Veterinary Parasitology 160 (2009) 60–65

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Effects of a transferring antibody against *Neospora caninum* infection in a murine model

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ARTICLE INFO

Article history:
Received 21 May 2008
Received in revised form 14 October 2008
Accepted 15 October 2008

Keywords:
Neospora caninum
Maternal immunity
Transferring antibody

ABSTRACT

We investigated the roles of a transferring antibody against *Neospora caninum* infection based on a murine model using recombinant vaccinia virus carrying NcSRS2 gene. Higher levels of anti-NcSRS2 antibody were detected in surviving offspring from vaccinated dams than controls while the transferring anti-NcSAG1 antibody was detected in the surviving offspring from unvaccinated dams. After mating, the female mice with transferring antibody against *N. caninum* were challenged with the parasites in mid-gestation. The transferring antibodies disappeared during pregnancy upon parasite infection. There was no significant difference on the parasite burden in dams and the survival rates of their offspring. Here, we have shown that *N. caninum*-specific transferring antibody does not control parasite infection in mice.

1. Introduction

Infection with the coccidian parasite *Neospora caninum* causes paralysis and death in livestock and companion animals (Dubey and Lindsay, 1996). In cattle, *N. caninum* infection is associated with abortions, stillbirth, and neurological disease in calves, making neosporosis an important economic agricultural concern (Dubey, 1999). Transplacental transmission from a naturally infected dam to her fetus appears to be the major, and only confirmed intraspecific, natural route of transmission of the parasite (Paré et al., 1996; Anderson et al., 1997; Schares et al., 1998). Repeated in utero infections can occur in the same dam during subsequent pregnancies, resulting in abortion or congenital infection (Barr et al., 1993; Thurmond and Hietala, 1997). Congenitally infected calves remain persistently infected and can pass the infection onto their offspring (Paré et al., 1996; Anderson et al., 1997). Cows may also become infected by ingestion of oocysts that have been shed by a definitive host, such as dogs (McAllister et al., 1998; De Marez et al., 1999).

There is accumulating evidence that some *N. caninum*-infected cows can develop a degree of protective immunity against abortion and/or congenital transmission, indicating the advantage of vaccination. Although prevention of abortion might be a more realistic goal for a vaccine, the ultimate objective for control of this disease must be to prevent vertical transmission of the parasite. Several groups have investigated induction of protective immunity using recombinant vaccines, especially those based on a *N. caninum* surface antigen, NcSRS2. Our previous results showed that *N. caninum* infection was controlled in BALB/c mice that were immunized with a recombinant vaccinia virus carrying NcSRS2 (Nishikawa et al., 2001a,b). In addition, vaccination of mice with recombinant NcSRS2 produced by *E. coli* or the native protein, induced protective immunity against *N. caninum* infection (Cannas et al., 2003; Haldorson et al., 2005; Pinitkiatisakul et al., 2005, 2007). NcSRS2 peptides induce specific T cell activation, interferon-gamma secretion of peripheral blood mononuclear cells and antibody production in cattle (Staska et al., 2005; Baszler et al., 2008).

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doi:10.1016/j.vetpar.2008.10.095

Although prevention of vertical transmission of the parasite is important, the roles of maternal immunity are not well known. Transferring antibody is the antibody derived from the dam's immune response to an antigen and transferred via placenta or colostrum to the offspring without need of the offspring's active immune response to the same antigen. We have addressed the following question: Can the transferring antibodies derived from the dam infected with *N. caninum* or immunized with vaccine control the vertical transmission of the parasites? To determine the effects of the parasite-specific transferring antibodies, we used a murine model using a recombinant vaccinia virus carrying NcSRS2. This study would provide important insights for prevention of neosporosis.

2. Materials and methods

2.1. Preparation of N. caninum tachyzoites

Neospora caninum tachyzoites of the Nc-1 isolate (Dubey et al., 1988) were maintained in monkey kidney adherent fibroblasts (Vero cells) cultured in Eagle's minimum essential medium (EMEM, Sigma, St. Louis, MO) supplemented with 8% heat-inactivated fetal bovine serum (FBS). For the purification of tachyzoites, parasites and host-cell debris were washed in cold PBS, and the final pellet was resuspended in cold PBS and passed through a 27-gauge needle and a 5.0-μm-pore filter (Millipore, Bedford, MA).

2.2. Preparation of recombinant vaccinia viruses

The recombinant vaccinia virus which expresses NcSRS2 was constructed as previously described (Nishikawa et al., 2000b). For preparation of viral inoculum, recombinant vaccinia viruses were propagated in rabbit kidney (RK13) cells in OPTI-MEM®I (OPTI-MEM, Gibco BRL, New York) without FBS.

2.3. Mice

BALB/c male and female mice, 6–7 weeks of age, were obtained from Clea Japan (Tokyo, Japan). Until their use at 7–8 weeks of age, mice were housed under specific pathogen-free conditions in the animal facility of the National Research Center for Protozoan Diseases at the Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan. Mice were cared for and used under the Guiding Principles for the Care and Use of Research Animals promulgated by the Obihiro University of Agriculture and Veterinary Medicine.

2.4. Vaccination, mating and challenge infection

Female mice, 6–7 weeks of age, were injected intraperitoneally (i.p.) with OPTI-MEM or 5×10^6 plaque forming units of recombinant vaccinia virus and boosted with the same inoculum at 2 weeks after the first inoculation. Female mice were housed with naïve males, 10 weeks of age, for 3 days (1 female with 1 male per cage) at 1 week after boost and were inspected twice daily for the presence of vaginal plugs. The first day a plug was noted was designated as day 0 of pregnancy for each individual. All pregnant dams were challenged on the same day with 1×10^5 Nc-1 tachyzoites at 6–9 days of gestation (4-week post-vaccination). The surviving offspring (F1) were used for investigation of maternal immunity (Fig. 1). The surviving F1 female mice or naïve female mice, 10 weeks of age, were mated with naïve males, 10 weeks of age, for 3 days (1 female with 1 male per cage) at 1 week. All pregnant dams were challenged on the same day with 1×10^5 Nc-1 tachyzoites at 6–9 days of gestation. The surviving offspring were named F2 (Fig. 1). Numbers and survival rates of F2 offspring were measured at 30 days after birth.

2.5. DNA isolation and PCR analysis

For DNA preparation, each whole brain of BALB/c mice was thawed in 10 time-volumes of extraction buffer (0.1 M Tris–HCl pH 9.0, 1% SDS, 0.1 M NaCl, 1 mM EDTA) and 1 mg/ml of Proteinase K at 55 °C. The DNA was purified by phenol-chloroform extraction and ethanol precipitation. The DNA concentration was adjusted to 100 μg/ml for the brain as a template DNA. The DNA amplified by PCR was suspended in 10 μl reaction mixture containing 2.5 μl of template DNA, 1 μl of 10× PCR buffer which contained 15 mM $MgCl_2$ (PerkinElmer, Boston, MA), 1 μl of 10 mM dNTP mix, 0.1 μl of 5 U/μl Ampli Gold™Taq DNA polymerase (PerkinElmer) and 2 μl of 10 pmol/μl *N. caninum* specific primers, Np6 and Np21 (Liddell et al., 1999). Amplification was done in a thermal cycler, GeneAmp PCR System 2400 (PerkinElmer) employing 40 cycles for denaturation (94 °C, 1 min), annealing (63 °C, 1 min) and primer extension (74 °C, 3.5 min). At the end of cycle reaction, a primer extension was continued for 10 min at 74 °C and then kept at 4 °C. The PCR products were visualized by electrophoresis in agarose gels.

2.6. Measurement of N. caninum specific antibodies

Serum (20 μl) was obtained from F1 mice at 30 days after birth and female F1 mice or naïve female mice at 0, 17 and 30 days after mating via tail vein for measuring *N. caninum*-specific antibodies by ELISA. To confirm the lack of an antibody response in unvaccinated and uninfected mice, control serum was taken from all the animals. The recombinant proteins of NcSAG1, NcSRS2, and NcGRA7 were expressed in *E. coli* as glutathione S-transferase (GST) fusion proteins and then purified using Glutathione Sepharose 4B (Amersham Pharmacia Biotech, Sweden) as described previously (Chahan et al., 2003; Gaturaga et al., 2005; Hara et al., 2006). The lysate of *N. caninum* (NLA) was prepared as previously reported (Liao et al., 2005). Fifty microliters of purified NcSAG1, NcSRS2, NcGRA7, and their control GST, as well as NLA at a final concentration of 5 μg/ml was coated on ELISA plates (Nunc, Denmark) overnight at 4 °C with a carbonate-bicarbonate buffer (pH 9.6). After blocking with PBS containing 3% skim milk (PBS-SM) for 1 h at 37 °C, the plates were washed twice with PBS containing 0.05%