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Protection Against Rinderpest Disease: A Vaccination and Challenge Study in Angus Calves

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Abstract: Humoral and Cell-Mediated Immune (CMI) responses of four Angus calves following vaccination and challenge with rinderpest viruses were investigated. Both of two calves vaccinated with the rinderpest bovine 'O' Kabete strain (RBOK) seroconverted with a mean peak percentage inhibition (PI) of 80.5 56 days post vaccination. Mean peak PI of 82 was demonstrated by sera of the vaccinated calves when challenged with the virulent rinderpest virus (RPV)-Saudi 1/81 strain 63 days post vaccination. Lymphoproliferative responses (LPR) of the peripheral blood mononuclear cells (PBMC) measured by cellular DNA {^{3}H}thymidine uptake revealed mean peak stimulation index (SI) of 10 at day 35 of vaccination. Mean peak SI of 13.2 was attained 2 days post challenge. PBMC of the infected control calves did not respond to stimulation. The two vaccinated calves did not succumb to challenge. Based on the pattern of the responses and reaction to challenge, protection against infection seemed to be regulated by the cell-mediated immune system rather than the humoral pathway alone.

Key words: Bovine calves, humoral immune response, cell-mediated immune response, c ELISA, rinderpest virus

INTRODUCTION

Rinderpest (RP) is an infectious viral disease that afflicts domestic and wild ruminants[1]. Cattle and buffaloes, however, are highly susceptible with varying degrees of morbidity and mortality.

Immunity against the virus has been known for many years and trials to immunize calves began as early as the year 1915[2]. Humoral Immune Response (HIR) to RP Virus (RPV) was soon recognized and vaccination and/or infection conferred solid life long protection against either parental or natural challenge[3]. Although resistance to infection with RPV was claimed to be associated with presence of the neutralizing Abs[4], recent evidence has demonstrated the involvement of cell-mediated immunity in protection against the disease[5-9]. The mechanisms involve the phagocytic activity of macrophages, the role of cytotoxic, helper and suppressor T cells and the non-specific effect of cytokines[10]. Such mode of defense is important in viruses that modify cell surface antigens and are released by budding, among which is RPV[11].

In an attempt to investigate further the humoral and CMI responses to vaccination with RPV vaccine strains and to interpret their possible involvement in protection against infection with virulent RPVs, susceptible calves of the Angus breed were used in this vaccination and challenge study.

MATERIALS AND METHODS

Experimental animals: Four five-month-old bovine calves of the Angus breed were used as experimental animals. These calves were kept at the Pirbright Laboratory, Institute for Animal Health (IAH); U.K. Animal welfare aspects for experimental purposes were followed according to the local Animal Ethics Committee.

Viruses: The Plowright rinderpest bovine 'O' Kabete (RBOK) kidney cell culture vaccine strain[12] was used for vaccination of the subject calves after adaptation in Vero cell lines. The highly virulent RPV-Saudi l/81 strain was used for challenge following IAH directions.

Immunization schedule: The experimental calves were randomLy allocated into two subject and two control calves. The subject subgroup received subcutaneously 10^2 CID50/mL of the RBOK vaccine strain. For challenge, vaccinated calves were given 10^4 TCID50/mL of RPV-

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Saudi 1/81 virulent strain at day 0 (63 days post vaccination), using the same route for vaccination. Control animals were challenged at the same time using the protocol adopted for the vaccinated calves.

Serum samples: Sera from all calves were collected before vaccination (day 0) and then from the subject subgroup on days 5, 7, 9, 12, 14, 21, 28, 35, 49 and 56 post vaccination. Post challenge serum samples from both subject and control animals were collected on days 0 (63 days post vaccination), 2, 5, 7, 9, 12 and 14. About 5-10 mL of blood were collected by venepuncture. Sera were separated within 24 h after centrifugation at 200 g for 10 min. All the samples were stored at -20 ^{0}C till used.

Isolation of peripheral blood mononuclear cells (PBMC): Heparinized venous blood from all calves were collected on days 0, 2, 5, 7, 12, 14, 21, 28 and day 35 of vaccination and on days 0 (day 63 of vaccination), 2, 5, 7, 12 and 14 of challenge. Initially, about 20-30 mL of the blood from each calf was centrifuged at 500-600 g for 10 min at 18-20°C. Collecting the buffy coat at the plasma-RBCs interface, the volume of each was brought up to 20 mL with sterile Hank's – balanced salt solution (HBSS) or phosphate-buffered saline (PBS) making an approximate dilution of 10%. PBMC were purified from the buffy coats by centrifugation over 10 mL histopaque 1083 solution (Sigma) at 800 g at 18-20°C for 30 min. Cells harvested at the interface were washed 3 times in HBSS or PBS at 400 g at 4°C for 10 min using 8 times the harvested PBMC volume. Prior to use, each PBMC pellet was dissolved into 2 mL RPMI-1640 medium containing L-glutamate, 25mM HEPES and 5% fetal calf serum or were stored into liquid nitrogen using lymphocyte storage medium (60% RPMI-1640 with L-glutamate and 25mM HEPES, 30% fetal calf serum, 10% dimethyl sulphoxide).

Determination of RPV antibodies (Ab) in sera: All reagents used were obtained in pre-titrated kit form from the Food and Agriculture Organization World Reference Laboratory for Rinderpest (FAO WRLRP), Pirbright, IAH, UK. A solid-phase microtitre competitive ELISA was used for quantitative and qualitative assay of the test sera using monoclonal antibodies (MAb) directed against RP haemagglutinin protein. Flat-bottomed polystyrene micro-ELISA plates (Nunc-immuno Maxisorb plates, Kopenhagen, Denmark) were used.

The test protocol followed that of[13] as described previously[14]. A multichannel spectrophotometric ELISA plate reader (Multiskan) fitted with an interference filter of 492 nm was used to read the test. Results were expressed in terms of percentage inhibition (PI) according to the formula:

PI=100- {(mean OD of test Wells/Mean OD of cm) x100}

where OD is the optical density value and cm refer to the MAb control. Inhibition values greater than 50% were regarded as positive sera.

Determination of the lymphoproliferative responses: Concentrations of 1.0 x 10^6 cells/mL of the separated PBMC were prepared from each PBMC sample using RPMI-1640 medium (containing L-glutamate, 25mM HEPES,100 IU/mL penicillin, 100 μg mL^{-1} streptomycin and 5% FCS). Triplicates of 1.0x10^5 cells/100μL/well of each PBMC sample were cultured in round-bottomed 96 well polystyrene tissue culture plates (Corning). One hundred microlitres of pre-determined concentrations of 10 μg mL^{-1}/well and 100 TCID50/mL of phytohaemagglutinin-P (PHA-P) (Sigma) and heat-inactivated RBOK vaccine strain, respectively, were incorporated into the triplicate sets of each PBMC sample. Unstimulated cells and mitogen-induced sets were included as controls.

All plates were incubated for five days at 37°C at 5% CO_2 tension and 95% atmospheric pressure. Towards the last 18 h of incubation all the plates were pulse-labeled with 20 μL/well of 10μci/mL {^{3}H}thymidine. Finally, all cells were harvested into printed filtermat A-glass fiber filters (Turku, Finland) using LKB scintillation machine harvester. LPR for the heat-inactivated RBOK vaccine strain and PHA-P were assessed by cellular DNA {^{3}H}thymidine uptake and were expressed as a stimulation index (SI) as follows:

$$SI_{Thymidine} = CPMs/CPMu$$

where;
CPMs: count per minute of stimulated cells
CPMu: count per minute of unstimulated cells
Stimulation indices (SIs) > 2.5 are regarded RPV positive cell responders.

RESULTS

Serum antibody levels of the vaccinated and challenged experimental calves: The serum antibody levels of the vaccinated and later challenged calves are shown in (Fig.1). Both calves (TQ94 and TQ95) responded positively to vaccination against RPV with a peak post vaccination PI of 79 and 82, respectively, 56 days later. Peak post challenge PI were 83 and 82, respectively.

Neither of the two susceptible control calves (TQ96 and TQ97) showed positive PI during vaccination or post challenge.

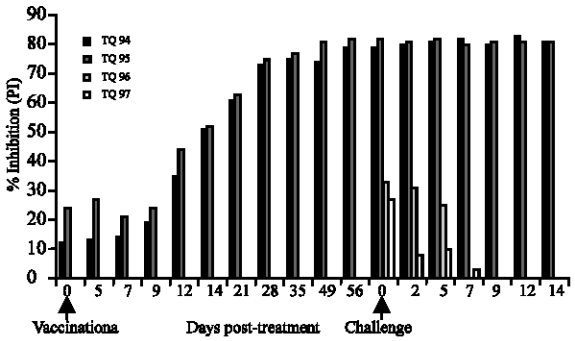

Fig. 1: Antibody levels of the vaccinated (TQ94 and TQ 95) and control (TQ 96 and TQ 97) calves after vaccination (day 0)and challange (days 63) with RBOK and RPV- saudi 1/81 strain respectivly

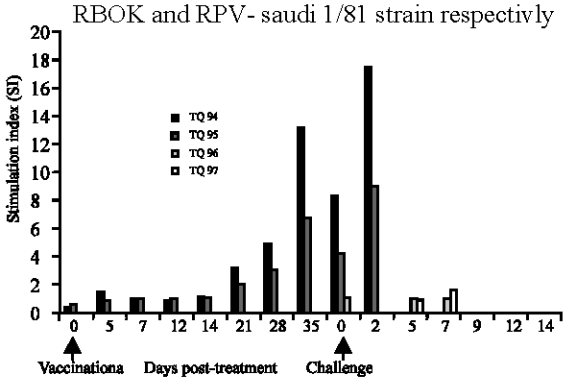

Fig. 2: Stimulation indicies (SI) of the vaccinated (TQ94 and TQ 95) and control (TQ 96 and TQ 97) calves after vaccination (day 0) and challange (day 63) with RBOK and RPV-saudi 1/81 strain respectivly

RPV-specific lymphoproliferative responses: Trypan blue exclusion staining of the harvested PBMC showed high populations of lymphocytes. Percentages of purity and viability of more than 97 and 95 were attained, respectively.

LPR of the vaccinated and later challenged subgroup is illustrated in Fig. (2). Peak SI of 13.2 and 6.8 were illustrated, respectively, on day 35 post vaccination. SI of 17.5 and 9.0 were detected, respectively, on day 2 post challenge. Neither of the two susceptible control calves showed positive LPR during vaccination or post challenge. Contrary to the vaccinated calves, both control calves succumbed to infection on challenge. Major clinical signs included pyrexia, leucopenia, profuse seromucopurulent conjunctivitis, vesiculoulcerative stomatitis, lymphadenitis, pasty blood-streaked diahrroea.

DISCUSSION

In this study, a slight insignificant rise in Ab titres in both vaccinated calves was detected on day 5 after vaccination. A gradual increase was observed up to day 9 of vaccination, then the pattern became sharp. The first positive Ab titre was detected at day 14 after vaccination when mean peak PI value of 51.5 was recorded. An observation similar to those of[15,16] albeit slight later than those showed by[17] when anti-RPV Abs were detected on day 11 post vaccination.

The increasing trend of Ab titre was continued among these calves and mean peak PI of 80.5 was demonstrated at day 56 after vaccination. Antibody titres were maintained at high levels after challenge in the vaccinated calves.

Our results have reinforced the fact that mitogens and viral antigens can stimulate and/or suppress both B and T cells *in vitro*[18-20]. Both vaccinated calves showed blastogenesis following *in vitro* stimulation of the PBMC with a heat-inactivated RPV-RBOK vaccine strain although only on day 21 was a positive LPR detected when mean SI of 2.6 was obtained. Maximum mean SI equivalent to 10 was reached at day 35 of vaccination, a finding similar to that of[9] although a bit earlier positive LPR (14 days post vaccination) was detected in all vaccinated animals. In contrast, an insignificant *in vitro* proliferation during the whole period of vaccination was reported by[21], an observation that might be attributed to the use of a recombinant vaccinia virus expressing RPV-H antigen alone.

The fact that the humoral and CMI responses were of similar pattern and demonstrated maximum values 35-56 days post vaccination in our investigation, suggests involvement of both the humoral and cell-mediated immune systems. Responses to PHA-P were tested and T cell DNA uptake of {^{3}H}thymidine was shown to be high. This was noted by the significant LPR of PBMC from both post-vaccination and challenge samples. Moreover, although the slight reduction in the initial total white blood cell count observed early post vaccination (data not shown) could be related to the immunosuppressive nature of the virus, this was not reflected in the pattern of the proliferative responses. This may be an explanation for the very low LPR values observed post challenge in the unvaccinated control calves that developed disease.

Given the increase in the peak post challenge Ab titres, although statstically insignificant, coupled with the retrieval of the viral genomes of the challenge strain from PBMC and ocular and nasal secretions of these calves[14],

the investigation suggested the occurrence of a systemic reaction requiring involvement of the CMI to modulate resistance to infection.

Although the study did not include any monitoring of the neutralizing Abs, the possibility of their involvement in protection remains.[5,7]denied the importance of such Abs in protection using a recombinant RPV-F vaccine strain, but[8] observed rapidly increasing neutralizing Abs and CMI responses on day 14 and 21 post challenge on vaccination with a recombinant RPV H vaccine. The investigation inferred that CMI is a common factor in modulating the immune response against RPV. The dominance and involvement of the CD4+ T cells in protection against infection had already been observed by[9].

Since little is known about the nature or specificity of T cell responses to RPV vaccine strains, further studies to investigate the effect of defined RPV antigens and their corresponding cytokines could be useful in understanding the role that could be played by the immunological cells and their subsets in protection against infection.

The failure of the control calves to respond immunologically although they were housed in contact with the subject subgroup shedding RBOK virus genomes during the vaccination period[14], unpublished data), could be attributed to the quantity of the virus shed and /or to the attenuation of the virus itself[22]. The fact that neither of the two vaccinated calves succumbed to challenge shows the solid immunity they gained against the disease.

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