RESPONSE TO ORAL CHALLENGE WITH *TOXOPLASMA GONDII* OOCYSTS

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ABSTRACT: *Neospora caninum* is a protozoan parasite that can cause severe disease in mammals. Two experiments were conducted to examine the effects of subcutaneous (s.c.) vaccination with Hank's balanced salt solution (HBSS), 1×10^5 *N. caninum* NC-1 strain tachyzoites or 1×10^5 *Toxoplasma gondii* TS-4 strain tachyzoites on challenge oral infections in mice with sporulated VEG strain *T. gondii* oocysts (1×10^3 oocysts exp. 1 and 5×10^3 oocysts exp. 2). An additional study, experiment 3, evaluated s.c. challenge with 2.5×10^3 tachyzoites of the highly virulent RH strain of *T. gondii* after vaccination with HBSS, NC-1 tachyzoites, or TS-4 tachyzoites. Mice vaccinated with NC-1 strain tachyzoites survived significantly ($P < 0.05$) longer than mice given HBSS in experiment 1, but not in experiments 2 and 3. Mice vaccinated with TS-4 strain tachyzoites survived significantly longer than HBSS-vaccinated mice in experiments 1, 2, and 3 and significantly longer than mice vaccinated with the NC-1 strain in experiments 2 and 3. *Toxoplasma gondii* tissue cyst numbers were significantly lower for mice vaccinated with TS-4 strain tachyzoites than mice vaccinated with HBSS or the NC-1 strain tachyzoites in experiment 1. No difference was observed in tissue cyst numbers in mice vaccinated with HBSS or NC-1 strain tachyzoites in experiment 1. No HBSS-vaccinated mice survived experiment 2, and the numbers of *T. gondii* tissue cysts were significantly lower for mice vaccinated with the TS-4 strain tachyzoites compared to NC-1 strain tachyzoites. No HBSS- or NC-1-vaccinated mice survived RH strain challenge in experiment 3. Results of these experiments indicate that infection with *N. caninum* provides some protection against fatal oral infection with *T. gondii* oocysts of a moderately pathogenic strain but not tachyzoites of a highly pathogenic strain. The protection provided by *N. caninum* is much less than that provided by previous exposure to *T. gondii,* and the numbers of tissue cysts in the brains of mice are not significantly ($P > 00.5$) lowered.

Neospora caninum is an apicomplexan parasite that was recognized as a cause of neuromuscular disease dogs in 1988 and subsequently determined to be a major cause of abortions in cattle (see Dubey and Lindsay, 1996). *Neospora caninum* is also associated with abortions and neonatal paralysis/disease in goats, sheep, black-tailed deer, Eld deer, and horses (see Dubey and Lindsay, 1996). *Toxoplasma gondii,* another important related parasite, is found in many animals that are susceptible to *N. caninum* infection.

We have previously demonstrated that outbred mice vaccinated subcutaneously (s.c.) with the NC-1 or NC-3 strains of *N. caninum* are not protected from fatal disease when s.c. challenged with tachyzoites of the highly virulent RH or GT-1 strains of *T. gondii* (Lindsay et al., 1990). The possibility exists that some cross protection may be present but overcome by the highly pathogenic strains of *T. gondii* used in our study.

The present studies were done to determine if prior *N. caninum* infection protects mice against fatal oral challenge with *T. gondii* oocysts of a moderately pathogenic strain, to determine if the numbers of *T. gondii* tissue cysts that are produced in *N. caninum*-infected mice are reduced compared to noninfected controls inoculated with oocysts, and to compare lesions in the brains of inoculated mice. Simultaneous studies were done with mice s.c. vaccinated with the nonpathogenic/nonpersistent TS-4 mutant (Waldeland and Frenkel, 1983) of *T. gondii* to compare relative protection afforded by NC-1 vaccination and *T. gondii* vaccination against oral oocyst challenge with *T. gondii.* Additionally, we examined a 1 log lower s.c. challenge dose of the RH strain to determine if mice vaccinated with the NC-1 strain would survive a lower s.c. *T. gondii* infection than in our previous study (Lindsay et al., 1990).

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MATERIALS AND METHODS

Parasite strains

Tachyzoites of the NC-1 strain (Dubey et al., 1988) of *N. caninum* and the RH strain (Sabin, 1941) and TS-4 strain (Pfefferkorn and Pfefferkorn, 1976) of *T. gondii* were grown separately in human foreskin fibroblast cells (Hs68, American Type Culture Collection, CRL 1635, Rockville, Maryland) at 37 C (NC-1 and RH) or 32.5 C (TS-4) in an incubator with 95% air–5% CO_2 atmosphere as previously described (Lindsay et al., 1993). The NC-1 strain of *N. caninum* and the TS-4 strain of *T. gondii* are avirulent in outbred mice, whereas the RH strain of *T. gondii* is highly pathogenic for mice. To obtain tachyzoites for inoculations, the growth medium was removed from the flask and replaced with Hanks' balanced salt solution (HBSS). Cells containing tachyzoites were scraped from the plastic growth surface using a plastic cell scraper. The suspension was then filtered through a sterile 3-μm filter and the numbers of tachyzoites present in the filtrate counted with the aid of a hemacytometer. The final volume of suspension was adjusted so that 0.5 ml contained 1×10^5 NC-1 or TS-4 strain and 2.5×10^3 RH strain tachyzoites for s.c. inoculations. Control mice were inoculated s.c. with 0.5 ml of HBSS.

Oocysts of the VEG strain (Parmley et al., 1994) of *T. gondii* were collected from the feces of experimentally infected cats (Dubey et al., 1996), sporulated in 2% (v/v) sulfuric acid solution, washed free of acid in HBSS, and stored in HBSS at 4 C until used. Bradyzoites and tachyzoites of the VEG strain are relatively nonpathogenic for outbred mice but oocysts are moderately pathogenic for outbred mice. Using a 22-gauge animal feeding needle, mice were fed 0.5 ml HBSS that contained 1×10^3 (experiment 1) or 5×10^3 (experiment 2) sporulated oocysts.

Mouse inoculations

Mice used were outbred HSD:ICR obtained from a commercial supplier (Harlan Sprague Dawley, Indianapolis, Indiana). Mice were 18–22-g females and housed in groups in plastic box cages and provided rodent chow and water ad libitum. Mice used to bioassay for the NC-1 strain of *N. caninum* only were given 4 mg methylprednisolone acetate (MPA) intramuscularly on the day of inoculation to enhance the possibility of *N. caninum* isolation (Lindsay and Dubey, 1989). Other mice used in this study did not receive MPA.

Experiment 1 contained 4 groups of 10 mice each, experiment 2

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TABLE I. Protocols for inoculation and challenge of mice and results of mortality and mean survival time postchallenge of mice in experiments 1, 2, and 3.

Experiment/ group	First/second vaccination*	Challenge inoculation†	Mean challenge (% mortality)‡	Mean survival (days ± SD)§
Experiment 1				
1/1	HBSS/HBSS	VEG/L	80	21 ± 19.1[a]
1/2‖	NC-1/NC-1	VEG/L	11	54 ± 5.0[b]
1/3	TS-4/TS-4	VEG/L	0	56 ± 0.0[b]
1/4#	NC-1/NC-1	None	0	NA
Experiment 2				
2/1	HBSS/HBSS	VEG/H	100	9 ± 1.7[a]
2/2	NC-1/NC-1	VEG/H	70	23 ± 22.7[a]
2/3	TS-4/TS-4	VEG/H	20	47 ± 19.8[b]
Experiment 3				
3/1	HBSS/HBSS	RH	100	10 ± 1.2[a]
3/2	NC-1/NC-1	RH	100	11 ± 1.5[a]
3/3	TS-4/TS-4	RH	10	52 ± 11.4[b]

* Vaccinations were subcutaneous (s.c.) and given on days 0 and 14 postinoculation (PI). HBSS = Hank's balanced salt solution; NC-1 = 1×10^5 tachyzoites of the NC-1 strain of *Neospora caninum*; TS-4 = 1×10^5 tachyzoites of the TS-4 strain of *Toxoplasma gondii*.
† Challenges were given orally (VEG/L and VEG/H) or s.c. (RH) on day 28 PI. VEG/L = 1×10^3 VEG strain *T. gondii* oocysts; none = nothing given; VEG/H = 5×10^3 VEG strain *T. gondii* oocysts; RH = 2.5×10^3 RH strain *T. gondii* tachyzoites.
‡ Percentage of mice that died during 56-day postchallenge (PC) period except for experiment 1, group 4, which was 64 days PC.
§ Mean number of days that mice survived PC ± standard deviation. Significant differences ($P < 0.05$) exist for groups with different alphabetical superscripts within an experiment.
‖ Data are from 9 mice only.
\# Mice in this group were examined at necropsy 64 days PC. NA = not applicable.

contained 3 groups of 10 mice each, and experiment 3 contained 3 groups of 10 mice each. The protocols for s.c. inoculations and oocyst feedings are given in Table I. Briefly, mice were s.c. inoculated with HBSS, NC-1, or TS-4 tachyzoites on days 0 (first vaccination) and 14 (second vaccination) postinoculation (PI). Mice were then challenged by feeding oocysts or HBSS, or by s.c. inoculation of RH strain tachyzoites or HBSS on day 28 PI. Blood was collected from the retro-orbital plexus of each mouse on day 28 PI and tested in an indirect immunofluorescent antibody test (IFAT, see below). The study was terminated on day 56 postchallenge (PC; 84 PI first inoculation) except for group 4 in experiment 1 that was inoculated with the NC-1 strain and not challenged. This group was examined 64 days PC of groups 1–3. Additional groups of mice were used to bioassay for the presence of tissue cysts in mice that were negative at necropsy.

To determine the influence of vaccination on mouse survival time PC, the survival days PC were compared for each challenged group within an experiment using a Kruskal–Wallis nonparametric test and a distribution-free multiple comparisons method in experiment 1 and a Mann–Whitney U-test in experiment 2 (Zar, 1984). Significance was established at a cutoff of $P < 0.05$ prior to analysis of the data.

Necropsy examinations and tissue cyst determinations

Lung, liver, or brain smears were made from the tissues of mice that died before the end of the study and examined unstained for tachyzoites or tissue cysts using bright-field microscopy. Surviving mice were bled from the retro-orbital plexus, killed, and examined at necropsy. The brain was removed from each mouse and the right side (cerebrum, cerebellum, etc.) fixed in 10% neutral buffered formalin. Tissue sections were obtained using routine histological techniques and stained with hematoxylin and eosin and used for microscopic lesion scoring (see below). The left side of the brain was placed in 2 ml of HBSS, ground with 5 strokes of a teflon-coated tissue grinder, and the number of tissue cysts present in 50 μl determined by counting using a bright-field microscope. If no tissue cysts were observed in the 50-μl sample then 1–1.5 ml of the homogenate was s.c. injected into an additional mouse, sera were collected, and the mouse was examined at necropsy 56 days PI. The numbers of tissue cysts present were evaluated using a Kruskal–Wallis nonparametric test and a distribution-free multiple comparisons method in experiment 1 and a Mann–Whitney U-test in experiment 2 (Zar, 1984). Significance was established at a cutoff of $P < 0.05$ prior to conduct of analysis of data.

Lesion scores

Hematoxylin- and eosin-stained tissue sections of brains were coded and brain lesions determined in a blinded fashion without knowledge of mouse treatment by 1 of the authors (S.D.L.). The brain lesions were scored based on the following 3 criteria: (1) number of inflammatory or necrotic foci, 0 foci = grade 1, 1–5 foci = grade 2, 6–10 foci = grade 3, >10 foci = grade 4; (2) mean size of foci, none = grade 1, ≤200 μm = grade 2, 201–500 μm = grade 3, >500 μm = grade 4; and (3) severity of lesions, none = grade 1, slight = grade 2, mild = grade 3, moderate = grade 4, and marked = grade 5. The mean of these 3 values was determined and represented a mean lesion score. The numbers of mice in each treatment with lesions was examined using Fisher's exact test. Mean lesion scores were evaluated using a Kruskal–Wallis nonparametric test and a distribution-free multiple-comparisons method in experiment 1 and a Mann–Whitney U-test in experiment 2 (Zar, 1984). Significance was established at a cutoff of $P < 0.05$ prior to analysis of the data.

Serology and western blotting

Sera from days 28 PI (day 0 PC) and 56 PC (64 PC for experiment 1, group 4) were examined for IgG antibodies to *T. gondii* and *N. caninum* by an IFAT as previously described (Cole et al., 1995). Tachyzoites of each species were used separately as antigen and sera were endpoint titered by doubling dilutions beginning at a 1:50 dilution. Sera from mice used to bioassay for tissue cysts was examined only at a 1:50 dilution for both *T. gondii* and *N. caninum*. Only tachyzoites that demonstrated complete surface fluorescence were considered positive.

For western blotting studies, 4×10^5 freshly isolated tachyzoites of the RH strain of *T. gondii* or NC-1 strain of *N. caninum* were lysed by the addition of sodium dodecyl sulfate (SDS) gel sample buffer and heating for 10 min at 95 C. The samples were applied to a 10% SDS-polyacrylamide gel electrophoresis (PAGE) gel and after electrophoresis were transferred to Immobilon membranes using an electrotransfer apparatus (BioRad, Hercules, California). Filters were rinsed in tris-buffered saline (TBS) and unreacted areas blocked by incubation in 2%

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(w/v) bovine serum albumin (BSA) in TBS (BSA/TBS) for 2 hr at room temperature (21–23 C). The filters were then placed in a multiscreen apparatus (BioRad) and incubated for 1 hr at room temperature in mouse serum diluted in TBS. The mouse serum was used at a dilution equlivant to its IFA titer. Fiters were washed once for 5 min in the apparatus in TBS and then removed and washed 3 times in 0.2% (w/v) BSA/TBS for 5 min each. The filters were then incubated in alkaline phosphatase-conjugated goat anti-mouse IgG diluted 1:3,000 in TBS for 1 hr at room temperature. Filters were washed 3 times for 5 min in TBS and once in alkaline phosphatase buffer before the reactive bands were visualized by development in nitroblue tetrazolium/BCIP buffer. After the bands became visible, the reaction was stopped by addition of acetic acid to 10% (v/v) and the filters washed in water and air dried before examination.

RESULTS

Experiment 1

The mice inoculated s.c. with HBSS (group 1-1) or TS-4 tachyzoites (group 1-3) did not develop clinical signs prior to oocyst challenge. One mouse in group 2 inoculated with NC-1 tachyzoites developed a head tilt on day 27 PI. Another mouse in group 2 had a swelling on its dorsal surface (indicative of abscess formation) first noticed 28 days PI. This mouse died 4 days PC with oocysts and was too autolytic for examination. It was concluded the mouse died from bacterial infection, and it was removed from the study. Therefore, only 9 mice were included in the analysis of data from group 1-2 mice. None of the mice inoculated with NC-1 strain tachyzoites in group 4 developed clinical signs. Eight of the group 1-1 HBSS-inoculated mice died between 8 and 12 days PC; 1 mouse in group 1-2 NC-1 inoculated died 41 days PC, and none of the mice in groups 1-3 or 1-4 died during the 56-day PC period. Stages of *T. gondii* were observed in smears from all mice that died and were examined. Results of mean survival days are presented in Table I. Challenged mice in groups 1-2 and 1-3 survived significantly longer than challenged mice in group 1-1.

Results of mean lesion scores and tissue cyst counts for mice in experiment 1 are presented in Table II. The mean lesion scores were significantly higher for mice vaccinated with HBSS or NC-1 strain tachyzoites and challenged with oocysts compared to mice vaccinated with the TS-4 strain and challenged with oocysts. The mean lesion scores of mice vaccinated with the NC-1 strain tachyzoites and not challenged with oocysts were significantly lower than mice vaccinated with HBSS or NC-1 strain tachyzoites and challenged with oocysts but not the TS-4-vaccinated oocyst-challenged mice. All tissue cysts observed in brain homogenates were thin-walled and considered to be *T. gondii*. All surviving mice in groups 1-1 (HBSS vaccinated) and 1-2 (NC-1 vaccinated) had tissue cysts. No *T. gondii* tissue cysts were observed in 7 of 10 mice in group 1-3 given TS-4 tachyzoites and fed VEG oocysts. All 7 had antibodies to *T. gondii* in their sera, and subinoculation of brain homogenate from these 7 mice indicated that all contained *T. gondii* tissue cysts. The numbers of *T. gondii* tissue cysts were significantly higher in mice in group 1-1 and group 1-2 than group 1-3. No significant difference was observed in the numbers of *T. gondii* tissue cysts in mice in groups 1-1 or 1-2. No tissue cysts (*N. caninum*-like or *T. gondii*-like) were observed in the 10 mice in group 1-4 given only NC-1 tachyzoites. One mouse died 33 days PI of brain homogenate and had lesions suggestive of neosporosis in tissue sections of brain. Three of the 9 other mice inoculated with brain homogenate were positive for *N. caninum* antibodies at a 1:50 dilution of serum.

Mice in group 1-1 did not have demonstrable IgG antibodies to *T. gondii* or *N. caninum* in the IFAT 28 days PI; the 2 surviving mice had IFAT titers of 1:12,800 and 1:25,600 to *T. gondii* but did not have IFAT titers to *N. caninum* 56 days PC. Mice in group 1-2 did not have demonstrable IFAT titers to *T. gondii* 28 days PC and had IFAT titers of 1:800 to 1:3,200 to *N. caninum*. The 8 surviving mice had IFAT titers of 1:3,200 to 1:25,600 to *T. gondii* and 1:200 to 1:800 to *N. caninum* 56 days PC. Mice in group 1-3 did not have demonstrable IFAT

TABLE II. Microscopic lesion scores and *Toxoplasma gondii* tissue cyst counts of mice in experiments 1 and 2.

Experiment/ group	1st/2nd vaccination*	Challenge inoculation	No. exam/ no. lesions‡	Mean lesion score§	Mean tissue cyst counts ± SD‖
Experiment 1					
1/1	HBSS/HBSS	VEG/L	2/2[a]	10.0[a]	77 ± 32[a]
1/2	NC/NC	VEG/L	8/8[a]	8.1[a]	74 ± 26[a]
1/3	TS/TS	VEG/L	10/1[b]	3.3[b]	2 ± 3[b]
1/4	NC/NC	None	10/3[b]	4.2[b]	NA#
Experiment 2					
2/1	HBSS/HBSS	VEG/H	NA/NA	NA	NA
2/2	NC/NC	VEG/H	3/3[a]	9.0[a]	71 ± 31[a]
2/3	TS/TS	VEG/H	8/3[a]	4.5[b]	14 ± 10[b]

* Vaccinations were subcutaneous (s.c.) and given on days 0 and 14 postinoculation (PI). HBSS = Hank's balanced salt solution; NC-1 = 1×10^5 tachyzoites of the NC-1 strain of *Neospora caninum*; TS-4 = 1×10^5 tachyzoites of the TS-4 strain of *Toxoplasma gondii*.

† Challenges were given orally (VEG/L and VEG/H), VEG/L = 1×10^3 VEG strain *T. gondii* oocysts; none = nothing given; VEG/H = 5×10^3 VEG strain *T. gondii* oocysts.

‡ Number of mice examined/number of mice with lesions. Significant differences ($P < 0.05$) exist for groups with different alphabetical superscripts within an experiment.

§ Mean lesion score. Significant differences ($P < 0.05$) exist for groups with different alphabetical superscripts within an experiment.

‖ Mean *Toxoplasma gondii* tissue cyst counts ± standard deviation. Significant differences ($P < 0.05$) exist for groups with different alphabetical superscripts within an experiment.

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titers to *N. caninum* 28 days PC and had IFAT titers of 1:800 to 1:12,800 to *T. gondii.* The 10 surviving mice did not have IFAT titers to *N. caninum* and had titers of 1:12,800 to 1:25,600 to *T. gondii* 56 days PC. Mice in group 1-4 did not have IFAT titers to *T. gondii* 28 days PI or 64 days PC and had titers of 1:800 to 1:3,200 to *N. caninum* 28 days PI and titers of 1:200 to 1:1,600 at 64 days PC.

Experiment 2

No clinical signs were noted in mice in experiment 2 prior to feeding of VEG oocysts. The group 2-1 HBSS control mice died 7–12 days after feeding of oocysts. Seven of 10 group 2-2 NC-1-vaccinated mice died 9–11 days PI and the remaining 3 survived for 56 days. Two of the group 2-3 TS-4-vaccinated mice died 9 days PI and the remaining 8 survived for 56 days. Results of mean survival days are presented in Table I. No difference in survival days were present in mice vaccinated with HBSS or NC-1 strain tachyzoites, but both were significantly lower than the mice vaccinated with the TS-4 strain and challenged with oocysts.

Results of mean lesion scores and tissue cyst counts for mice in experiment 2 are presented in Table II. Lesion scores were not obtained from mice in group 2-1 because they all died before the end of the study. Mice vaccinated with the NC-1 strain tachyzoites and fed oocysts had significantly higher lesions scores compared to mice vaccinated with TS-4 strain tachyzoites and fed oocysts. All tissue cysts observed in brain homogenates were thin-walled and considered to be *T. gondii.* All surviving mice in groups 2-2 (NC-1 vaccinated) and 2-3 (TS-4 vaccinated) had tissue cysts, and the mice in group 2-2 had significantly more tissue cysts than did the mice in group 2-3.

Mice in group 2-1 did not have demonstrable IgG antibodies to *T. gondii* or *N. caninum* in the IFAT 28 days PI. Mice in group 2-2 did not have demonstrable IFAT titers to *T. gondii* 28 days PC and had IFAT titers of 1:100 to 1:1,600 to *N. caninum.* The 3 surviving mice had IFAT titers of 1:1,600 to 1:3,200 to *T. gondii* and 1:400 to 1:800 to *N. caninum* 56 days PC. Mice in group 2-3 did not have demonstrable IFAT titers to *N. caninum* 28 days PC and had titers of 1:100 to 1:400 to *T. gondii.* The 8 surviving mice did not have IFAT titers to *N. caninum* and had IFAT titers of 1:800 to 1:6,400 to *T. gondii* 56 days PC.

Experiment 3

No clinical signs were noted in mice in experiment 3 prior to challenge inoculations with RH tachyzoites. The group 3-1 HBSS control mice died 7–11 days PC with tachyzoites. All 10 of the group 3-2 NC-1-vaccinated mice died 9–14 days PC. One of the group 3-3 TS-4-vaccinated mice died 20 days PC and the remaining 9 survived for 56 days. Two of the 9 mice in group 3-3 had lesions and the mean lesion score was 3.8. No tissue cysts were observed in the brains of the 9 surviving mice in group 3-3. Infectious stages of the RH strain of *T. gondii* were present in 6 of 9 of the surviving mice in group 3-3; subinoculation of brain homogenate from these mice produced mortalities in 5 mice 14–27 days PI and 1 mouse survived for 56 days. Tissue cysts were observed in brain smears from this mouse, and a mouse inoculated with brain homogenate from this mouse died from toxoplasmosis 16 days PI.

Mice in group 3-1 vaccinated with HBSS did not have IFAT titers to *N. caninum* or *T. gondii* 28 days PI and none survived the challenge with RH strain tachyzoites. Mice in group 3-2 vaccinated with NC-1 strain tachzyoites had IFAT titers of 1:800 to 1:6,400 to *N. caninum* but did not have IFAT titers to *T. gondii* 28 days PI, and none survived the challenge with RH strain tachyzoites. Mice in group 3-3 vaccinated with TS-4 strain tachzyoites did not have IFAT titers to *N. caninum* but had IFAT titers of 1:100 to 1:800 to *T. gondii* 28 days PI. The 9 surviving mice did not have IFAT titers to *N. caninum* but had IFAT titers of 1:3,200 to 1:12,500 to *T. gondii* at 56 days PC.

Western blot analysis

Sera from mice not vaccinated did not produce reactions in western blots to tachyzoites of either *N. caninum* or *T. gondii.* Sera from mice vaccinated with *N. caninum* recognized numerous peptides in western blots of *N. caninum* tachyzoites, but no or inconsistant reactivity was observed with tachyzoites of *T. gondii.* Sera from mice vaccinated with *T. gondii* recognized numerous peptides in western blots of *T. gondii* tachyzoites but no or inconsistant reactivity was observed with tachyzoites of *N. caninum.*

DISCUSSION

The present study indicates that vaccination with *N. caninum* can increase the survival time for mice fed oocysts of a moderately pathogenic strain of *T. gondii.* The mechanism of this induced protection is not known. Humoral antibodies do not appear to play an important role because none of the NC-1-vaccinated mice had demonstrable serum IgG antibodies to *T. gondii* in the IFAT.

There is little consensus on cross-reactive antigens recognized by *N. caninum* antiserum on *T. gondii* tachyzoites and *T. gondii* antiserum on *N. caninum* tachyzoites in western blot analysis of animal sera (Bjerkås et al., 1994; Björkman et al., 1994; Paré et al., 1995; Baszler et al., 1996). Bjerkås et al. (1994) found that rabbit *N. caninum* antisera recognized antigens of 45, 66, and 85 kDa on *T. gondii* tachyzoites, whereas rabbit *T. gondii* antisera recognized antigens of 46, 88, and 97 kDa on *N. caninum* tachyzoites. Björkman et al. (1994) found that rabbit *T. gondii* antisera recognized a 40-kDa antigen on *N. caninum* tachyzoites. Paré et al. (1995) did not find reactivity of bovine *N. caninum* antisera with *T. gondii* proteins in western blots. Baszler et al. (1996) found that bovine *T. gondii* antisera recognized *N. caninum* tachyzoite antigens that ranged from 11 to 80 kDa if a 1:100 serum dilution was used, but that if a 1:10,000 dilution was used, then only *N. caninum* tachyzoite antigens of 14 and 37 kDa were recognized.

Cell-mediated immunity is likely to be involved in the protection observed in *N. caninum*-vaccinated mice and may be stimulated in some nonspecific manner by vaccination. Khan et al. (1997) have shown that splenocytes from mice inoculated with the NC-1 strain of *N. caninum* will undergo proliferation in response to soluble *T. gondii* (strain PLK) lysates. The host response responsible for prolonging the survival of NC-1-vaccinated *T. gondii*-challenged mice does not appear to influence *T. gondii* tissue cyst formation because tissue cyst numbers were not significantly different from nonvaccinated controls.

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Mouse and *T. gondii* strain are known to influence tissue cyst production and resistance to primary *T. gondii* infection in inbred mice (Suzuki et al., 1989, 1991, 1993; Brown and McLeod, 1990; Blackwell et al., 1993).

No protection was provided from s.c. challenge with the RH strain of *T. gondii* in *N. caninum*-vaccinated mice. This is identical to our previous findings (Lindsay et al., 1990) and was obtained with a log lower dose of RH strain tachyzoites used as challenge inoculum.

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