Vaccination of cattle with attenuated rinderpest virus stimulates CD4$^+$ T cell responses with broad viral antigen specificity

Brett T. Lund,1† Ashok Tiwari,2 Sareen Galbraith,2‡ Michael D. Baron,2 W. Ivan Morrison3 and Tom Barrett2

1, 2Division of Immunology1 and Division of Molecular Biology2, Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK
3Division of Immunology, Institute for Animal Health, Compton Laboratory, Compton, Nr Newbury, Berkshire RG16 0NN, UK

The immune responses of cattle inoculated with either a virulent or an attenuated vaccine strain of rinderpest virus (RPV) were examined by measuring the proliferation of peripheral blood mononuclear cells (PBMC) to whole RPV antigen preparations and to individual RPV major structural proteins expressed using recombinant adenoviruses. Responses to the T cell mitogen concanavalin A (ConA) were also measured as a control to monitor non-specific effects of infection with RPV on T cell responses. Infection with the vaccine strain of RPV was found to induce a strong CD4$^+$ T cell response. A specific response was detected to all RPV proteins tested, namely the haemagglutinin (H), fusion (F), nucleocapsid (N) and matrix (M) proteins, in animals vaccinated with the attenuated strain of the virus. No one protein was found to be dominant with respect to the induction of T cell proliferative responses. As expected, vaccination of cattle with an unrelated virus vaccine, a capripox vaccine, failed to produce a response to RPV antigens. While profound suppression of T cell responses was observed following infection with the virulent strain of RPV, no evidence of impairment of T cell responsiveness was observed following RPV vaccination, or on subsequent challenge of vaccinated animals with virulent virus.

Introduction

Rinderpest, or cattle plague, is an economically important disease of domestic and wild ruminants which is enzootic in parts of eastern Africa, the Middle East and southern Asia (Barrett & Rossiter, 1999). Mortalities can be as high as 100% in susceptible herds infected with highly virulent strains of the virus (Taylor, 1986). Rinderpest is caused by a virus (RPV) of the genus Morbillivirus; other members of this genus include peste des petits ruminants virus (PPRV), which infects sheep, goats and other small ruminants (Scott, 1990), human measles virus (MV), canine distemper virus (CDV), which infects carnivores, and morbilliviruses found in marine mammals (Barrett, 1999). Control of RPV and PPRV over the last 40 years has relied on the use of a live attenuated vaccine, originally developed by prolonged passage of a virulent virus in primary bovine cells in vitro (Plowright & Ferris, 1962); this vaccine provides life-long protection from disease. Successful immunization against experimental infection with RPV has also been achieved using recombinant vaccinia and capripox viruses expressing the envelope glycoproteins (Yilma et al., 1988; Belsham et al., 1989; Yamanouchi et al., 1993; Romero et al., 1994a, b). While vaccination with the attenuated rinderpest vaccine invariably results in a strong neutralizing antibody response, a proportion of animals immunized with recombinant viruses expressing the F glycoprotein are protected despite failing to produce detectable levels of neutralizing antibody, indicating that induction of such an antibody response is not essential for protection (Belsham et al., 1989; Romero et al., 1994b).

Very little is known about the nature or specificity of T cell
of adenovirus, were grown and maintained in RPMI 1640 with HEPES buffer containing the same amounts of antibiotics and FCS. Cells were grown at 37 °C in an atmosphere of 5% CO₂. RPV stocks were titrated in Vero cells. Tenfold dilutions of virus were seeded on semi-confluent monolayers in 96-well tissue culture plates and the titre calculated using the method of Reed & Muench (1938).

**Antibody assays.** Serum was collected at weekly intervals following vaccination, and stored at −20 °C. The titre of antibody produced was assayed in a microneutralization test, on Vero cells for rinderpest-specific antibody or LT cells for capripox-specific antibody (Romero et al., 1994a).

**Isolation of peripheral blood mononuclear cells (PBMC) and CD4⁺ T cells.** PBMC were isolated from heparinized venous blood. Blood was diluted 1:3 with PBS and then centrifuged at room temperature at 250 g for 12 min to obtain buffy coat cells. PBMC were purified from buffy coats by centrifugation at 400 g over Histopaque 1083 (Sigma) for 35 min at room temperature. Cells collected at the interface were washed three times in PBS supplemented with 1% (v/v) FCS prior to being used in the assays.

CD4⁺ T cells were purified from PBMC by magnetic cell sorting (Miltenyi Biotech). PBMC were incubated with the anti-CD4⁺ monoclonal antibody (MAb) CC8 (IgG2a: Bensaid & Hadam, 1991) for 30 min at 4 °C, washed twice with PBS and then incubated with paramagnetic bead-coupled anti-mouse IgG for 20 min at 4 °C. Cells were washed with PBS and antibody-labelled cells were purified according to the manufacturer’s instructions.

**Flow cytometry.** PBMC were harvested from in vitro assays and washed twice in PBS supplemented with 1% (v/v) FCS and 0.1% (v/v) sodium azide (PBA) prior to FACS analysis. The cells were analysed by single-colour fluorescence for CD4⁺, CD8⁺ and the αβ T cell marker WC1 using MAbs CC8 (IgG2a: Bensaid & Hadam, 1991), CC63 (IgG2a: MacHugh & Sopp, 1991) and CC15 (IgG2a: Clevers et al., 1990), respectively, and by two-colour fluorescence for these markers and CD25, detected by MAb IL-21 (IgG1: Naessens et al., 1992). For single-colour immunofluorescence, 10⁶ cells were incubated with MAbs, at the predetermined optimal concentrations, for 30 min at 4 °C. The cells were washed twice with PBA and then incubated with phycoerythrin-labelled Fab₂ goat anti-mouse whole immunoglobulin at predetermined optimal concentrations, for 30 min at 4 °C. The cells were then washed twice and resuspended in 100 µL of PBA prior to analysis. For two-colour immunofluorescence, 10⁵ cells were incubated with the MAbs, at the predetermined optimal concentrations, for 30 min at 4 °C. Cells were washed twice and then incubated with both phycoerythrin-labelled Fab₂ goat anti-mouse IgG2a and fluorescein-labelled Fab₂ goat anti-mouse IgG1 at predetermined optimal concentrations, for 30 min at 4 °C. The cells were then washed twice and resuspended in 100 µL of PBA prior to analysis.

**Preparation of rinderpest antigen.** The Saudi 1/81 strain of RPV was passaged three times in B95a cells to prepare a high titre stock and this stock was used as for subsequent antigen preparation. Cells infected with the virus were harvested when cytopathic effect became extensive. The cells were lysed by freeze-thawing and the cell debris was pelleted at 18,000 g for 60 min at 4 °C, and the supernatant was recovered, the virus pelleted at 18,000 g for 60 min at 4 °C, and then resuspended in TE buffer (10 mM Tris–HCl, pH 7.6; 1 mM EDTA). The resuspended pellet was layered over a 15–60% (v/v) sucrose gradient in TE and centrifuged at 18,000 g for 90 min at 4 °C. The band of virus protein was collected and resuspended in TE before being pelleted once more at 18,000 g for responses to RPV, their role in protective immunity, or indeed if RPV vaccination results in immunosuppression. RPV is known to be highly lymphotropic, with virulent strains causing a severe leukopenia with destructive pathology of lymphoid tissues (Brown & Torres, 1994; Wohlsein et al., 1993, 1995). The related morbillivirus MV has been reported by many authors to cause immunosuppression (Schnorr et al., 1997; Hirsch et al., 1984; Dagan et al., 1987; Tamashiro et al., 1987; McChesney et al., 1989; McChesney & Oldstone, 1989; Gans et al., 1999), with some effects seen following vaccination with otherwise attenuated strains (Smedman et al., 1994; Pala et al., 1998). It has been suggested that RPV vaccination could result in transient immunosuppression (Jeggo et al., 1987) which, if true, would be of great practical importance since vaccination could lead to an increase in mortality in the field due to flare-up of intercurrent infections as a consequence of vaccination.

As a first step to addressing these issues, a study was undertaken to examine the effects of RPV vaccination and infection on bovine T cell responses and to define the viral antigen-specificity of any responding T cells. Vaccination was found to induce a strong CD4⁺ T cell response, with an equivalent response to all of the major structural proteins. The RPV vaccine virus did not cause a detectable suppression of the immune system in the target host animals as judged by their ability to respond to a T cell mitogen.

**Methods**

**Animal vaccination and challenge.** Groups of three Friesian cross Aberdeen Angus calves, age 6–12 months, were vaccinated subcutaneously in the pre-scapular region with 10⁶ TCID₅₀ of the Plowright vaccine strain of rinderpest (RBOK) (Plowright & Ferris, 1962). Two separate groups (animals A–C and G–I) were vaccinated with RBOK. Control animals received 10⁵ p.f.u. of the Kenya sheep-1 (KS-1) vaccine strain of capripox. (animals D–F). Four weeks after vaccination all animals were challenged subcutaneously with 10⁴ TCID₅₀ of the Saudi 1/81 strain of RPV (Taylor, 1986). Throughout the experiment, animal body temperatures and white blood cell counts were recorded at regular intervals. After vaccination and following challenge with RPV, cattle were examined daily for clinical signs of rinderpest infection. All animals showing clinical signs of rinderpest were euthanized by captive bolt on day 9 following challenge with the RPV Saudi 1/81 strain.

**Viruses.** The RBOK rinderpest vaccine virus was propagated in Vero cells and the Saudi 1/81 RPV used for challenge was obtained as freeze-dried spleen extract from a previously infected animal. Lamb testes (LT) cells up to the 16th passage were used to propagate the KS-1 strain of capripox. These cells were maintained in Dulbecco’s minimal essential medium (DMEM) supplemented with 10% (v/v) foetal calf serum (FCS), containing 100 U/ml benzylpenicillin and 100 µg/ml streptomycin (DMEM-complete). Other cell lines, namely 293, BHK, MDBK, MDCK, HeLa, HT-COS, HEP, BSC, RK-13, RS-2 and bovine skin fibroblasts, which were used to test the growth of adenovirus, were grown in the same medium. A mammoot lymphoblastoid cell line, B95a (Kobune et al., 1991), was used to propagate RPV Saudi 1/81 for antigen preparation. These cells, along with CHO cells, which were also used to test growth...
Preparation of adenovirus recombinants. Recombinant adenoviruses were produced using a slight modification of previously published techniques (McGrory et al., 1988; Wilkinson & Akrigg, 1992). We created a combined adenovirus transfer and expression plasmid (pAH1) by ligating the HindIII–HindIII fragment from pMV100 (Wilkinson & Akrigg, 1992), which contains the HCMV immediate early promoter, to HindIII-digested pMV31 (Wilkinson & Akrigg, 1992), which contains the adenovirus sequences required for recombination. The open reading frames for the various RPV proteins (Baron et al., 1993, 1994; Baron & Barrett, 1995; Evans et al., 1994) were excised from their respective plasmids, blunt-ended, and cloned into the unique BamHI site just downstream of the CMV promoter in pAH1. Inserts present in the correct orientation were identified by restriction analysis. Recombinant adenoviruses were produced in 293 cells as described by McGrory et al. (1988). Recombinant adenoviruses were checked for expression of the expected proteins before preparing recombinant virus stocks. The titres were then determined by measuring the TCID\textsubscript{50} in 293 cells.

Preparation of adenovirus-derived rinderpest antigens. B95a cells were infected with recombinant adenoviruses expressing the different RPV genes, or a recombinant adenovirus expressing bacteriophage T7 RNA polymerase, at an m.o.i. of 500. The cells were grown for 72 h following infection, at which time crude preparations of rinderpest antigens were made. For this, B95a cells from each infection were harvested and resuspended in 2 ml of buffered sucrose (8% sucrose, 1 mM EDTA, 20 mM Tris–HCl pH 7.8). Cells were passed 20 times through a 28 gauge needle and cell debris, remaining whole cells and nuclei were pelleted by centrifugation at 6000 r.p.m. for 1 min in a microcentrifuge. The whole process was repeated on the supernatant and the final post-nuclear supernatant was centrifuged at 14000 r.p.m. for 30 min at 4 °C in a microcentrifuge. Preliminary experiments with the resulting pellet and supernatant fractions indicated that the supernatant, comprising the cytosol fraction plus plasma membrane, and most non-nuclear internal membrane fractions, contained the majority of rinderpest antigen in all infections with recombinant adenoviruses, irrespective of the RPV gene expressed. Therefore, this supernatant fraction of each different rinderpest or control recombinant antigen preparation was used in assays of antigen-specific T cell proliferation.
Proliferative and cellular responses to antigen. Antigen-specific proliferation of PBMC and purified CD4+ T cells was assessed in a 96 h assay measuring [3H]thymidine incorporation. Purified PBMC were resuspended at 10^6/ml in RPMI medium supplemented with 10% (v/v) FCS, 100 U/ml benzylpenicillin, 100 µg/ml streptomycin and 10-3 M β-mercaptoethanol (RPMI-complete). 100 µl of the PBMC suspension was added to the wells, in triplicate assays, in the presence of optimal concentrations of the different antigens, 5 µg/ml ConA, or medium alone, in flat-bottomed 96-well plates. In cultures with CD4+ T cells the proliferative response of 10^6 cells per well was measured in the presence of the various antigens, with 10^5 gamma-irradiated (3000 rads) PBMC as antigen-presenting cells. Cultures were pulsed with 1 µCi per well of [3H]thymidine during the last 12–15 h of the proliferation assay and the cells were harvested on glass-fibre filter paper and counted in a scintillation counter.

Optimal dilutions of whole rinderpest antigens, and all recombinant adenovirus-expressed rinderpest antigen preparations, were determined in preliminary experiments using PBMC from rinderpest-vaccinated animals. The proliferative response of PBMC was determined, in triplicate, over a wide range of serial dilutions of the antigen preparations and the optimal dilution was determined as that which gave the maximal proliferative response. In all experiments using recombinant adenovirus-expressed rinderpest antigens, the antigen-specific proliferative response was determined using the optimal dilution. This response was compared to that obtained using the same dilution of the supernatant fraction from B95a cells infected with a recombinant adenovirus expressing the bacteriophage T7 RNA polymerase.

Results

Clinical signs following vaccination and challenge

There was no change in body temperature following vaccination with RBOK (Fig. 1A); however, there was a transient decrease in the white blood cell count by 40–50% between days 4 and 10 post-vaccination (Fig. 1C). In contrast, following infection with KS-1 an increase in body temperature was observed between days 4 and 9 post-vaccination (Fig. 1B), but no change was noted in white blood cell count (Fig. 1D). Following challenge with virulent RPV, no clinical disease was seen in the animals that had been vaccinated with RBOK. In contrast there was a rapid increase in body temperature after the sixth day in animals vaccinated with KS-1 (Fig. 1B). This was accompanied by a dramatic decrease in the white blood cell counts (Fig. 1D), and the onset of clinical signs of rinderpest infection. All animals showing signs of rinderpest were humanely killed on the 9th day following challenge before the disease progressed to the severe stages. These results are similar to those previously reported following a known course of vaccination or disease in cattle (Romero et al., 1994a, b; Wohlsein et al., 1993).

Serum antibody responses

RPV-specific neutralizing antibodies were first detected 14 days after vaccination with RBOK (Fig. 2). The titre, which varied from animal to animal, increased during the course of the experiment. No RPV-specific neutralizing antibody was detected in animals vaccinated with KS-1 virus (data not shown).

Proliferative T cell responses to rinderpest antigen

Antigen-specific T cell proliferative responses to whole rinderpest antigen were monitored at intervals after vaccination. A proliferative response was first observed in PBMC isolated from some RBOK-vaccinated animals at 7 days post-vaccination (Fig. 3) and was evident in all animals by day 14. The intensity of the response varied from animal to animal. However, by day 28, the response to rinderpest antigen was consistently high in all RBOK-vaccinated animals. There was no significant response to the irrelevant antigen (KS-1). In addition, no effect on the ConA-stimulated proliferation of cells isolated from these animals was observed, suggesting that vaccination was not accompanied by a general immuno-suppression.

The proliferative response to rinderpest antigen remained strong following challenge on day 28, but no obvious boosting of the response was apparent in most of the vaccinated animals. In contrast, PBMC from all animals vaccinated with KS-1 failed to proliferate in response to rinderpest antigen, following either vaccination or challenge (Fig. 4). PBMC from these animals, however, showed a strong proliferative response to capripoxvirus antigen, which was detectable by 14 days post-infection in all animals. Following challenge on day 28 with virulent RPV, the proliferative responses of PBMC were dramatically reduced in all animals; by day 6 there was little or...
no response detected to capripoxvirus antigen and a significantly reduced response to the mitogen ConA. This loss of response was associated with the onset of disease and correlated with the decreasing lymphocyte counts.

**Rinderpest-specific proliferative responses are mediated by CD4\(^+\) T cells**

Immunofluorescence analysis of cells from cultures that gave a strong proliferative response to rinderpest antigen demonstrated an increase in the numbers of large CD4\(^+\) T cells and CD4\(^+\) cells that co-expressed CD25 (data not shown). The number of CD8\(^+\) and \(\gamma\delta\) TCR\(^+\) lymphoblasts, and the percentages of these that expressed CD25, did not change significantly after culture with antigen. To confirm that the response was mediated primarily by CD4\(^+\) T cells, the rinderpest-specific proliferative responses of purified populations of CD4\(^+\) T cells were examined. Fig. 5 shows the results obtained with purified CD4\(^+\) T cells from animal B 6 weeks post-vaccination. These cells proliferated strongly in response to rinderpest antigen but no response was elicited to an irrelevant antigen. The response of the purified CD4\(^+\) cells was greater than that of unfractionated cells. These data indicated that CD4\(^+\) T cells were the predominant component of the PBMC that responded to rinderpest antigen in vitro.

**Expression of rinderpest antigens by adenovirus recombinants**

In order to further characterize this T cell response in the vaccinated cattle, we expressed four RPV proteins individually in an adenovirus vector. The proteins were expressed in mammalian cells to ensure that they undergo normal post-translational modifications, particularly in the case of the F and H glycoproteins. Protein bands of the correct molecular masses were immunoprecipitated from lysates of metabolically
labelled cells following infection with the appropriate adenovirus recombinant (data not shown), confirming the presence of the correct cDNA insert and that the proteins were reacting with antibodies specific for the different RPV proteins.

The various RPV antigens were produced in B95a cells since preliminary experiments showed that expression of RPV H was higher in these cells than in any other of a range of cell lines tested (Vero, MDCK, Cos1, Rs-2, HeLa, BSC 40, Hep2, MDBK, CHO, BHK bovine skin fibroblasts and bovine lymphoblasts) (data not shown). Expression of RPV H on the cell surface was increased by increasing the m.o.i. of the infecting adenovirus; however, only a marginal increase in expression was found when the m.o.i. was raised from 500 to 1000. All other antigens were therefore produced from cells infected at an m.o.i. of 500.

**Proliferative responses to rinderpest antigens following vaccination**

Antigen was prepared from B95a cells infected with RPV H, F, N and M adenovirus recombinants and control antigen from cells infected with a recombinant adenovirus expressing bacteriophage T7 RNA polymerase. Fig. 6 shows that a proliferative response could be detected to all of the different rinderpest antigens in one animal on day 7 and in all animals from day 14 onwards following vaccination. A response to each rinderpest antigen was detected at every time-point.

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**Fig. 4.** Proliferative responses of PBMC from KS-1-vaccinated animals. PBMC were isolated from cattle at the time-points indicated after vaccination and subsequent challenge, on day 28 post-vaccination, with the virulent Saudi 1/81 strain of RPV. The proliferative responses of PBMC in the presence of RPV antigen, in medium alone or in the presence of mitogen (ConA) are included as controls. Results are expressed as the mean c.p.m. of triplicate cultures of 10⁵ cells. Each value represents the proliferative response obtained with an optimal concentration of the relevant antigen as determined in preliminary studies.
T cell responses to rinderpest virus

Discussion

There have been many reports of suppression of immune responses following MV infection (Schnorr et al., 1997; Hirsch et al., 1984; Dagan et al., 1987; Tamashiro et al., 1987; McChesney et al., 1989; McChesney & Oldstone, 1989; Gans et al., 1999). In addition, a variety of in vivo and in vitro T cell-mediated responses, including in vitro T cell proliferative responses to measles antigen, have been shown to be suppressed for several weeks following infection with MV (Starr & Berkovich, 1964; Coovadia et al., 1974; Wesley et al., 1978; Whittle et al., 1978; Hirsch et al., 1984; Tamashiro et al., 1987). A number of different reasons have been suggested for this MV-induced immunosuppression (Karp et al., 1996), including a role for the cell–cell interaction of infected and uninfected cells. This response was maintained throughout the course of the experiment. In contrast, no response was detected to antigen prepared from uninfected cells (data not shown) or to the control antigen (bacteriophage T7 RNA polymerase). Animals vaccinated with KS-1 showed no response to any of the rinderpest antigens at any time (data not shown).
uninfected factors (Schlender et al., 1996; Schnorr et al., 1997), soluble factors (Fujinami et al., 1998; Sun et al., 1998), a Th2-bias in the CD4+ T cell response to MV (Crespi et al., 1988; Ward et al., 1991; Griffin et al., 1992; Griffin & Ward, 1993; Ward & Griffin, 1993) and cell cycle arrest (McChesney et al., 1987, 1988; Schnorr et al., 1997). Although RPV is highly lymphotrophic, growing equally well in B cells, $\alpha/\beta$ or $\gamma/\delta$ T cells (Rossiter et al., 1993; B. T. Lund & T. Barrett, unpublished observations), and infection is known to induce a profound leukopenia (Anderson et al., 1996), less is known about the amount or nature of any immune suppression caused by this virus. In this study we found that infection of unvaccinated calves with wild-type RPV clearly resulted in both leukopenia and a marked immunosuppression of the surviving PBMC, as indicated by a rapid reduction in the responses of these cells to either the capripoxvirus antigen to which they had previously been sensitized, or the mitogen ConA.

Immune suppression has been noticed following immunization with the vaccine strain of MV (Smedman et al., 1994; Pala et al., 1998). It has also been suggested that RPV vaccination could result in transient immunosuppression and hence to an increase in mortality following RPV vaccination in the field (Jeggo et al., 1987). In the case of RPV vaccination, the present study showed a rapid in vitro CD4+ T cell proliferative response to RPV antigen, together with unaltered responses to the mitogen ConA, despite the transient leukopenia caused by the RPV vaccine. This leukopenia is presumably caused by the low-level replication of the virus necessary for triggering the immune response. These data indicate that the RPV vaccine does not result in biologically significant immunosuppression. The results are in agreement with a recent field investigation into the frequency of infection with trypanosomosis in vaccinated and unvaccinated cattle in Kenya, where no increased risk of infection was noted in the vaccinated cattle (Stevenson et al., 1999). This contrasts with the immunosuppression observed following MV vaccination in humans. It may therefore be possible to develop other morbillivirus vaccines, including MV vaccines, which cause less immunosuppression in the host. Further studies are required to investigate the cytokine profile of the rinderpest-specific CD4+ T cell responses to determine whether or not they are similar to those induced by MV, and if these responses have the potential to subvert cellular responses to other pathogens.

There was also no evidence of immunosuppression following challenge of RBOK vaccinated animals with the highly virulent RPV: in vitro proliferative responses to rinderpest antigen and ConA were maintained at normal levels. In addition, no increase was seen in the T cell proliferative responses to RPV antigen. This is likely to be a consequence of the solid immunity induced by this vaccine, as reflected by the absence of any clinical signs following challenge. However, an anamnestic response in serum antibody titres was seen in all animals following challenge, indicating that the virus must have replicated to some extent in the vaccinated animals.

A T cell proliferative response to crude RPV antigen was readily detected in animals vaccinated with the attenuated RPV strain. The response was detectable as early as 7 days in some animals and was consistently high from 28 days onwards. As anticipated, when using an inactivated antigen in the in vitro assay, this response was mediated mainly by CD4+ T cells, as demonstrated by immunofluorescence staining of the responding cells and testing the responses of purified CD4+ T cells. This finding does not exclude the possibility that RPV also stimulates a CD8+ T cell response, as the inactivated antigen used in the assay is unlikely to undergo processing by the endogenous route, which is required for stimulation of CD8+ T cells. Examination of cattle vaccinated with an unrelated vaccine (capripox KS-1) was carried out as a control and these animals showed a similar immune response to crude KS-1 antigen.

Studies of the antigen-specificity of the CD4+ T cell response focussed on the F, H, N and M structural proteins of the virus. The first three proteins have each been implicated in stimulating protective T cell responses following infections by MV and other paramyxoviruses (Bellini et al., 1981; Rose et al., 1984; Ilonen et al., 1990; Muller et al., 1993; Pette et al., 1993; Hickman et al., 1997) and the M protein of MV has also been identified as a target for T cell responses (Bellini et al., 1981). In the case of RPV, the N protein has been shown to induce a low level of protection and synthetic peptides based on the N protein sequence have been shown to stimulate T cells in vaccinated cattle and in cattle recovered from infection with a mild strain of the virus (Ohishi et al., 1999a, b). The aim of the present experiments was to determine whether the response in animals is biased to particular proteins and, if so, whether the bias in specificity varies between animals. Such data would be valuable when designing new recombinant vaccines for RPV or other morbilliviruses. Adenovirus recombinants were used to produce individual RPV proteins for analysis of the specificity of virus-specific CD4+ T cell responses. This expression system allows the production of relatively high levels of antigen with protein folding and post-translational modification properties similar to those of the native viral proteins. Uptake and processing of the proteins by antigen-presenting cells should be similar and any biological effects the molecules might have on immune cell types should be retained. The T cell responses detected using these recombinants showed a high degree of specificity for RPV antigens, as there were no detectable responses to either the adenovirus or host cell proteins present in the preparations. This was demonstrated by the lack of responses to adenovirus-derived bacteriophage T7 polymerase antigen prepared in an identical manner to that of the individual rinderpest antigens. No bias in the response was observed to any of the rinderpest antigens; responses of similar magnitude and kinetics were detected in all the animals examined. A similar broad specificity of the CD4+ T cell response to gel-purified MV proteins has been reported (Bellini et al., 1981).
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References


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