

Cell-mediated immune responses in cattle vaccinated with a vaccinia virus recombinant expressing the nucleocapsid protein of rinderpest virus

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Rinderpest virus (RPV) is a member of the genus *Morbillivirus* in the family *Paramyxoviridae* which causes an acute and often fatal disease in large ruminants. To examine the immune response to the virus nucleocapsid (N) protein, a recombinant vaccinia virus expressing RPV nucleocapsid protein (rVV-RPV-N) was used to vaccinate cattle. The recombinant vaccine induced low levels of non-neutralizing anti-N antibodies. RPV-specific cell-mediated immunity induced by the recombinant was assessed by measuring both the lymphocyte proliferation and cytotoxic T-lymphocyte responses. The protective immune response was examined by challenging the vaccinated cattle with either a highly virulent (Saudi 1/81) or a mild (Kenya/eland/96) strain of the virus. The vaccinated cattle were not protected against challenge with the virulent RPV strain, except they showed a slight delay in the onset of disease when compared with the unvaccinated controls. In cattle challenged with the mild strain, apart from a transient fever, no clinical signs of rinderpest infection were seen in the vaccinated cattle. One out of two control cattle showed a similar response but the other died from classic rinderpest disease. Virus-neutralizing antibodies were induced more quickly following challenge with the mild strain in vaccinated cattle compared to the control animals. These data suggested that the cell-mediated immunity induced by rVV-RPV-N could stimulate the rapid production of neutralizing antibodies following RPV challenge but this response was not sufficient to protect against challenge with a virulent strain of the virus. Protection was seen in one of three animals challenged with a mild strain of the virus; however, a greater number of animals would need to be tested to estimate the significance of the protection afforded by the N protein.

Introduction

Rinderpest virus (RPV) is a member of the genus *Morbillivirus* of the family *Paramyxoviridae*, and is most closely related to measles virus (MV). The genus also includes canine distemper virus, which infects carnivores, and peste des petits ruminants virus, which infects sheep and goats. New morbilliviruses have recently been discovered in marine mammals (Barrett *et al.*, 1993). Morbilliviruses have six structural proteins, namely the attachment or haemagglutinin protein

(H), the fusion protein (F), the matrix protein (M), the phosphoprotein (P), the polymerase protein (L) and the nucleocapsid protein (N). Recombinant poxviruses expressing either the RPV H or F protein have been shown to protect cattle from challenge with a lethal dose of virulent RPV, indicating the importance of the virus surface glycoproteins in generating a protective immune response (Belsham *et al.*, 1989; Yamanouchi *et al.*, 1993; Yilma *et al.*, 1988). In some cases, cattle with very low neutralizing antibody titres following vaccination, or even some which were without detectable neutralizing antibody, were shown to be protected against the challenge. This strongly suggested the involvement of cell-mediated immunity in the protection which followed vaccination with recombinant poxviruses (Yamanouchi *et al.*, 1993). In other virus systems, such as influenza, rabies and

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respiratory syncytial viruses, the internal structural proteins have been shown to be involved in the induction of a protective cell-mediated immunity (Bangham *et al.*, 1986; Fu *et al.*, 1991; McMichael *et al.*, 1986; Townsend *et al.*, 1984). In the case of morbilliviruses, the significance of N protein-induced T-cell immunity in protection was demonstrated in an MV-induced encephalitis rodent model system, using either a recombinant vaccinia virus expressing MV N (Bankamp *et al.*, 1991) or a recombinant adenovirus expressing MV N (Fooks *et al.*, 1995). However, the replication of a neuro-adapted MV strain inoculated intracerebrally as a challenge virus in these experiments was limited to the central nervous system. Rinderpest infection, as a system to study protective immunity, has the great advantage that the natural host (domestic cattle) can be used and, in which, typical systemic infection can be produced following virus inoculation. In this study we report on the immune response generated in cattle following vaccination with the viral N protein expressed in a vaccinia virus recombinant (rVV-RPV-N).

Methods

Animals and experiment design. Friesian cross Aberdeen Angus calves were used for two series of vaccination and challenge experiments (Experiments I and II) in the high security animal accommodation available at the Pirbright Laboratory. In Experiment I, two cattle (SP46 and SP47) were vaccinated subcutaneously with 10^8 p.f.u. of rVV-RPV-N in the shoulder region. Seven weeks later they received the second vaccination of 5×10^7 p.f.u. of rVV-RPV-N. Three animals were left unvaccinated as controls. They were challenged 18 weeks after primary vaccination with 10^4 TCID₅₀ of the virulent Saudi 1/81 strain of RPV injected subcutaneously. In Experiment II, three cattle (nos 64, 65 and 66) were vaccinated with 10^8 p.f.u. of rVV-RPV-N followed 2 weeks later with a booster dose of 5×10^7 p.f.u. Seven weeks after primary vaccination the three vaccinated cattle and two of the control cattle were challenged with a mild strain of RPV (Kenya/eland/96) recently isolated from an eland in Nairobi National Park (Barrett *et al.*, 1998). Following challenge the animals were observed daily for clinical signs of rinderpest disease and rectal temperatures were recorded. A temperature of 39.5 °C, or higher, was considered pyrexia. RPV infection was confirmed in all the cattle using a standard agar gel immunodiffusion test to detect virus antigen.

Tissue culture cells. Vero cells were maintained in Dulbecco's minimum essential medium (DMEM) containing 5% foetal calf serum (FCS) (First Link, batch no. FCS1031) and 100 mg/l of kanamycin (Sigma). B95a (Kobune *et al.*, 1991) cells, which are a marmoset-derived lymphoblastoid cell line highly sensitive to RPV, were maintained in RPMI 1640 medium containing the same serum and antibiotic supplements.

Virus strains. The recombinant vaccinia virus expressing RPV N under the control of the vaccinia virus early/late p7.5 kDa promoter was constructed as described previously using an attenuated strain of vaccinia virus (LC16mO) as a vector (Ohishi *et al.*, 1998). The Saudi 1/81 strain of RPV used as a challenge virus in Experiment I was an homogenate of spleen material from a previously infected animal. This avoided possible attenuation of the virus by tissue culture passage. A dose of 10^4 TCID₅₀ is known to kill all infected cattle within 10 days and is the most severe form of the disease known (Taylor, 1986). A strain of RPV recently

isolated in Kenya (Kenya/eland/96), which was shown to be very mild in domestic cattle but highly virulent in wildlife species such as buffalo, kudu and eland, was used as challenge virus in Experiment II (Barrett *et al.*, 1998). A freshly prepared 2 ml suspension (10% w/v) of lymph node material derived from a cow infected with this virus and stored at -70 °C was used as the challenge virus. Prior to use in Experiment II, the same dose of this virus stock was shown to induce a mild form of rinderpest disease, consisting of high fever along with mild conjunctivitis and gingivitis, in two cattle and in one uninoculated animal kept in the same pen as a contact control (Colin Dunne, personal communication).

Lymphocyte proliferation assays. Virus antigens used for lymphocyte proliferation assays were prepared using the RBOK vaccine strain of RPV and the LC16mO strain of vaccinia virus. Briefly, Vero cells were infected with RPV for 3 days and the cells lysed by freeze-thawing. The cell lysate was pelleted by centrifugation at 15 000 g for 30 min at 4 °C, resuspended in FCS-free DMEM and the virus titrated on Vero cells. Vaccinia virus antigen was prepared as previously described (Ohishi *et al.*, 1990). Lymphocyte proliferation was assessed by [³H]thymidine incorporation into DNA. Peripheral blood lymphocytes (PBLs) were prepared from buffy coat cells obtained by centrifuging heparinized blood at 1000 g for 10 min at 4 °C. They were purified by centrifuging through Histopaque 1083 (Sigma) at 800 g for 25 min at room temperature without using the centrifuge brake. The cells were cultured (2×10^5 cells per well in a 96-well microtitre plate) in RPMI 1640 medium supplemented with 5% FCS (Advanced Protein Products, batch no. AF1211) for 6 days in the presence of either UV-irradiated RPV (TCID₅₀ of $10^{3.6}$ per well), vaccinia virus (1.4×10^3 p.f.u. per well) or concanavalin A (ConA) at a final concentration of 5 µg/ml. The cells were pulse-labelled with [³H]thymidine (Amersham) using 0.2 µCi per well for 16 h, collected on a glass filter and the bound radioactivity was measured by liquid scintillation counting. All assays were carried out in triplicate cultures. The stimulation index (SI) was calculated as (mean c.p.m. of lymphocytes with antigen)/(mean c.p.m. without antigen). Values higher than 2.5 were considered significant.

Cytotoxic T-lymphocyte (CTL) assays. PBLs from the vaccinated cattle were purified as described above and stimulated for 7 days *in vitro* with stimulator cells. Autologous PBLs, infected with rVV-RPV-N at an m.o.i. of 5 for 16 h and then irradiated with UV (500 mJ/cm²) to kill the virus, were used as stimulator cells. Autologous skin cells, previously obtained by skin biopsy, were used as target cells. The target skin cells were infected with RPV RBOK strain at an m.o.i. of 10 TCID₅₀ or with adenovirus expressing RPV N at an m.o.i. of 20 TCID₅₀ (kindly provided by A. K. Tiwari, Institute for Animal Health) for 14 h and then labelled with 0.2 mCi/ml Na₂⁵¹CrO₄ for 30 min. The effector cells were incubated for 6 h with ⁵¹Cr-labelled target cells and the radioactivity released into the cell supernatant was measured using a gamma counter. The percentage specific lysis was calculated as [(experimental release - spontaneous release)/(maximum release - spontaneous release)] × 100. The maximum release was determined from wells treated with 10% Triton X-100. The spontaneous release was determined from wells without effector cells and it was < 12% of the maximum release.

Antibody assays. Virus-neutralizing antibody titres to RPV were assayed in a microneutralization test on Vero cells (Sato *et al.*, 1981). Briefly, RBOK RPV strain was diluted to a concentration of 2×10^3 TCID₅₀/ml with DMEM. Volumes of 50 µl of serial twofold dilutions of test sera in the microtitre plate were mixed with an equal volume of the virus suspension and incubated for 1 h at 37 °C. Vero cells were then added at 5×10^3 per well and the plates incubated at 37 °C in an atmosphere of 5% CO₂. They were observed for cytopathic effects (CPE) on days 5 and 7. The titre was expressed as the reciprocal of the highest serum dilution which inhibited 50% of the virus CPE.

Anti-vaccinia virus antibodies were measured using an ELISA. UV-irradiated vaccinia virions (10^7 p.f.u./ml) were used as the antigen. Virus suspension (50 μ l) was added to each well of 96-well microtitre plates and allowed to adsorb at 4 °C overnight. The plates were then washed with PBS containing 0.05% Tween-20 and incubated for 1 h with test sera, diluted 1/1000. The plates were again washed with the same buffer, followed by the addition of HRP-conjugated rabbit anti-cow IgG antibody for 1 h. *O*-Phenylenediamine solution was used as substrate to detect the bound conjugate and the colour development was measured at the optimal wavelength of 492 nm in an automatic ELISA reader.

The specificity of the antibodies induced following vaccination was analysed by a standard immunoprecipitation assay. Radioactively labelled virus proteins were prepared by the addition of 35 S-labelled L-methionine to B95a cells infected with the RBOK strain of RPV. The labelled cell lysate was incubated with normal bovine serum for 30 min followed by the addition of protein G-Sepharose to eliminate non-specific precipitation reactions. The precipitated complexes were removed by centrifugation and aliquots of the supernatant were incubated with test sera for 1 h. The specific antigen-antibody complexes were again bound to protein G-Sepharose and washed five times in lysis buffer followed by two washes with PBS. The tightly bound protein in the final pellet was eluted by boiling the samples in gel loading buffer and analysed by SDS-PAGE. The precipitated radioactive proteins were detected by autoradiography.

Results

Experiment I

Two cattle (SP46, SP47) were vaccinated with rVV-RPV-N as detailed in Methods. The two vaccinated cattle and three controls (4378, 4404, 4406) were challenged with the virulent Saudi 1/81 strain of RPV. From day 4 post-challenge all cattle showed a typical rinderpest prodromal fever (Fig. 1). This was followed by other more specific clinical signs, such as erosive stomatitis, conjunctivitis and severe diarrhoea. Control cattle showed conjunctivitis and stomatitis from 4 days after the challenge; however, in the two vaccinated cattle these symptoms were seen 1 or 2 days later. All cattle showed diarrhoea from day 9. The control cattle were euthanized on days 9 and 10 post-challenge and the two vaccinated cattle were euthanized on day 10.

Experiment II

Three cattle (nos 64, 65 and 66) were vaccinated with rVV-RPV-N as outlined in Methods. Seven weeks after the primary vaccination, the three vaccinated cattle and two control unvaccinated cattle (TE41, TE42) were inoculated subcutaneously with a mild strain of RPV recently isolated from an eland in Nairobi National Park, Kenya (Kenya/eland/96). Rectal temperatures following challenge are shown in Fig. 2. With the exception of a mild transient fever in animals 65 and 66, none of the vaccinated cattle showed any clinical signs of rinderpest infection. One control animal (TE42) showed a temperature profile similar to the two vaccinated febrile animals but no other clinical signs were observed in this animal. In contrast, the second control animal (TE41) went on to develop

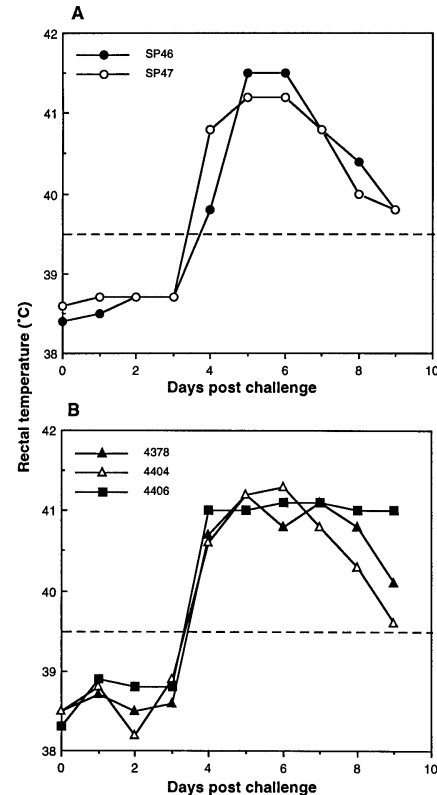


Fig. 1. Rectal temperatures in cattle following challenge with the virulent Saudi 1/81 strain of RPV (Experiment I). Temperatures higher than 39.5 °C were considered pyrexia. (A) Vaccinated cattle SP46, SP47; (B) control cattle 4378, 4404, 4406.

clinical signs characteristic of rinderpest disease and died on day 21 post-challenge. The pyrexia, which began on day 12 and was maintained until its death, coincided with a reddening of the buccal mucosa and conjunctivitis, while shallow erosions were seen on the lower gum on day 17. On post-mortem examination numerous haemorrhagic erosions were seen in the intestines and typical 'zebra' striping was seen in the colon and rectum.

Antibody responses

No neutralizing antibodies to RPV were detected in the sera from vaccinated cattle before or after challenge in Experiment I. In Experiment II, three vaccinated cattle (64, 65 and 66) developed neutralizing antibodies (titres of 64, 64 and 16, respectively) 2 weeks following challenge, whereas the two control animals (TE41 and TE42) only developed detectable antibodies (titres of 16) at 3 weeks post-challenge. Significant anti-vaccinia virus antibody responses were detected by ELISA in all the vaccinated cattle, confirming that the vaccine virus had replicated in the animals (data not shown).

Immunoprecipitation assays showed protein bands corresponding in position to the N protein (60 kDa) with the sera from the three vaccinated cattle in Experiment II before

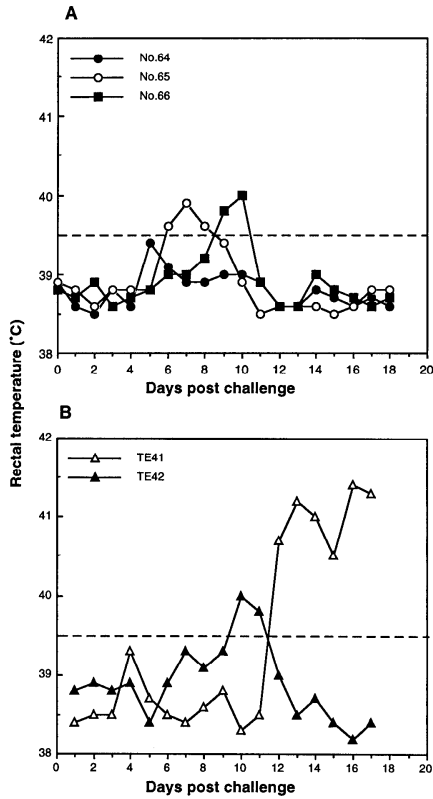


Fig. 2. Rectal temperatures in cattle following challenge with the mild Kenya/eland/96 strain of RPV (Experiment II). Temperatures higher than 39.5 °C were considered pyrexia. (A) Vaccinated cattle nos 64, 65, 66; (B) control cattle TE41, TE42.

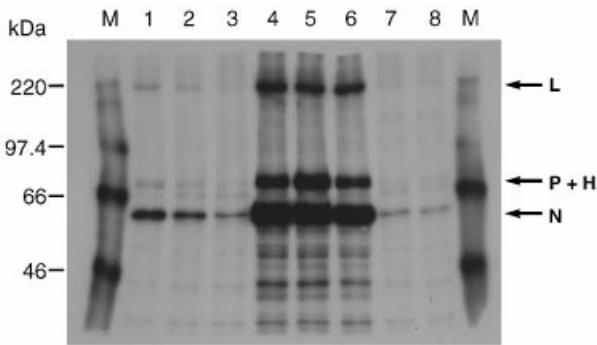


Fig. 3. Analysis of antibodies induced following vaccination and challenge in Experiment II. ³⁵S-labelled B95a cell lysates infected with RBOK RPV strain were immunoprecipitated with the sample sera as detailed in Methods. Lanes 1–3, sera taken just before challenge from animal nos 64, 65 and 66, respectively. Lanes 4–6, sera taken 2 weeks after challenge from animal nos 64, 65 and 66, respectively. Lanes 7 and 8, sera taken 2 weeks post-challenge from animals TE42 and TE41, respectively. Lane M, protein molecular mass markers.

challenge (Fig. 3, lanes 1–3). At 2 weeks post-challenge, the production of anti-N antibodies was greatly stimulated and protein bands around the positions expected for the H and P

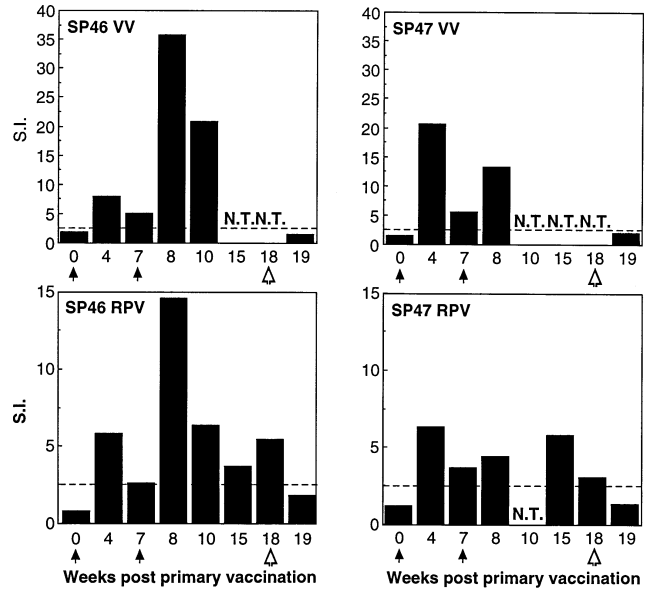


Fig. 4. Lymphoproliferative responses to either RPV or vaccinia virus (VV) antigen stimulation in PBLs derived from animals in Experiment I. The stimulation index (S.I.) was calculated as (mean c.p.m. of lymphocytes with antigen)/(mean c.p.m. without antigen). Values higher than 2.5 were considered significant. \blacktriangle , Vaccination day; \triangle , challenge day; NT, not tested.

proteins (72–74 kDa) and the L protein (220 kDa) were observed (Fig. 3, lanes 4–6). The control sera at 2 weeks post-challenge demonstrated only weak protein bands corresponding to the position of the N protein (Fig. 3, lanes 7–8).

Lymphocyte proliferative responses

In Experiment I, RPV-specific lymphocyte proliferation was observed in both SP46 and SP47 cattle 4 weeks after primary vaccination, which then increased following the second vaccination. Vaccinia virus-specific proliferative responses were also seen at high levels in both cattle after the primary vaccination, which also increased following the second vaccination. However, at 1 week post-challenge with the virulent virus, proliferative responses to both RPV and vaccinia virus were abolished (Fig. 4). The c.p.m., with or without antigen, before challenge were in the range 1076.6–26569.4 and 247.1–3704.8, respectively. They decreased to 77.3–118.8 and 51.5–62.9 after challenge. Similarly, the proliferative responses to stimulation with a non-specific mitogen (ConA) were also abolished 1 week following challenge (data not shown).

In Experiment II significant proliferative responses to RPV antigen were only detected after the second vaccination, which was given 2 weeks after the primary vaccination. Animal no. 64 showed a strong proliferative response before the challenge, at 7 weeks after primary vaccination, which was depressed following challenge, while the other two vaccinated animals

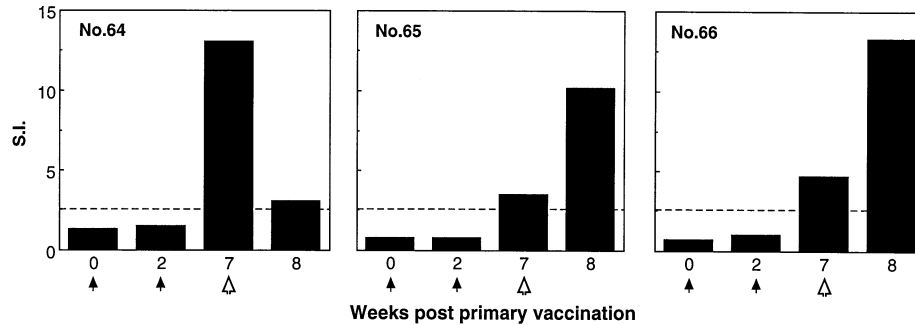


Fig. 5. Lymphoproliferative responses to RPV antigen stimulation in PBLs derived from animals in Experiment II. PBLs were purified from nos 64, 65 and 66 at the indicated weeks post-vaccination and stimulated with RPV antigen. The stimulation index (SI) was calculated as (mean c.p.m. of lymphocytes with antigen)/(mean c.p.m. without antigen). Values higher than 2.5 were considered significant. \blacktriangle , Vaccination day; \triangle , challenge day.

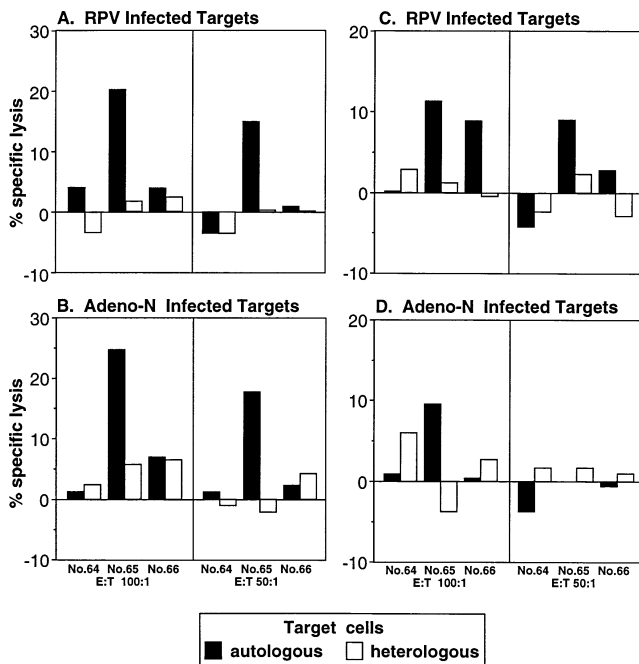


Fig. 6. Cytolytic activity of lymphocytes from the vaccinated cattle from Experiment II. Lymphocytes were purified from cattle nos 64, 65 and 66 at 3 weeks post-vaccination (A, B) and 1 week post-challenge (C, D). They were used in a standard 6 h ^{51}Cr -release assay. Autologous (solid bars) and heterologous (open bars) target skin cells were infected with either RPV RBOK strain (A, C) or a recombinant adenovirus expressing RPV N (B, D). Effector/target cell (E:T) ratios of 100:1 and 50:1 were tested.

(nos 65 and 66) showed a greatly increased proliferative response after RPV challenge (Fig. 5). The c.p.m., with or without antigen, were in the range 135.3–1814.7 and 63.6–447.9, respectively.

Cytotoxic T-cell responses

CTL responses were only measured in animals from Experiment II. PBLs from animal no. 65 showed significant

cytotoxic activity directed to autologous target cells 3 weeks after the first vaccination, i.e. 1 week after the second vaccination. The activity was detected against target cells infected with either RPV or the adeno-N recombinant virus (Fig. 6 A, B). CTL activity in PBLs from animal no. 65 was also confirmed 4 weeks after primary vaccination (data not shown). No CTL activity could be demonstrated in the PBLs derived from the other two vaccinated cattle (nos 64 and 66). One week after challenge, animal no. 65 also showed weak CTL activity against targets infected with either RPV or adeno-N, while animal no. 66 showed weak CTL activity, but only to RPV-infected cells at an effector to target ratio of 100:1 (Fig. 6 C, D). In all cases CTL activity was shown only against autologous target cells, indicating that the cytotoxic activity was restricted by MHC class I molecules.

Discussion

The significance of cell-mediated immunity induced by internal virus proteins for protection against systemic RPV infection was investigated in cattle following vaccination with a recombinant vaccinia virus expressing the RPV N protein. The recombinant failed to protect cattle against challenge with a highly virulent form of RPV. All the vaccinated and control cattle showed typical clinical signs of rinderpest disease, i.e. high fever, severe erosive stomatitis, ocular/nasal discharge, conjunctivitis and severe diarrhoea, following challenge with the Saudi 1/81 strain of the virus. However, there was a slight delay in the onset of clinical signs in the vaccinees. A recent report, where a recombinant bacille Calmette–Guérin (BCG) expressing the N protein of MV was used as a vaccine, also showed that it did not prevent systemic MV infection in rhesus monkeys, although a significant reduction in lung inflammation after challenge was observed in the vaccinated animals (Zhu *et al.*, 1997). Severe immunosuppression in all cattle following virulent RPV challenge was demonstrated by the failure of PBLs to respond to specific virus antigen or to non-specific mitogen stimulation. Gross pathology on post-mortem examination was typical of severe rinderpest infection (Wohlsein *et*

al., 1993) in both vaccinated and control animals. Similar results were reported for rabbits experimentally infected with the lapinized strain of RPV (Yamanouchi *et al.*, 1974) where the vaccinia virus N recombinant failed to protect rabbits against systemic infection with this virus (Nakamura *et al.*, 1998).

The discrepancy between our results for RPV and the reported successful protection of rats and mice from fatal encephalitis by recombinant vaccines expressing MV N (Bankamp *et al.*, 1991; Fooks *et al.*, 1995) may be due to the different routes of infection, i.e. limited infection confined to the central nervous system vs systemic infection involving lymphoid tissues and organs. Vaccination with the same dose of a recombinant vaccinia virus expressing the virus H protein (rVV-RPV-H), made using the same LCI6mO strain as a vector, induced measurable levels of neutralizing antibodies and protected cattle against the same RPV challenge dose (Yamanouchi *et al.*, 1993). These results demonstrate the importance of the H protein compared to the N protein as a protective immunogen against systemic infection with a highly virulent, highly lymphotropic and epitheliotropic strain of RPV.

On the other hand, cattle vaccinated with the N recombinant appeared to have some level of protection from infection with a mild strain of the virus. This was shown by the absence of any clinical signs of rinderpest infection following challenge in one of the three vaccinated cattle, and only mild transient fever in the remaining two cattle. Mild strains of the virus have a longer incubation period (Wamwayi *et al.*, 1995) and result in 20–30% mortality compared to 90–100% mortality upon infection with highly virulent strains of the virus (Taylor, 1986; Wamwayi *et al.*, 1995). Only one out of four unvaccinated cattle inoculated with this strain of the virus succumbed to typical rinderpest disease, as expected for a mild strain. However, it will require a larger number of animals to be tested to be able to conclude with certainty that the N recombinant affords protection against a mild strain of the virus. In another bovine paramyxovirus infection (bovine respiratory syncytial virus, BRSV), Taylor *et al.* (1997) found that recombinant vaccine viruses expressing the F, G and N protein of respiratory syncytial virus gave some protection against the development of pneumonic lesions in calves following virulent virus challenge. BRSV infection in cattle is probably more equivalent to infection with a mild strain of RPV in cattle.

In these experiments, we demonstrated the induction of cell-mediated immunity by rVV-RPV-N. PBLs from all rVV-RPV-N-vaccinated cattle showed significant RPV-specific proliferative responses to antigen stimulation. Since no detectable virus-neutralizing antibodies were present in the sera of any of the vaccinated cattle before the challenge, cell-mediated immunity was considered to have contributed to the slight delay in the onset of clinical signs following challenge with the virulent strain. In Experiment II, animal no. 64, which showed no febrile response on challenge, demonstrated the

highest SI at the time of challenge. The other two vaccinated animals (nos 65 and 66) also had high SI values which, in contrast to no. 64, were greatly increased following challenge. PBLs from the control animal which survived the RPV infection (TE42) showed a similar increase in stimulation (SI of 11·8) after challenge, while PBLs from the animal which succumbed to the infection (TE41), failed to respond to antigen stimulation (SI of 1·1). This suggested that the ability of lymphocytes to proliferate in response to antigen stimulation following challenge might determine whether or not the animal is protected. Preliminary FACS analysis showed that the proliferating cells against RPV in our assay were mainly CD4⁺ T-cells and that $\gamma\delta$ T-cells were also activated (data not shown). Activated T-cells can help stimulate antibody production and they secrete many kinds of cytokines which stimulate effector cells, suppress the replication of viruses or directly attack the infected cells.

Following challenge of vaccinated cattle with the mild strain of RPV, there was a rapid rise in the level of anti-N antibodies, as measured in immunoprecipitation assays, and virus-neutralizing antibodies were detected at 2 weeks. It took a further week for the non-vaccinated control animals to develop detectable neutralizing antibodies. This suggested the N-protein-primed T-cells helped not only to induce non-neutralizing anti-N-antibody-producing B-cells, but also to induce virus-neutralizing antibody-producing B-cells on challenge. A similar enhancement of the production of neutralizing antibodies by T-cells primed by internal component of a virus following virus challenge has been observed in influenza and rabies virus infections (Russell & Liew, 1979; Scherle & Gerhard, 1986; Ertle *et al.*, 1989; Fu *et al.*, 1991).

In Experiment II we could detect significant CTL activity in one of three vaccinated animals (no. 65) prior to, and following virus challenge. CTL activity was directed against autologous skin cells expressing either all RPV antigens or the RPV-N antigen alone. No CTL activity was directed against similarly infected heterologous skin cells. This result clearly demonstrated that the N protein is one of the target antigens to which MHC-restricted CTLs are directed. However, this animal was not the one which showed complete clinical protection following challenge with the mild strain and so the significance of CTLs for protection remains unclear. A weaker CTL activity was also detected in animal no. 66 following challenge, but this was only demonstrated in cells expressing all virus antigens, suggesting that this CTL activity was directed against another RPV antigen. Thus far, the role of CTLs in controlling morbillivirus infection has only been examined in MV-induced encephalitis models in rodents in which virus growth is limited to the central nervous system (Beauverger *et al.*, 1993; Niewiesk *et al.*, 1993). Other authors have questioned the role of CTLs in protection against MV-induced encephalitis in mice and emphasized the importance of CD4⁺ T-cells (Finke & Liebert, 1994). Vaccination with rVV-RPV-N did not induce detectable CTL responses against RPV N protein in the one

animal fully protected from mild virus challenge; however, it is possible that CTLs directed against other virus antigens are of critical importance in induction of protective immunity to RPV. These protocols designed to study cell-mediated immune responses in cattle infected with RPV, the natural host for this morbillivirus, will enable us to determine the role CTLs generated against other virus antigens play in the protection mechanism and provide a better understanding of the pathogenesis of morbillivirus infections.

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