BABESIA DIVERGENS VACCINE

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A vaccine strategy against Babesia divergens bovine babesiosis was successfully developed after perfecting of an efficient in vitro culture. Crude supernatants and purified fractions were able to induce a vaccine protection in gerbils against B. divergens infection. More, supernatants induced an effective vaccine protection in cattle. The role of B. divergens exoantigens of 17, 37, 46, 70 and 90 kDa in the development of the immune response was clearly demonstrated in gerbils, cattle, and man.

Key words: Babesia divergens - in vitro culture - exoantigen - vaccine

Several Babesia species infect cattle throughout the world (Telford et al., in press). B. divergens is the main agent of bovine babesiosis in Europe and is responsible for important economic losses. Severe B. divergens human infections have also been reported.

Different vaccine strategies have been considered to protect cattle against babesiosis: 1/ using live attenuated vaccines, 2/using plasma-derived exoantigens and 3/using antigens derived from erythrocytic stages. The attenuated vaccines currently available provide effective protection but have the usual restricting features of such vaccines. Our group is developing a vaccine strategy against B. divergens bovine babesiosis.

In this respect, a method of long term in vitro culture of B. divergens in human erythrocytes was achieved (Gorenflo et al., 1991). It resulted in high levels of parasitemia that routinely reached 30 to 40%. It was possible to generalize this in vitro culture system to any bovine isolate or human isolate. A human iso-

late (Rouen, 1987) has been maintained from four years (435 subcultures) without apparent modification of the virulence.

A vaccine protection had been observed with immunogens derived from in vitro cultures of B. bovis, B. bigemina and B. canis, but no protection had been reported with supernatants of in vitro culture of B. divergens in bovine erythrocytes. In order to evaluate immunoprotection induced by B. divergens antigens contained in the supernatants of in vitro culture in human erythrocytes, vaccine trials were performed in Gerbils (Meriones unguiculatus) a convenient laboratory rodent highly susceptible to B. divergens and in cattle.

Protection against homologous challenge -
The effects of vaccination with concentrated supernatants from B. divergens Rouen 1987 in vitro culture (30 to 40% parasitemia) were examined in 100 gerbils challenged by the homologous B. divergens isolate (Gorenflo et al., 1990). Quil A saponin was used as adjuvant. Cumulative mortality rates in controls varied from 85% (animals injected with 9% NaCl) to 90% (animals immunized by 1.5ml unparasitized culture supernatant equivalent (UPCSE). In contrast, all gerbils vaccinated with two or three injections of a whole vaccine dose (1.5 ml parasitized culture supernatant equivalent (PCSE)) or with 1.5 vaccine dose (0.3 ml PCSE) survived except one animal twice injected with 0.3ml PCSE.
High protection of gerbils that was obtained with culture supernatants did not correlate with high levels of anti-B. divergens serum antibodies as detected by immunofluorescence assays (antibody titers: 1/300 - 1/600).

Protection against heterologous challenge by B. divergens isolates from different geographic areas (France, United-Kindom, Germany) – The cross protective capacity of culture derived soluble immunogens from the B. divergens Rouen 1987 isolate was tested in 80 gerbils against isolates from different geographic areas. (Pré cigout et al., 1991). Vaccination procedure consisted of two injections of 1.5 ml PCSE. Results showed complete protection against the 7107b french isolate and substantial protection against the Weybridge 8843 english isolate (80% protection) and the Munich 87 german isolate (60% protection).

Vaccination trial in cattle – Several attempts demonstrated that it was very difficult to induce a severe and reproducible experimental B. divergens infection in cattle. Therefore a vaccination trial was performed in splenectomized calves bred in a tick free area. This experiment clearly demonstrated that a crude B. divergens in vitro culture supernatant could induce protective protection in cattle, even after immunosuppression by splenectomy (Valentin et al., in press).

Characterization of protective antigens – The B. divergens Rouen 1987 isolate exoantigens inducing an antibody response in ox, gerbil and man were identified. The exoantigens of 37, 46, 70 and 90kDa were the immunodominant polypeptides whatever the host (Gorenflot et al., 1990). The occurrence of numerous epitopes shared between proteins of various B. divergens isolates (Rouen 1987, 7107b, Weybridge 8843 and Munich 87) was detected by immunoprecipitations performed on (35)S methionine radiolabelled babesial proteins of each isolate immunoprecipitated by the homologous gerbil antisera. Immunoprecipitations of radiolabelled babesial proteins by ox, gerbil and human antiserum against the various B. divergens isolates showed that a heat shock protein hsp 70 was an immunodominant babesial antigen whatever the infected host (Carcy et al., 1991). Thus, the hsp 70 appeared to be a valence antigen in a vaccine.

In order to facilitate the identification and purification of the B. divergens polypeptides contained in in vitro culture supernatants, a semi-defined medium which supported the growth of B. divergens without serum was developed (Valentin et al., 1991). Several fractions were purified from this semi-defined medium and were used to immunize gerbils. One of these fractions conferred strong protective immunity to gerbils against homologous challenge. The immunoprotection induced by the fraction was identical to that observed with seric or aseric whole supernatant. A monoclonal antibody (MAB DG7) was produced against a 17Da polypeptide present in this fraction. Such a 17kDa polypeptide could be detected in B. divergens isolates from different geographic areas. Furthermore, it was always present in vitro culture supernatants of these isolates (Pré cigout et al., submitted).

Immunization trials in cattle and/or gerbils clearly assessed the capacity of B. divergens exoantigens (crude supernatants or purified fractions) from in vitro culture to induce a vaccine protection against life homologous or heterologous infection. Demonstration of heterologous protection induced by babesial exoantigens and comparative analyze of the immunogenic B. divergens polypeptides among the different isolates suggested that a subunit vaccine should protect cattle against B. divergens isolates from different geographic areas.

REFERENCE


