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CELL CULTURE-DERIVED *ANAPLASMA MARGINALE* VACCINE : SEQUENTAL CHALLENGE EXPOSURE OF PROTECTIVE IMMUNITY DURING A 3-MONTH POST VACCINATION PERIOD

M. J. Haider, N. Arain and N. Qureshi

Department of Zoology, Federal Urdu University of Arts, Sciences and Technology, Gulshan campus , Karachi-75300.

ABSTRACT

Cell culture-derived soluble *Anaplasma marginale* vaccine admixedwith a saponin adjuvant was administered in single dose, 2 weeks between doses , to 6 months rats. An additional 10 rats served as non-vaccinated controls. Comparable groups of these rats were challenge exposed at 90 and 100 days after vaccination, using 1X 10^5 *A. marginale* organisms contained in freshly collected blood from a splenectomized rats with ascending parasitemia. Challenge exposure led to no deaths of vaccinated rats, whereas 5 of the control (non-vaccinated) rats died by *A. mrginale*. All clinical and hematologic variables examined indicated that the vaccinated rats had gained immunologic protection in comparison with the control group. An immune recognition as manifested by an anamnestic humoral response to challenge exposure was in evidence in the vaccinated rats.

Key words: Cell culture, *A. marginale* , vaccine, challenge exposure, protective immunity, vaccination period.

INTRODUCTION

In a previous short-term study, soluble, tissue culture-derived *Anaplasma marginale* vaccine induced a marked immunologic and protective response in 6 months rats (Haider, 1990 a). The animals were challenge exposed with 1 X 10^5 *A. marginale* organisms 48 days after the 1st vaccinal dose was given. It was concluded that this type of vaccine would offer several advantages over presently used premunizing techniques (Haider, 1990 b, 1991) if a sufficient level of vaccine protection would persist for a significant time.

Soluble *Anaplasma* antigens derived from the supernatant of erythrocytic cultures are believed to be free of RBC (Red blood carpuscles) stromal antigens (Haider, 1990 b); thus they should not produce the isoantibodies associated with neonatal isoerythrolysis (Haider, 1991). Being non-replicating

Antigens, there is no risk of the severe reaction that is occasionally observed with premunizing vaccines. Furthermore, the storage and transportation of a lyophilized products are simpler and more economical than the handling of infected blood. This report extends the previous vaccine study by using a larger number of rats and 2 challenge exposed at 90 an 100 days after vaccination.

MATERIALS AND METHODS

The *Anaplasma marginale* vaccine consisting of a soluble antigen derived from tissue culture and combined with Quil-A, saponin adjuvant, has been described, (Haider, 1990 c). The vaccine was administered sub-cutaneously in two 1ml doses, 2 weeks between doses, to 6 months rats weighing an average of 500 ± 20 g. Ten similar rates weighing an average of 490 ± 30 g were used as non-vaccinated controls.

The challenge inoculum contained an estimated 1 X 10^5 *A. marginale* organisms in freshly collected citrated blood from a splenectomized rats showing an ascending parasitimea. For each challenge-exposure period, a rat was infected by needle inoculation of a frozen, tick-transmitted *A. marginale* stabilate originally isolated in Karachi five vaccinated rats and two non-vaccinated rats were inoculated 1M (Intramuscular) with the challenge material at 90 days after they were given the 1st vaccinal dose. The 2nd group of 10 vaccinated rats and 4 non-vaccinated control rats were similarly challenged at 100 days after the 1st vaccinal dose.

Tests to monitor vaccine and challenge exposure responses:

Blood samples for hematologic determinations and serologic tests were obtained by veinipuncture from all rats as follows: once a week before vaccination, once a week after vaccination, and once a day after the onset of patent infection as indicated by a temperature response 35 °C or higher after challenge exposure. Packed cell volumes (PCV) were determined. Sera obtained from clotted samples and clarified by centrifugation were tested with the complement fixation (CF) and indirect fluorescent antibody (IFA) tests using *A. marginale* antigens as previously described (Haider, 1991). Giemsa-stained thin blood smears were made and examined for evidence of parasitemia.

Rectal temperatures were recorded before challenge exposure and daily thereafter. Animals weights recorded each month before vaccination, and recording was continued 1 or 3 months after challenge exposure for evidence of weight gains and losses associated with challenge infections.

The hematologic and serologic test changes were examined for evidence of significance, using an analysis of variance. A linear regression analysis was conducted on the rate of PCV decreases, beginning on the day of patent infection and continuing until the day that the low PCV was reached. At various times comparisons were made of blood and serum values of animals being challenge exposed to evaluate the level and duration of immunity.

RESULTS

A comparison of challenge exposure in 15 vaccinated rats (results of both the 90 and 100 day challenge) and the 6 non-vaccinated controls is presented (Table 1). Most measured values favour the assumption of vaccine protection, and these differences were highly significant in the case of average low PCV(Packed cell volume), percentage of reduction I PCV, and in the rate of PCV regression after patent infection. Among the vaccinated group no deaths occurred, whereas 3 of 6 controls died of acute anaplasmosis, as determined by clinical findings, *Anaplasma marginale* parasitemia, and necropsy results. Among the controls, calculations of weight loses and duration of anemia were made only on surviving animals, resulting in relatively few observations, thus influencing the statistical evaluation. In addition, the most severely affected control animals were removed from the data pool after 10 days or less of patency so that all data for the controls after that time are biased by the absence of the least resistant individuals.

Serologic response of the 15 vaccinated rats before challenge exposure was similar to the previous study (Haider, 1991), in that a CF (Complement-fixation) response was not seen before infection occurred, but marked IFA (Indirect fluorescent antibody tests) serum titers were recorded after vaccination. These reached their peak 2 weeks after the 3rd vaccination and declined thereafter. A straight line regression coefficient calculated from day 50 to day 90 indicates that by day 100, the serum titer would drop to 100, below which sera are not recognized as serologically positive. The average IFA and CF titers after challenge exposure are recorded. Highly significant difference among IFA titers of vaccinated and non-vaccinated rats are noted, whereas CF titers, while slightly higher in the vaccinated rats, are not significantly different.

Tables of temperature response were not included because few differences were detected between vaccinated and non-vaccinated rats. Prepatent periods were calculated on the appearance of febrile response, which was marked in all challenge animals, similar in many respects to that recorded in my previous study (Haider, 1992). *Anaplasma marginale* parasitemias were observed in all challenge exposure rats, but the cell counts were low, and efforts to quantitate them were unsuccessful.

DISCUSSION

The results of the present study confirmed those of the previous short-term vaccine experiment in that the inactivated cell culture-derived *Anaplasma marginale* vaccine is suitable for induction of protective immunity against rodent anaplasmosis (Haider, 1990 c, 1991). It is evident from the results of this 2nd experiment, which used greater numbers of susceptible rats, that the vaccinal immunity, approximately 5 months after vaccination, is sufficient to prevent mortalities. Also, other hematologic and serologic values were significantly in favour of vaccinated rats. The results of these studies are comparable with those of a similar experiment in which rats were challenged by exposure to *A. marginale* infected *Boophilus microplus* larvae (Haider, 1992). Consequently, the duration of protection induced by the vaccine appears sufficiently long to allow for safe introduction of vaccinated rats into enzootic areas and provides for the development of a long-term protection after natural exposure. The vaccine may be useful for vaccination of rats I enzootic zones assuming it is used before natural exposure of rats to *A. marginale* in these areas occurs. Seroepizootiologic studies should be used to establish the suitable vaccinal age of animals in enzootic areas.

The mechanisms of protection and relative importance of humoral as opposed to cellular immune system in anaplasmoses are not clearly understood. The mean IFA (Indirect fluorescent antibody tests) antibody titer of vaccinated animals increased significantly and greatly exceeded that of control rats after challenge exposure. Similar findings were seen in experiments with tick challenge exposure, (Haider, 1991). More significantly, however, is the observation that vaccinated rats were still immune to anaplasmosis when circulating antibody titer was below detectable levels, as determined by the IFA test. Prompt humoral immune response after challenge exposure at 5 months after rats were vaccinated may indicate there was participation of T-helper cells by providing recollection of immunologic memory. Recent unpublished in vitro studies have demonstrated that antibodies produced in response

to vaccination may prompt activation of blood macrophage by interaction as cytophilic antibodies with these cells, indicating that the mechanism of immunity induced by the antigen may be more complex than strictly a direct interaction between antibody and the organism. In addition, it has been reported that rodent mononuclear leukocytes also may act as effector cells mending the destruction of parasitized erythrocytes in an antibody-dependent cell method cytotoxic mechanism (Haider, 1991).

The strain of *A. marginale* used for production of the vaccine and for challenge exposure originated from Karachi representing a homologous system. The question frequently raised is whether there is immunologic sharing among isolates of *A. marginale* from various geographic regions. To that effect, Mahoney *et al.* (1979) demonstrated cross protection between heterologous strains and suggested that the mechanism of cross-immunity is based on priming of he host's immune system by protective antigens of the vaccine strain so that a secondary response against the heterologous strain occurred soon after challenge. This hypothesis is now being investigated by a collaborative experiment abroad, using 2 isolates of *A. marginale* obtained from various geographic regions.

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