Vaccine Development against Neospora caninum Infection

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ABSTRACT. Neospora caninum is a recognized protozoan parasite of a wide range of mammalian hosts, and was reported for the first time in 1988. The isolation of its oocysts in dog’s feces in 1998 led to its establishment as a parasitic species undergoing typical coccidian life cycle. Infection with N. caninum causes paralysis and death in young livestock and companion animals, and is associated with abortions and stillbirth in cattle, and neurologic disease in calves. Considering the economic and agricultural importance of neosporosis, there is the urgent need to develop biological control measures aimed at preventing its transmission, infection, as well as reducing severity of the disease. In this paper, we have reviewed the progress made to date on the parasite-host immunology and on vaccine development including its prospects, and discussed possible strategies in the formulation of vaccine(s) against neosporosis.

KEY WORDS: Neospora caninum, neosporosis, vaccine.


Neospora caninum is an intracellular protozoan parasite, closely related to Toxoplasma gondii [20]. The predicted coccidian nature of the parasite was recently confirmed when its oocysts were found in dog’s feces [46, 54]. Neospora caninum is a frequently diagnosed cause of epidemic and endemic abortion worldwide [25]. Vertical transmission contributes to the spread of N. caninum, and most congenital cases result in the birth of healthy calves [10, 65, 67]. Abortion is an important disease problem in the cattle industry owing to significant negative economic impact on revenues due to decreased milk production and calf loss. Because of the prevalence and economic importance of neosporosis, a vaccine that is safe and efficacious needs to be developed and made readily available to help reduce economic losses in the livestock industry. In this paper, we present a review of available information to date on the immunology and progress of vaccine development against neosporosis.

Immunological study: Experimental neosporosis has been reported in cattle [7, 18], fox [55, 69], sheep [12, 13, 53], pigs [36], goat [5, 44], coyotes [45], dogs [22], cats [21], bird [4], rats [43] and monkeys [5]. Transplacental transmission or vertical propagation appears to be a major route of N. caninum infection in animals [27]. This has been documented in several species of dams over several generations, such as cattle [7, 24], sheep [12, 23, 53], goats [44], dogs [17, 22], cats [21], monkeys [6] and pig [36]. Although it would be desirable to study N. caninum immunity in every susceptible host species, the cost involved is prohibitive coupled with certain limitations in the availability of animals and reagents needed. The availability of mice with selective genetic/immunologic defects which allows N. caninum to grow, is advantageous in studies on the immune mediation of N. caninum infection [25, 66] and in the isolation of the parasite from tissues of naturally infected animals [26]. Vertical transmission of the parasite has been demonstrated in mice [16, 47]. Mice experimentally exposed to N. caninum resulted to infection of the brain, lungs, liver, spleen, kidney and heart [42, 59]. Tachyzoite propagation has been observed in the placenta and uterus of immunodeficient mice with signs of tissue inflammation and is believed to be associated with occurrences of abortion [72, 77]. Considering all these earlier findings, mice undoubtedly represent an excellent biological experimental model in studies on the biology, immunology and chemotherapy of N. caninum infection. Progression of neosporosis in mice however, is influenced by the mouse strain used. Outbred Swiss Webster adult mice do not develop clinical neosporosis. Inbred BALB/c mice are relatively susceptible to chronic infection, develop encephalomyelitis, and tachyzoites persist in chronic lesions at the late stage of infection. Severe clinical neosporosis can develop in immunodeficient mice such as nude mice, SCID mice, B cell (μMT)− and interferon-gamma (IFN-γ)-deficient mice [19, 26, 29, 72].

A thorough understanding of the host immune response is important in the continued search for effective vaccine(s) against N. caninum. Cell-mediated immunity and parasite-specific antibody responses play crucial role in protective immunity against parasite infection. The spleen cells of infected BALB/c mice initially produce interleukin-12 (IL-12), and then IFN-γ [38]. These two cytokines are apparently important in natural protection against infection since mice treated with either anti-IL-12 or anti-IFN-γ antibody succumb to lethal infection as do IFN-γ-knock-out mice [8, 26, 38]. At the late stage of infection, host protection is principally mediated by CD4+ and/or CD8+ T cells [75]. The major histocompatibility complex (MHC) class II-up-regulated macrophages and CD4+ cells also play a crucial roles in the IFN-γ-mediated host immune responses to infection [63]. Administration of exogenous recombinant IL-12
results in decreased severity of early clinical cases, but it does not appear to alter the course of long-lasting disease, suggesting that IFN-γ is a major mediator of resistance during acute infection [8]. In other related studies, the relative susceptibility of mice seems to be related to IL-4 levels, as resistance of B10.D2 mice is associated with lack of production of IL-4 by splenocytes. The susceptibility of both C57BL/6 and BALB/c mice to infection shows a correspondingly higher level of splenocyte IL-4 [8, 48]. There has been no association reported between IL-10 and transient immunosuppression [8, 48]. There seems to be a functional relationship between up-regulation of serum IL-6 and IFN-γ in infected athymic nude mice with inflammatory response following infection [72]. High level of the IgG2a isotype, and low to undetectable levels of IgG1 isotype are produced following infection [8, 29, 48], suggesting the role of Th1 cell type in immunity of resistant mice. B-cell deficient C57BL/6 mice are sensitive to infection [29], implying that these cells play in developing protective immunity. Activation of macrophages with IFN-γ results to increased production of nitric oxide (NO) and killing activity against N. caninum [74], suggesting the influence of NO production on mice resistance to infection. Also, IFN-α, -β and -γ and tumor necrosis factor (TNF)-α are essential in in vitro cultivation of N. caninum in host cells [34, 61, 78]. Furthermore, IFN-γ-induced increase of FasL expression and down-regulated Bcl-2 expression in N. caninum-infected cells have been associated with apoptosis in vitro [62].

During infection with N. caninum, several components of the innate immune system are activated. Macrophages, NK cells and dendritic cells respond by releasing cytokines such as IL-12, TNF-α and IFN-γ. Within this milieu of pro-inflammatory cytokines, T cells recognize the appropriate combination of parasite antigen, MHC and co-stimulatory molecules. Since B cells have been established as essential components controlling/suppressing N. caninum infection, regulation of the Th1/Th2 cell type balance is crucial in protective immunity against the parasite. Molecular study: The surface proteins of all obligatory intracellular parasites are believed to play a critical role in infection. These proteins may collectively serve a number of important functions because they represent the initial interaction with the host cell and components of the host immune system. In N. caninum tachyzoites, approximately six surface molecules have been identified [68], and among these, two surface proteins, NcSRS2 and NcSAG1 have recently been cloned [30–33]. These proteins are functionally involved in the adhesion and invasion process [30, 58]. Moreover, low molecular weight tachyzoites proteins, about 30 kDa can stimulate in vitro proliferation of CD4+ T cells from calves experimentally infected with N. caninum, suggesting the potential usefulness of these proteins in studies on vaccine development against neosporosis [52]. Other proteins show promise, such as NTP3/NTPase-I [3], GRA2 [28], GRA6 [41] and GRA7 [40], microneme protein (38 kDa) [73], MIC2 [50], a subtilisin-like serine protease (Nc-pe65) [49] and N. caninum 14–3–3 gene [39], nevertheless their function(s) have yet to be fully worked out. An indepth understanding of the parasite-host interaction based on molecular studies may reveal still unknown mechanisms utilized by the parasite in exploiting host cell pathways and diverting host organelle functions. These novel modifications could also be potential targets in generating other possible control strategies against N. caninum infection.

Vaccine development: Immunological studies have indicated that both humoral and cell-mediated immune responses are important components of protective immunity against N. caninum. In the case of cattle, cellular immune responses also have a crucial in protective immunity [51]. Putative targets for vaccines may be selected to prevent infection, transmission, disease or abortion. To limit economic losses in the cattle industry, prevention of vertical transmission is necessary. There are advantages and disadvantages in the use of live versus attenuated or killed parasites as vaccine components against neosporosis. A live vaccine is advantageous, for it can stimulate both humoral and cell-mediated immunity. Live parasite infection results in the presentation of parasite antigen with MHC class I antigens, which is required to stimulate CD8+ T cell response [37]. The disadvantage lies in the danger of causing chronic infection in the animal host, which may result in persistent vertical transmission. On the other hand, the use of attenuated or killed parasites is safer and does not result in persistence of infection in the host animal. Immunization of dams with single inoculation of a crude lysate of N. caninum tachyzoites has been reported to induce complete protection of offsprings of BALB/c mice against infection [42]. Pregnant sheep administered Toxovax® that prevents abortion due to toxoplasmosis [11], were not protected against challenge with N. caninum [35]. There are certainly many other challenges in designing an attenuated or killed vaccine including selection of an appropriate immunogen. Inoculation with killed vaccine formulated with Bay R1005 adjuvant triggers humoral immune response and is safe in cattle [15]. Induction of IFN-γ production in cattle immunized with a POLYGEN-adjuvant killed N. caninum tachyzoite preparation had been reported, however this immunization failed to prevent foetal infection in pregnant cattle following experimental tachyzoite challenge [1, 2]. Also, immunization with killed N. caninum antigen, and either liposoidal or Freund’s adjuvant induce a type-2 immune response that has been associated with worsened disease in BALB/c mice [9]. Considering all these earlier findings, the need for continuous search for specific N. caninum antigens and other antigen-delivery systems that will elicit effective protective immunity against neosporosis is warranted.

Recombinant virus vector is an excellent candidate of an antigen-delivery system for vaccine development. Recombinant herpes [71] or vaccinia [14, 32, 64, 70, 76] virus has been demonstrated to be an effective antigen delivery system for protozoan parasite infections. Dogs immunized with recombinant canine herpesvirus (CHV) expressing N. caninum surface protein NcSRS2 produce IgG antibody to N. caninum. Dogs inoculated with recombinant CHV do not
develop clinical symptoms, and they undergo complete remission from infectious CHV [56]. Vaccination with recombinant vaccinia virus which expresses N. caninum surface protein NeSAG1 or NeSRS2 could effectively protect mice from parasite invasion. This study showed that high level of IgG1 Ab production to parasite is important for parasite clearance at the early stage of infection, and at the later stage of disease progression, T cell response is essential against intracellular infection [57, 59]. Moreover, the vaccination of dams with recombinant vaccinia virus expressing NeSRS2 appear to confer effective protection against vertical transmission in offspring of BALB/c mice [60]. Since the most realistic target for vaccination is to try to prevent disease or abortion, the recombinant virus may be suitable for a vaccine against neosporosis.

Conclusion: Vaccine development based on attenuated or killed parasites has demonstrated safe and efficacious results. However, this strategy requires supply and purification of a large number of tachyzoites, and its formulation with adjuvants. On the other hand, live recombinant viral vaccine vehicles are presently being tried out in vaccine studies against N. caninum. Vaccination with a recombinant virus expressing the parasite surface protein could elicit strong and protective immune response against infection. Live antigen delivery systems hold many advantages for a large-scale development of vaccines, including ease of production, resistance to environmental extremes, and affordable cost. Moreover, the reduced pathogenicity of certain viruses as potential vaccine vectors is advantageous. Identified and cloned relevant parasite molecules can be used in the development of new control strategy. Data generated from immunologic studies on N. caninum point to the important role of the regulated-cytokine production and of both cellular and humoral immune responses in suppressing neosporosis. Several distinct cell types such as CD4+ and CD8+ T lymphocytes, γδ T cells, and non-T cells such as macrophages, dendritic cells, and neutrophils, are important in triggering and sustaining the Th1/Th2 balance. It is likely that distinct parasite proteins are involved in activation of these different cell types. Moreover, greater understanding of the parasite-host cell interaction may provide additional information/data useful in vaccine research. To achieve the appropriate and desired protective immune response against N. caninum infection, the determination of particulate vaccine carriers (eg. bacteria, other viruses, liposomes, immune-stimulating complexes and genetic immunization) and suitable antigens is crucial.

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