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Immunization with native surface protein NcSRS2 induces a Th2 immune response and reduces congenital *Neospora caninum* transmission in mice ☆

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Abstract

NcSRS2, a tachyzoite surface protein of *Neospora caninum*, is an immunodominant protein with respect to induction of antibody production and has a role in attachment and invasion of host cells. Native NcSRS2 was isolated from whole tachyzoite lysate antigen by affinity chromatography using NcSRS2 specific monoclonal antibody and used to immunize BALB/c mice in a congenital transmission study. NcSRS2 was a highly conserved protein as indicated by comparison of deduced amino acid sequence obtained from NcSRS2 gene sequences of 10 geographically distinct *N. caninum* isolates. Mice immunized with purified native NcSRS2 produced antigen-specific antibody, primarily of IgG 1 subtype. Following challenge during gestation with 10^7 tachyzoites, immunized mice had a statistically significant decreased frequency of congenital transmission compared to non-immunized mice ($P \leq 0.05$) or mice inoculated with adjuvant alone ($P \leq 0.01$). Decreased congenital transmission among immunized mice correlated with a predominately Th2 immune response compared to non-immunized mice as indicated by an increased ratio of interleukin 4 (IL-4) to interferon gamma (IFN-γ) secretion from antigen-stimulated splenocytes. The results provide a rationale for NcSRS2 as a candidate subunit vaccine antigen for reduction of *N. caninum* congenital transmission. Furthermore, the studies suggest that a Th2 immune response, if directed against an appropriate antigen, may induce protection against *N. caninum* congenital infection in mice.

Keywords: *Neospora caninum*; Mouse; Immunity; Congenital transmission; SRS2

1. Introduction

Neospora caninum is an apicomplexan parasite closely related to *Toxoplasma gondii*. The parasite infects a wide rage of species, though to date, the only known definitive hosts for *N. caninum* are domestic dogs (McAllister et al., 1998) and coyotes (Gondim et al., 2004). While many mammalian species can be infected based upon evidence of seroconversion, clinical disease occurs primarily in dogs and cattle. In cattle, clinical disease is manifested as parasite-induced abortions (Dubey, 2003). Congenital transmission, resulting in a clinically normal but infected calf, occurs and is likely the most common means of perpetuation of infection within a given herd (Dubey, 2003). Therefore, development of an effective vaccine to prevent congenital transmission would prove beneficial for control of neosporosis in cattle herds.

Research towards development of effective vaccines for the prevention of neosporosis to date shows mixed results. Clearly, immunity to fetal infection and abortion induced by *N. caninum* can occur in experimentally and naturally

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infected cattle (Innes et al., 2001; Williams et al., 2003). Immunity to experimental neosporosis in mice is associated with a T helper 1 (Th1) immune response dominated by production of interferon gamma (IFN-γ) and IgG2a antibody induction and a similar response may be protective in cattle (Khan et al., 1997; Long et al., 1998; Baszler et al., 1999). Immunization of mice with various recombinant surface and dense granule proteins has demonstrated protection against systemic disease (Cho et al., 2005). In addition, immunization of mice with whole killed tachyzoites followed by experimental infection results in reduction of congenital transmission but the mechanism of immunity is unknown (Liddell et al., 1999). However, in an encephalitis model in mice, immunization with tachyzoite sonicate can result in exacerbation of disease suggesting that identification of specific protection-inducing antigens may be important (Baszler et al., 2000). In ruminants, evaluation of whole *N. caninum* tachyzoite protein vaccines shows similar equivocal results. A study in 2000 showed that vaccination of cattle with a whole tachyzoite antigen did not reduce congenital transmission rates following subsequent experimental challenge (Andrianarivo et al., 2000). A later study with a commercially available whole tachyzoite vaccine under field conditions demonstrated a reduction in general abortion rates in vaccinated cattle, although precise causes of the abortions and adjuvant effects were not determined (Romero et al., 2004). In sheep, one study using whole tachyzoite immunizations detected no change in abortion rate, but reduced congenital transmission (O'Handley et al., 2003), while a second study resulted in improved fetal survival, but no effect on congenital transmission rate (Jenkins et al., 2004).

Methods for improvement of *N. caninum* vaccines may require subunits that direct the immune system toward specific antigens or epitopes that induce a protective response. In this regard, much research is directed toward *N. caninum* surface proteins in attempt to target the immune responses toward antigens accessible during the extracellular phase of parasitemia, or toward proteins crucial for parasite transmission and survival. One of these surface proteins, *N. caninum* SAG1 related sequence 2 (NcSRS2) has previously been shown to have a role in attachment and invasion of host cells (Nishikawa et al., 2000a). Most work with NcSRS2 as an immunogen has focused upon DNA vaccines and/or recombinant proteins. Nishikawa and coworkers (Nishikawa et al., 2000b) have developed recombinant vaccinia virus vectors that express the NcSRS2 gene. Immunization with the vaccinia virus that expresses NcSRS2 resulted in reduced parasite load in tissues following challenge compared to animals immunized with vector alone (Nishikawa et al., 2001a). Similarly, immunization with this recombinant virus resulted in reduction in congenital transmission in females vaccinated before pregnancy and challenged during pregnancy (Nishikawa et al., 2001b). In a cerebral infection model, immunization with recombinant NcSRS2 resulted in production of antibodies to recombinant, but not native protein, and only slight reduction in infection relative to adjuvant controls. However, when recombinant NcSRS2 was combined with the eukaryotic expression plasmid pcDNA3 containing a NcSRS2 cDNA insert, antibodies to both recombinant and native protein were produced and cerebral infection following subsequent challenge was significantly reduced (Cannas et al., 2003). These findings suggest that protein in its native state may be required to induce an effective immune response.

The purpose of the current work is to determine whether or not native NcSRS2 would induce protective immunity to congenital neosporosis and to correlate that protection to a specific immune response. The results indicate that immunization of mice with native NcSRS2 prior to pregnancy induced protective immunity to parasite challenge given during pregnancy. Surprisingly, protective immunity correlated with a Th2 immune response.

2. Materials and methods

2.1. NcSRS2 sequence comparison

2.1.1. Parasites

Ten geographically distinct *N. caninum* isolates obtained from naturally infected hosts were used as a source of NcSRS-2 DNA for sequence comparison. Sources of isolates were as follows: BPA1, bovine isolate from the USA (Conrad et al., 1993); BPA6, bovine isolate from the USA (Barr et al., 1993); JPA4, bovine isolate from Japan (Yamane et al., 1998); JPA5, bovine isolate from Japan (Yamane et al., 1998); NC1, canine isolate from the USA (Dubey et al., 1988); NC2C, canine isolate from the USA (Hay et al., 1990); NC5, canine isolate from the USA (Dubey et al., 1988); NCLiv, canine isolate from the UK (Barber et al., 1995); SweB1, bovine isolate from Sweden (Stenlund et al., 1997); and DubeyB1 (NC-beef1), bovine isolate from the USA (McAllister et al., 2000). The Dubey B1 isolate (also known as NC-beef1) was isolated in the laboratory of one of us (JPD) in equine dermal cells inoculated on February 28, 1998 with brain homogenate of a beef calf that was born prematurely. Tachyzoites were first seen in the flask 31 days later and subpassaged to new flasks at that time. Thus, this strain had been cultured in vitro for only a few passages before use in the present study. For all isolates, parasite tachyzoites were propagated in Vero cells using Dulbeco's modified essential medium (DMEM), 10% fetal calf serum (FCS), at 37 °C in 95% air/5% CO_2 atmosphere. Vero cell cultures were grown to confluency and infected with *N. caninum* and allowed to lyse. Separation of parasites from Vero cell debris was accomplished by passage through 10 μm nylon filters (Millipore). The parasites were washed several times in PBS and centrifuged at 800 g for 20 min. The pellets were stored at −20 °C.