Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87*, Suppl. III, 279-281, 1992

BABESIA DIVERGENS VACCINE

A. GORENFLOT; E. PRECIGOUT*/+; A. VALENTIN*/++; G. BISSUEL**; B. CARCY*; P. BRASSEUR***; Y. MOREAU** & J. SCHREVEL*/****

Laboratoire de Biologie Cellulaire, Faculté de Pharmacie, 15 Avenue Charles Flahault F-34060 Montpellier Cedex 1, France *Laboratoire de Biologie Cellulaire, URA CNRS 290, F-86022 Poitiers Cedex **Laboratoire IFFA, Rhône-Mérieux, F-69342 Lyon Cedex 07 ***Laboratoire de Parasitologie, Hôtel Dieu, F-76031 Rouen ****Muséum National d'Histoire Naturelle, 61 rue Buffon, F-75007 Paris, France

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 – exoatingen – 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 (Gorenflot 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 isolate (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 supernantants 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 (Gorenflot 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.

+Present address: Laboratoire de Biologie Cellulaire, Faculté de Pharmacie, 15 Avenue Charles Flahault, F-34060 Montepelier.

++Present address: Laboratoire d'Immunologie/Parasitologie, Faculté de Pharmacie, 15 Avenue Charles Flahault, F-34060, Montepelier.

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-Kingdom, 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. (Précigout et al., 1991). Vaccination procedure consisted of two injections of 1.5ml 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 effective 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 antiserum. Immunoprecipitations of radiolabelled babesial proteins by ox, gerbil and human antisera raised 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 (Précigout et al., submitted).

Immunization trials in clattle 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 heretologous 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

CARCY, B.; PRECIGOUT, E.; VALENTIN, A.; GORENFLOT, A.; REESE, R.T. & SCHREVEL, J., 1991. Heat shock response of *Babesia divergens* and identification of the hsp 70 as an immunodominant early antigen during ox, gerbil and human babesiosis. *Biol. Cell, 72:* 93-102.

GORENFLOT, A.; PRECIGOUT, E.; BISSUEL, G.; LECOINTRE, O.; BRASSEUR, P.; VIDOR, E.; L'HOSTIS, M. & SCHREVEL J., 1990. Identification of major *Babesia divergens* polypeptides that induce protection against homologous challenge in gerbils. *Infect. Immun., 58:* 4076-4082.

GORENFLOT A.; BRASSEUR, P.; PRECIGOUT, E; L'HOSTIS, M.; MARCHAND, A. & SCHREVEL, J., 1991. Cytological and immunological responses to *Babesia divergens* indifferent hosts: ox, gerbil, man. *Parasitol. Res., 77:* 3-12.

PRECIGOUT, E.; GORENFLOT, A.; VALENTIN, A.; BISSUEL, G.; CARCY, B.; BRASSEUR, P.; MOREAU, Y. & SCHREVEL, J., 1991. Analysis of immune resposes of different hosts to Babesia divergens isolate from different geographoic areas

and capacity of culture-derived exoatingens to induce efficient cross-protection. *Infect. Immun., 59:* 2799-2805.

TELFORD, S.R.; GORENFLOT, A.; BRASSEUR, P. & SPIELMAN, A. (In press.)_. Babesial infections in man and wildlife. In J.P. Kreier, *Parasitic protozoa.* Academic Press, New York.

VALENTIN, A.; RIGOMIER, D.; PRECIGOUT, E.; CARCY, B.; GORENFLOT, A. & SCHREVEL, J., 1991. Demonstration of a lipid transfer between high density lipoproiteins and *Babesia divergens* infected human erythrocytes. *Biol. Cell.*, in press.

VALENTIN, A.; PRECIGOUT, E.; L'HOSTIS, M.; CARCY, B.; GORENFLOT, A. & SCHREVEL, J., 1992. Cellular and humoral immune responses induced in cattle by vaccination with *Babesia divergens* culture-derived exoantigens correlate with protection. *Infec. Immun.*, in press.