Evaluation of Humoral Immune Response to Foot-and-Mouth Disease Vaccination in Experimentally Infected Guinea Pigs with *Trypanosoma evansi*

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Summary

The primary and secondary antibody responses of *Trypanosoma evansi* infected guinea pigs, all lacking foot-and-mouth disease (FMD) antibodies, to an inactivated FMD vaccine containing O_1, A-Mardabad and $Asia_1$ virus strains was evaluated. In experiment 1, guinea pigs (group A) were infected and vaccinated against FMD simultaneously. In experiment 2, guinea pigs (group B) were infected then vaccinated on posttreatment day 7. Booster doses were injected 28 days after primary vaccination. Blood samples were obtained 21 days after primary vaccination and 15 days after the second in both experiments. Body weight gains were diminished significantly in experiment 2, whereas body weight gains of guinea pigs in experiment 1 did not differ from that in control guinea pigs. Only in experiment 2, the infected animals showed a significant suppression ($P<0.05$) of humoral immune response to FMD virus after primary vaccination but the antibody titers were not significantly depressed until after secondary vaccination. The results indicate that *T.evansi* can depressed the immunity against FMD virus in guinea pigs. The animals in experiment 2 failed to achieve protective antibody titers of 1/16 after a primary does and secondary antibody responses of the some infected animals required more time to reach pick titers.

***Key words*:** foot-and-mouth disease, immunization, immunosuppression, guinea pig, *Trypanosoma evansi*

Introduction

Immunity of FMD in cattle appears to be mainly dependent on serum neutralizing antibody levels present at the time of exposure to infection. It is probable that breeding, age, nutrition and concurrent disease may play a role in influencing the magnitude of the antibody response to vaccination. Certain concurrent disease present in the animal at the time of vaccination can reduce immune response. Bovine viral diarrhoea, bovine leukaemia, infectious bovine rhinotracheitis, *Pasteurella haemolytica*, Trypanosoma sp., *Babesia bovis, Theileria parva* and *Th. annulata* are listed as known immunosuppression agents (Muneer *et al* 1988). During the course of protozoal infections, the host's immunologic response to FMD virus is often depressed (Sharpe *et al* 1982, Sharpe & Langley 1983, Ahmad *et al* 1991, Scott *et al* 1977).

Surra is a term for the disease produced in cattle by *Trypanosoma evansi* (Asian pathogenic trypanosomes). *T.evansi* is consistently of high virulance for cattle and is often capable of causing disease in other livestock species as well. Infection in cattle is usually chronic or asymptomatic and results in production losses. Clinical manifestations are intermittent fever, severe anemia, leg weakness, incoordination, prostration, and nervous symptoms including circling movement, excitation, jumping, aggressive behavior, lateral recumbency, convulsion; sudden collapse and finally death. Decreased milk yield, abortion at late stages of pregnancy or premature parturition and congenital transmission in cattle have been recorded. Economic losses due to surra are estimated to be considerable (Manuel *et al* 1998, Tuntasuvan *et al* 1997, Ahmed *et al* 1991, Luckins *et al* 1998, Xichen *et al* 1998, Cheah *et al* 1999, Pholpark *et al* 1999).

Although many of the clinical and pathologic features of the disease are well recognized, the role of immunosuppression of *T.evansi* in the outcome of FMD vaccine in cattle and animal model is poorly studied. The objective of the present

study was to examine further the immunologic capacity of guinea pig, as a laboratory model, to FMD vaccination undergoing experimental infections with *T.evansi*. Guinea pigs were inoculated with FMD vaccine, using differing schedules so that the following were compared between infected-vaccinated and control animals: (1) primary and (2) secondary antibody responses to FMD virus vaccine injected simultaneously and after the onset of infection with *T.evansi*. Its effect on the humoral immune response to FMD virus vaccination up to 50 days was discussed.

Materials and Methods

Trypanosoma. *Trypanosoma evansi* was isolated from a camel, Iran, and was passaged twice in rats. After 2-4 days, when the rats showed rising parasitemia by examination of wet film prepared from the tail blood, they were exsanguinated and blood was extracted by cardic puncture using EDTA as anticoagulant. The *T.evansi* was purified from the host blood cells by anion exchange chromatography using DEAE-cellulose as described by Lanham & Godfrey (1970).

Vaccine. Inactivated trivalent FMD vaccine including types O_1, A-Mardabad and $Asia_1$ strains with aluminium hydroxide and saponin adjuvants (Razi Institute, Karaj) was used for vaccination. The 50% protective does of the vaccine in guinea pig potency test was estimated at 0.5ml.

Guinea pigs. Fifty-five guinea pigs, weighing between 450 and 511 g, were randomly assigned to two groups of 20 (A, B) and three control groups of 5 (C, D, E). The control groups were considered as FMD vaccinated (C), parasite infected (D) and, non infected and unvaccinated (E) groups. They had free access to feed and water. Guinea pigs were weighted at the start of experiments and then weekly.

Experimental design. Motile trypanosomes (1×10^5), resuspended in 0.5ml aliquots of phosphate saline glucose (PSC) pH8.0, were injected intrapretonelly into each of forty guinea pigs in groups A, B and five in control group D. At the same time each guinea pig of groups A and C was vaccinated against FMD at the does rate of 0.5ml subcutaneously (Experiment 1). Group B was vaccinated seven days later, when fulminating infection developed (Experiment 2). Group E was considered as non-

infected and unvaccinated control. The mean daily rectal temperatures, white blood cell count, blood cell volume (PCV) and weekly body weight of both the infected-vaccinated groups and control groups were recorded. Experiments 1 and 2 were done concurrently, and data from the 15 control guinea pigs was used in analysis of both experiments. Blood samples were taken by cardic puncture from guinea pigs anesthetized with carbon dioxide on posttreatment day (PTD) 21, and 28 only for group B and then 15 days after secondary vaccination. Collected serum was stored at –20°C.

Serological assay. Serum samples were tested for antibodies to FMD virus using the micro-serum neutralizing test as described by Rweyemamu *etal* (1978). Briefly, doubling dilution from 1/8 to 1/64 of the three type specific antisera in Eagle's cell growth medium were made in tissue culture grade microtiter plates, using 0.05ml diluting loops and to each of these was added 0.05ml undiluted test virus ($10^2TCID_{50\%}$) sample. The serum-virus mixtures were left at room temperature for 1h. Then 0.05ml of 1.5×10^6 BHK monolayer cells per ml was added to each well. Suitable controls were set up for cell growth, virus cytopathogenecity and possible serum cytotoxicity. The plates were incubated at 37°C and read at 48h after inoculation. The highest dilution of sera protecting more than 50% cells was taken as the end point titer as calculated by Reed & Muench formula.

Results

Two series of experiments were carried out to investigate the role played by *T.evansi* on the humoral immunity response to FMD vaccination at the different times of exposure. All guinea pigs inoculated with *T.evansi* had detectable numbers of parasites in the peripheral blood within the 1st week. Four peaks of parasitemia were attained on the day 7, 18, 27 and day 32 after the injection of approximately 1×10^5 parasites (Figure 1). Clinical signs in infected guinea pigs were loss of appetite, increased pulse and respiration, increased temperature (to 39°C) and anemia. The number of lymphocytes decreased at the first week. The PCV value fell after the first wave and persisted at low until death and/or the end of experiments. Infected animals

Figure 1. *Mean of parasitemic levels in T.evansi infected guinea pigs*

lost an average of 10-34g of body weight by the day 14. The non-infected guinea pigs (C, E) gained 11 to 45g during the same interval. Fourteen animals died after 25 and 28 days during a severe parasitemic episode. They developed acute disease marked by clear waves of parasitemia. The number of parasites decreased to undetectable levels (10 parasites/ml) by day 36 after inoculation.

Antibody assays. The primary immune responses of guinea pigs in experimental groups and secondary immune responses in groups A, B and C following vaccination with FMD vaccine are shown in figure 2. Twenty-one days after primary vaccination the mean antibody level of the trypanosoma-infected animals in group A was equal, or slightly below, related to type of virus, than that of the non-infected guinea pigs (C). The levels were above those considered necessary to confer 95% protection to needle challenge with FMD virus. The mean antibody levels of the trypanosoma-infected animals in group B were depressed significantly ($P<0.05$) twenty-one days after primary vaccination.

Fifteen days following the second vaccination the antibody levels of animals in group A were again similar those of the control group. The mean antibody titer of the infected animals in group B was also below that of the non-infected control animals.

Although, this reduction in antibody titer was not statistically significant ($P<0.05$).

Figure 2. *Mean of antibody titers against FMDV types in treated guinea pigs. group A, group B, group C, group D, group E*

Discussion

The capability of an animal to mount a primary immune response is dependent on the functional capacity of T- and B-lymohocytes, and antigen-presenting cells. The only previous report of the suppression effect of *T.evansi* infection on immune response was from Ahmad *et al* (1991). They were able to demonstrate suppression of FMD antibody response in guinea pigs following single injection of the parasite on day of vaccination against FMD virus subtype A_{22}. The infected animals showed a significant suppression of both types of immune response, humoral and cell-mediated immunity. It is often suggested that trypanomoes might interfere with vaccination campaigns and infected animals have a reduced level of protection to virus challenge after vaccination (Holmes *et al* 1974). It has previously shown that some suppression of both primary and secondary response to FMD virus occurring in cattle vaccinated appeared 10 to 13 days after infection with *T.congolense* (Sharpe *et al* 1982).

The present results indicate that *T.evansi* can temporarily depress the humoral immune responses of guinea pigs to FMD virus vaccination, only when the acute

infection was occurred. The disease is usually characterized by high fluctuating parasitemias and death within 5 weeks or more. Guinea pigs developed a prolonged, relapsing type of infection, with 2-4 cycles of parasitemia before death after a mean period of 45 days (Ibadullaev & Khalikov 1972, Sarmah *et al* 1985, Bhattacharyya & Sinha 1974, Monzon & Villavicencio 1990). Clearly under these conditions, there is often marked depression of humoral antibody responses to the antigen injected into the infected animals. Immunodepression is dependent on the time of antigen injection and is maximal after the peak of injection and coincides with parasite elimination and/or the reduction of parasite load. In compared to group A, the treatment animals in group B mount poorer antibody responses to FMD virus vaccine, both primary and secondary. But the antibody titers were not significantly depressed on secondary vaccination. Maximal suppression occurred at time of peak, resulted in primary vaccination, although humoral responses to FMD virus were lower at all stages of trypanosome infection, resulted in secondary vaccination. Infection of guinea pigs at the time of vaccination did not significantly decrease the antibody response to primary vaccination. The time taken to induce depression varies between the two groups and seems to be dependent on the parasite load, so that *T.evansi* did not markedly affect the humoral antibody production against FMD when the two antigens were injected simultaneously. Only in the case of A-Mardabad it was low, but not significantly depressed. This was followed by a normal response two weeks later, on secondary vaccination. In fact immunodepression effect of *T.evansi* is correlated with the presence of its circulating antigen and the immune response to FMD virus probably reflects the absence of parasite antigen in the host's blood.

In group B, there was a decrease in the antibody response to FMD virus, possibility the depression was apparent between 7 and 18 days following injection, corresponding to the period of peak clinical reaction. The difference in the antibody titer between treatment and control (C) guinea pigs was statistically significant ($P<0.05$). The response was associated with an increase in the parasite percentage in these infected animals. In parallel with the second peak on day 18, there was a decrease in the antibody titer on day 21. The period of immunodepression may began

at the first peak parasitemia on day 7 and was maximal 11 days later on day 18. The ultimate limitation of the number of parasites to undetectable levels was observed by day 36 after infection. Subsequently, after second vaccination, the responses to FMD virus returned to normal levels. Because of variation of guinea pigs in their ability to regain full immunocompetence some of them showing depressed responses, after day 36, it seems that they had a patent parasitemia.

The depression of *T.evansi* on cell-mediated immune response can be evaluated by counting the number of T-cells by erythrocyte (E) rosette technique that have been reported by Ahmad *etal* (1991). The percentages of E-rosettes was gradually decreased in all infected animals, while it was increased in non-infected, vaccinated guinea pigs up to 28 days and finally decreased on day 48. These changes were accompanied by a rise in parasitemia have been observed between 7 and 28 days following injection. Their study has confirmed that *T.evansi* causes immunosuppression and the infected animals do not produce optimal response to FMD vaccination. However, in contrast with our study, they found a depression effect on FMD vaccination at the time of infection. This difference may be related either to the virulence of the trypanosomes used or to the strain of the FMD virus.

Our study indicates that immunodepression caused by the trypanosome may interfere with FMD vaccination and affect the duration of antibody achieved, however, further studies are required to evaluate the possible immunosuppression role of the parasite in cattle.

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