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Immunity to *Anaplasma marginale* and Recent Advances in Vaccine Development

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Abstract

Anaplasma marginale is the etiological agent of bovine anaplasmosis, one of the most prevalent tick-borne diseases of cattle in tropical and subtropical regions that causes significant economic losses to cattle industry. Cattle that survive infection remain persistently infected for life. Immunity against *A. marginale* infection seems to require both humoral and cellular mechanisms. Antibodies against *A. marginale* neutralize bacteria by interacting with Major Surface Proteins (MSPs). For this reason, MSPs are believed to be one of the best candidate antigens for vaccine development. Research carried out in the last few years has helped us to understand the antigenic composition of *A. marginale* and to develop new potential vaccine formulations. Desirable bovine anaplasmosis vaccine must induce protective immunity as well as prevent infection and transmission.

Keywords: Anaplasmosis, *Anaplasma Marginale*, Immunity, MSP, Immunization

Introduction

Anaplasma marginale is a gram-negative obligate intraerythrocytic bacteria of ruminants [1, 2] belonging to the order *Rickettsiales*, family *Anaplasmataceae* and genus Anaplasma [3]. It was first identified by Sir Arnold Theiler in 1910 who observed "marginal points" in stained red blood cells of sick cattle [4, 5] and can be easily identified in blood smears as basophilic inclusions measuring up to 0.85 μm in erythrocytes [6]. This bacterium is the etiological agent of bovine anaplasmosis, a severe hemolytic disease [7] and one of the most prevalent tick-borne disease of cattle [8, 9]. Bovine anaplasmosis is widely distributed worldwide, especially in tropical and subtropical regions, and causes significantly economic losses to beef and dairy industries due to the low weight gain, decreased milk production, abortion, treatment costs and death of the animals [10].

Clinical signs include progressive prostration, fever, weight loss, jaundice, hepatosplenomegaly, occurrence of abortion and death of animals in acute infection. Cattle may also develop hemolytic anemia, which occurs due to macrophages activity in an attempt to remove infected erythrocytes from the blood. Hemolysis occurs both extravascular and intravascularly, being more intense in splenectomized animals. Animals up to eight months of age usually are resistant and exhibit subclinical disease [11], however become chronically infected [12]. In advanced stage of the disease, cattle usually develop gastrointestinal atony, rumen stasis and constipation due to dehydration and weight loss. Some animals undergo neurological deficits end icterus. Mortality rates in young animals are of about 50-60% [13].

Currently prevention strategies are still limited and rely on tick control, administration of antibiotics and the use of a live vaccine. Tick control is not efficient since it can lead to selection of tick resistant populations and acaricide residues can be found in meat and milk being a serious public health problem [14]. Administration of antibiotics is difficult and expensive in large herds, [15] and also increases the incidence of resistant strains. The available vaccine only prevents clinical disease, yet does not prevent persistent infection, causing animals to become a reservoir for *A. marginale* [16]. Therefore, development of an effective strategy to protect cattle and prevent transmission is urgently needed.

This review focused on providing a comprehensive overview on immunity against *A. marginale* and potential vaccine targets. In addition, recent studies in vaccine development are discuss.

Transmission and Life Cycle

Transmission of *A. marginale* occurs mechanically, via arthropods of the genera *Tabanus, Stomoxys*, and several mosquito species or blood contaminated fomites, and mainly biologically, involving at least 20 species of ticks [17, 18]. In South America, bacterium is transmitted mostly by *Rhipicephalus (Boophilus) microplus* ticks [11], in temperate regions of North America by *Dermacentor* spp. ticks and by *Boophilus* spp. or other genera in other regions [19]. In addition, *A. marginale* can also be transmitted via transplacental, during gestation. Animals are born healthy but remain persistently infected [12].

The life cycle of *A. marginale* begins when ticks ingest infected erythrocytes by the blood feeding on infected hosts. Bacteria enters midgut epithelium and the first cycle of replication begin. After development of the pathogen in tick gut cells, rickettsia migrates to tick salivary glands epithelium end initiate the second cycle of replication. In the salivary glands, *A. marginale* has access to saliva, where it is easily disseminated to the next host during the blood feeding of the tick [20].

In tick cells, *A. marginale* develops within vacuoles (or colonies) bound to cell membrane. The first form of development is the reticulated form, or also called vegetative, that multiplies by binary fission. This initial form then turns into a dense form, the infective form, which exhibit a limited period of time of surviving outside the host cells. Inside bovine erythrocytes, *A. marginale* develops within inclusion bodies, or also called initial bodies, bound to the membrane. The number of parasitized erythrocytes grows exponentially. Inclusion bodies may contain 4 to 8 rickettsia and can be found in up to 70% of erythrocytes during the acute phase of

infection [17].

Incubation period for cattle varies from 7 to 60 days, with an average of 28 days, depending on the dose of rickettsia [21]. The severity of disease is age-dependent: young animals rarely come up with clinical signs or even less death, on the other hand, cattle over two years old are more predisposed to develop acute disease and often leading to death. Animal that survives acute infection becomes persistently infected, acting now as a source of *A. marginale* for ticks [22, 23].

Immune Response against *Anaplasma Marginale*

Understanding how the host's immunity against the parasite occurs is extremely important to define effective targets that can be used in the development of a vaccine capable of protecting cattle and preventing transmission. Immunity against *A. marginale* infection was propose to require humoral and cellular mechanisms. In the presence of rickettsia, bacterial antigens activate antigen-presenting cells (APCs) to produce and secrete interleukin-12 (IL-12) and interferon-gamma (IFN-γ). Such cytokines activate helper T lymphocytes (CD4 +) [24] to secrete IFN-γ, which in turn enhances IgG2 production by B lymphocytes leading to a Th1 pathway [25]. It is presumed that immunoglobulins act by neutralizing bacteria still in the extracellular environment preventing invasion on new erythrocytes and by opsonizing bacteria which promotes phagocytosis by macrophages. In addition, it is believed that IgG2 play a hole in the control of acute rickettsemia. Moreover, IFN-γ activate macrophages to produce nitric oxide (NO), a bactericidal molecule [26], and stimulate the expression of Fc receptors, which facilitates phagocytosis and phagosome-lysosome fusion [25]. Immune response of cattle to infection with *A. marginale* has shown a strong IgG1 and IgG2 response with titers of 3,000 to 100,000 in acute (9 days) and persistent infection (up to 1 year) [27].

Major Surface Proteins (Msps)

Antibodies neutralize the initial corpuscles of *A. marginale* by interacting with Major Surface Proteins (MSPs) [26]. Due to their fundamental role in the survival of rickettsia, such as adherence and invasion of erythrocytes, these proteins are exposed on the surface of the bacteria, which makes them easily recognized by the host immune system. The membrane of the initial corpuscles of *A. marginale* has six MSPs already identified: MSP1a, MSP1b, MSP2, MSP3, MSP4 and MSP5 [28].

MSP1 is a complex formed by two proteins, MSP1a of 105 kDa and MSP1b of 100 kDa, covalently bound by disulfide bounds [29]. MSP1 was shown to be an act as adhesin for bovine erythrocytes, playing a fundamental role in the process of invasion and transmission of *A. marginale* [30, 31]. MSP1a was also shown to be an adhesin for tick cells, being involved in the survival of bacteria in the vector [32, 33]. Serum from bovines previously immunized with native MSP1 showed similar antibody titers against MSP1a and MSP1b, predominating the IgG class [34].

MSP2 is one of the immunodominant outer membrane proteins of *A. marginale* and it is found as monomers or multimers bound by disulfide bonds [29]. Analysis of MPS2 transcripts showed a hypervariable region encoding B-cell epitopes flanked by conserved N and C terminals. These variants may play an important role in the process of evading host immune system and bacteria persistence [35, 36].

MSP3 is also an immunodominant protein on the surface of A. marginale. Immunization of cattle with native MSP3 showed a delay in the onset of rickettsemia after challenge, but there was no difference in the peak of parasitemia or in the degree of anemia [37].

MSP4 is an immunodominant highly conserved protein [38]. Although its function is unknown [39], cattle immunized with native MSP4 were protected against *A. marginale* challenge [40].

MSP5 is also an immunodominant highly conserved protein, but its function is unknown [39]. It is found as monomers and multi-

mers bound by disulfide bounds on the membrane of *A. marginale* [29]. Immunization of cattle with native MSP5 was not able to protect the animal against challenge [37].

Advances in Vaccines Development

Vaccination is an effective and low-cost way to control infectious diseases worldwide. Research carried out in the last few years has helped us to understand the antigenic composition of *A. marginale* and to develop new potential vaccine formulations. Unfortunately, so far none have been worldwide accepted. Thus, the development of a vaccine capable of protecting cattle and preventing transmission is urgently needed.

The first attempt was in the early 1900s, with the isolation and administration of A. centrale, a less virulent strain with induces cross protection against A. marginale. This strategy has been used for over 100 years in several countries meanwhile it does not provide fully protection [14].

One of the best candidate antigens for vaccine development against anaplasmosis is MSP family due to both neutralization sensitive and immunodominant epitopes [41]. Several studies focused on using recombinant proteins of MSPs of *A. marginale* as vaccine antigens. Recombinant MSPs were incorporated into immunostimulating complex (ISCOM) and ISCOMATRIX and inoculated in BALB/c mice to evaluate the humoral immune response. Immunization with the formulations induced higher levels of antibodies (total IgG, IgG1, and IgG2a) compared to control groups [42]. Immunization of calves with recombinant MSPs (rMSP1a, rMSP1b, rMSP4 and rMSP5) incorporated into ISCOMATRIX also induced high levels of antibodies (total IgG, IgG1, and IgG2) in contrast to control groups. However, the study doesn't evaluated protection afforded by this formulation [43]. Subolesin (SUB) is a conserved protein discovered in Ixodes scapularis as protective antigen of ticks. Immunization with purified bacterial membrane-bound SUB-MSP1a chimeric antigen showed enhanced immunogenic than the membrane-free SUB-MSP1a and SUB antigens in BALB/c mice, rabbits and domestic pigs, although this was not tested in cattle [44].

Purified recombinant fragment of MSP1a were covalent attached to multiwalled carbon nanotubes (MWCNTs) and induced equivalent level of antibody, higher levels of CD4+/CD44+ and CD4+/CD62L+ lymphocytes, higher levels of proinflammatory cytokines TNF-α and IFN-γ and higher proliferative rate of splenocytes compared to BALB/c mice immunized with recombinant protein without the nanoparticles [45]. In calves, immunization with the nanoformulation elicited increase in total number of leukocytes, NK cells, lymphocyte populations and enhanced levels of antibodies compared to animals immunized with inactivated vaccine AmUFMG2. In addition, MWCNTs did not induced significant changes in biochemical profile, suggesting no potential renal and hepatic disorders. However, it is still needed to evaluate protection of animals from clinical disease [46].

Other studies focused on epitopes-based vaccines against anaplasmosis. Immunization of BALB/c mice with hybrid protein containing epitopes of MSP1a and common epitopes of outer membrane proteins (OMPs) OMP7, OMP8 and OMP9 protected animals against challenge, showing strong reduction in rickettsemia and no signs of anaplasmosis or hepatic lesions [47]. In a recent study, BALB/c mice were immunized with MSP1a functional motif noncovalently attached to oxidized MWCNT and showed a balanced Th1 and Th2 immune response. Immunization with the nanovaccine lead to a nearly undetectable levels of bacteremia and induced equivalent level of antibodies and better cell-mediated immune responses compared to the immunization with MSP1a functional motif without the nanoparticles [48]. Both studies showed strong protection of mice against A. marginale, being a potential candidate to anaplasmosis vaccine. Evaluation of these formulation on cattle are highly needed.

Beyond MSPs, others outer membrane proteins are under investigation as potential vaccine antigens. AM854 and AM936 is known to play a role in host cell internalization. Cattle immunized with purified recombinant AM854 and AM936 showed similar IgG and IgG2 responses to both proteins. However, the recombinant proteins elicited higher bacteremia after challenge compared to control groups, suggesting that utilization of specific antigens may exacerbate disease [49]. AM779 is a conserved protein present in outer membranes and surface complexes. However, immunization of cattle with recombinant AM779 did not protect ani-

mal from disease [50]. VirB9-1 and VirB10 are immunogenic proteins of the outer membrane type IV secretion system. Purified recombinant VirB9-1 and VirB10 were bound to silica vesicles (SV), also called SV-100 nanoparticles, and inoculated in C57BL/6j mice to evaluate immune response. Antigens adsorbed on the SV-100 induced higher antibody responses compared to proteins without the nanoparticle and cell mediated immune responses. Both proteins were capable to stimulate bovine T-cells, but the protection against infection was not evaluated [51].

Conclusion and Perspectives

At the moment, there are no effective control strategy for *A. marginale* infection and transmission. Conventional vaccine approaches have not shown promising results. Desirable bovine anaplasmosis vaccine must induce protective immunity as well as prevent infection and transmission. Many efforts have been done to find and characterized antigen targets and there are a lot of works that provide relevant results. Subdominant antigens alone seem to be not sufficient to induce protective immunity, but they may be part of vaccine composition with other antigens or nanoparticles. New technologies of drug delivery systems, such as nanoencapsulation, may be considered to ensure to enhance immunity leading to protection against infection and prevention of transmission. Different nanosystems can be developed, such as nanoemulsions, liposome, lipid nanoparticles (SLN/NLC), polymer nanoparticles and cyclodextrin. Further research is required in order to understand how each antigen will behave alone or in nanocomplexes in inducing immune response.

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