Recognition of foot-and-mouth disease virus and its capsid protein VP1 by bovine peripheral T lymphocytes

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The role of T cells in immunity to foot-and-mouth disease virus is still poorly defined compared to that of the humoral response. In this paper we describe a systematic, longitudinal study on the cellular recognition of FMDV and its subunit protein VP1 by bovine peripheral blood T lymphocytes. Multiple vaccination with a single virus serotype induced a serotype cross-reactive proliferative T cell repertoire that varied in magnitude between individual animals and with the serotype of the vaccine used. Primary proliferative T cell responses of vaccinated and acutely infected cattle were weak relative to the magnitude of responses determined for the same animals after boosting. In contrast, the level of circulating antibody produced after both primary and secondary exposure to virus was good. Phenotypic analysis of lymphocytes from vaccinated or infected cattle showed a small increase in CD8+ T cells after infection compared to vaccination. However, in general the profiles of circulating lymphocytes elicited were similar. Thus, we were not able to use proliferative or phenotypic analyses to distinguish between vaccinated and convalescent cattle. T cell recognition of VP1 by multiply-vaccinated cattle was serotype-specific implying that the cross-reactive responses observed with whole virus may be attributed to proteins other than VP1. In contrast to other studies, immunization with recombinant VP1 induced only low levels of neutralizing antibody and failed to elicit profound proliferative responses or protection even after two immunizations.

Introduction

Foot-and-mouth disease is an acute and highly contagious virus disease of cattle and other cloven-hoofed livestock. The causative agent is an aphthovirus of which there are seven serotypes: O, A, C, Asial and South African Territories (SAT) 1-3. Each virion consists of a single-stranded, positive sense RNA genome enclosed within a non-glycosylated icosahedral capsid comprising 60 copies each of four structural polypeptides VP1-4 (Burroughs et al., 1971; King & Newman, 1980; Acharya et al., 1989).

The importance of neutralizing antibody in protection against FMDV has been recognized for many years (Laporte et al., 1973; Bachrach et al., 1975, 1979, 1982; Kleid et al., 1981, 1984; Bittle et al., 1982; Cartwright et al., 1982; Strohmaier et al., 1982). As a consequence, considerable research effort has been invested in the development of vaccines capable of inducing reproducibly high titres of FMDV-specific antibody. In cattle, protection has been correlated with the titre of neutralizing antibody induced by aqueous vaccine (van Bekkum, 1969; Pay & Hingley, 1992). Infection and vaccination both elicit serotype-specific neutralizing antibodies although infection is reported to induce higher titres (Meloen, 1979a, b) and longer lasting protection (Cunliffe, 1962; Garland, 1974) than vaccination.

Various residues of the surface-exposed polypeptides VP1, VP2 and VP3 contribute to the antigenic sites recognized by neutralizing antibodies. However, only isolated VP1 is able to induce virus-neutralizing antibodies and protection (Bachrach et al., 1975; Laporte et al., 1973). Recently, protection has been achieved using recombinant VP1 and recombinant fusion proteins and synthetic peptides containing the continuous B cell epitopes located in the G-H loop (residues 134-160) and the carboxy terminus (residues 200-213) of VP1 (Yansura et al., 1983; Francis et al., 1985; DiMarchi et al., 1986; Broekhuysen et al., 1987).

In contrast to the well characterized role of humoral immunity in FMD, the role of the cellular response and,
in particular, the role of T cells in the development of B cell responses in natural host species is poorly understood. Early studies in a guinea-pig model failed to demonstrate any correlation between T cell effector cell function, as defined by delayed hypersensitivity, and protection (Knudsen et al., 1979). Subsequent studies have demonstrated dose-dependent, serotype cross-reactive FMDV-specific proliferative T cell responses in cattle (Wardley et al., 1979; Collen & Doel, 1990; Collen et al., 1991; van Lierop et al., 1992) and pigs (Saiz et al., 1992) and recent studies in the guinea-pig model (Bartels et al., 1994) further support these findings.

In this paper we present a systematic, longitudinal study of cellular and humoral immune responses to FMDV and its structural protein VP1 for naive, vaccinated and infected cattle. The responses to virus were found to be in agreement with previous studies although responses to VP1 were poor, even after two immunizations with recombinant protein.

**Methods**

**Virus strains.** Cattle were infected with FMDV strain O1 Lausanne, or vaccinated with strains O1 Lausanne, A24 Cruzeiro, Cl Oberbayern or Asial India 8/79. In vitro assays were performed with purified FMDV strain O1 BFS 1860, A24 Cruzeiro, Cl Noville or Asial Iran 1/73.

Cloning, expression and purification of recombinant VP1 (P1D). The P1D genome region of FMDV cDNA (pMR15) (Ryan et al., 1989) was amplified by PCR to create an exact copy of P1D flanked by unique BglII restriction enzyme sites. The PCR product was restricted with BglII and ligated into the pGEX-2T plasmid (Pharmacia). The construct thus created (pGEX-1D) encodes a fusion protein consisting of VP1 linked to the carboxy terminus of glutathione S-transferase (GST) via a thrombin-digestible sequence.

*E. coli* strain JM109 was transformed with pGEX-1D and grown overnight at 37°C with shaking. Cultures were diluted 1:10 with fresh Luria Broth containing ampicillin (100 µg/ml) and incubated for 2-5 h at 37°C with shaking. Protein expression was induced by the addition of IPTG to a final concentration of 0.1 mM and the cultures were incubated for a further 2-5 h. Cultures were pelleted by centrifugation, resuspended in Tris-HCl (50 mM, pH 8.0), sucrose (15%, w/v) and Triton X-100 (0.12%, v/v) and disrupted by sonication. The lysate was centrifuged at 15,000 g for 3 min and the pellet resuspended in Tris-HCl (50 mM, pH 8.0), Triton X-100 (0.12%, v/v) and EDTA (10 mM). The resulting suspension was sonicated and pelleted as described above and the pellet resuspended in Tris-HCl (50 mM, pH 8.0), and EDTA (10 mM). Inclusion bodies were pelleted by centrifugation at 15,000 g for 3 min, resuspended in TS buffer containing CaCl₂ (2.5 mM) and incubated with thrombin (human plasma; Boehringer Mannheim) at a final concentration of 20 U/ml for 2 h at room temperature.

The insoluble capsid protein, GST and uncleaved fusion protein were harvested by centrifugation and separated by SDS-PAGE. African swine fever virus fusion protein expressed in pGEX-2T (donated by Dr Sun, IAH) and pGEX-2T with no insert were used as controls. Lysate (1 mg) was diluted with SDS sample buffer [Tris–HCl (125 mM, pH 6.8), 2-mercaptoethanol (600 mM), SDS (4%, w/v), glycerol (10%, v/v) and bromophenol blue (0.02%, w/v)] and dissociated by heating for 3 min at 100°C. Lysate proteins were resolved in a discontinuous 10% SDS-PAGE system (Laemmli, 1970). Gels were stained with Coomassie brilliant blue, destained and equilibrated with tap-water and protein bands excised. Identical gels from several gels were loaded into the same tube of a Bio-Rad electro-eluter (model 422) and electrophoresed in a continuous buffer system [Tris–HCl (25 mM, pH 8.3), glycine (192 mM) and SDS (0.1%, w/v)] at 10 mA per tube for 4-5 h at room temperature. Eluted proteins were aspirated from the dialysis membrane caps and precipitated by the addition of 4 vols of cold (−70°C) acetone followed by incubation in dry-ice-ethanol for 1 min. The samples were pelleted at 17,500 g for 5 min, air-dried and resuspended in sterile medium. Samples were analysed by SDS–PAGE and Western blotting and stored at −70°C until required.

**Immunization and sampling of animals.** Friesian cattle were immunized against FMDV by infection or by subcutaneous inoculation of a standard cattle dose of inactivated virus in either aluminium hydroxide gel (0.5%, v/v) and saponin (1.7 mg/ml) (aqueous) or Montanide ISA 206 (incomplete oil) as adjuvant and used as a source of serum and immune lymphocytes. Blood was taken for serum and was allowed to clot for 30 min at 37°C. The clots were allowed to contract overnight at 4°C and the serum aspirated, clarified by centrifugation and stored at −20°C. Blood for the isolation of peripheral blood mononuclear cells (PBMC) was collected into preservative-free heparin to give a final concentration of 10 U/ml heparin and was used immediately.

**Group 1. Multiple virus vaccination.** Pairs of cattle were immunized against FMDV O1 (RV68 and RV95), A24 (RV70 and RV96), Cl (RV93 and RV94) and Asial (RV91 and RV92) by subcutaneous inoculation of a standard cattle dose of commercial aqueous vaccine. The animals were revaccinated three more times at 3 monthly intervals using aqueous O1, A24, Cl and Asial vaccines prepared by the International Vaccine Bank (IVB), Pirbright Laboratory, UK. The first boost was performed with incomplete oil vaccine and the second and third boosts utilized aqueous vaccine.

**Group 2. Longitudinal study.** Cattle were infected with FMDV O1 either by intradermal tongue inoculation of 10⁶ TCID₅₀ (tissue culture infective doses) of virus (SC79, SC80 and SC81) or by subcutaneous inoculation of a standard cattle dose of incomplete aqueous vaccine (SC85, SC86 and SC87) prepared by the IVB. Five months later, both groups were challenged on the tongue with 10⁶ TCID₅₀ of O1 virus.

**Group 3. Recombinant VP1 vaccination.** Cattle were immunized by subcutaneous and intramuscular inoculation of 0.5 mg F-VP1 (SG42, SG43 and SG44), 0.25 mg of GST (SG45, SG46 and SG47) or 0.25 mg of r-VP1 (SG48, SG49 and SG50) emulsified in incomplete oil and splits equally between each of the routes. After 39 days, all of the animals were boosted with the same dose and formulation of antigen used for primary immunization. Three naive cattle were immunized with 0.5 mg F-VP1 (SG60, SG61 and SG62) or a standard cattle dose of O1 virus vaccine (SG63 and SG64). One month later all of the animals were challenged by intradermal tongue inoculation of 10⁶ TCID₅₀ of O1 virus.

**Virus-neutralizing antibody assay.** The neutralizing antibody activity of serum from immunized cattle was determined in a 96-well flat-bottomed microwell plate assay (Golding et al., 1976) using Instituto Biologico renal swine (IB-RS-2) cells. Wells were observed for cytopathic effect and the neutralization titre of a serum expressed as the reciprocal of the final dilution of serum that neutralized 50% of the virus activity.

**ELISA.** Nunc immunoplates were coated with virus at 4°C overnight or with protein or peptide (2 μg/ml, 50 μl/well) at 37°C overnight. Proteins and peptides were fixed with methanol (50 μl/well) and the solvent allowed to evaporate. Plates were washed three times with PBS.
were washed and then incubated for 1 h at 37 °C with a 1:2000 dilution of rabbit anti-bovine IgG-horseradish peroxidase conjugate (Dakopatts) diluted in 'blocking buffer'. After washing, bound conjugate was visualized using *ortho*-phenylenediamine (OPD) and H₂O₂ as the chromogen-substrate and colour development was stopped by the addition of sulphuric acid (10%, v/v). Changes in absorbance were determined at 492 nm. The antibody titre was determined as the reciprocal of the last serum dilution with a value twice that of a negative background serum.

**Western blotting.** Protein samples were separated in a discontinuous 10% SDS-PAGE system (Laemmli, 1970) and transferred to nitrocellulose membranes. The membranes were washed with distilled water and blocked with PBS containing 'Marvel' dried skimmed milk powder (5%, w/v) for 1 h at 37 °C. Titrated antisera and conjugate were diluted in PBS containing 'Marvel' (5%, w/v) and the blots were washed three times in PBS for 5 min each wash with agitation during each step. Blots were incubated with rabbit or bovine anti-O1 virus for 1 h at 37 °C, washed and then incubated with swine anti-rabbit (Dakopatts, P217) or rabbit anti-bovine (Dakopatts, P159) IgG-horseradish peroxidase conjugate (1:500) for 1 h at 37 °C. Protein bands were visualized using PBS containing diaminobenzidine (DAB; Sigma D5637: 1 mg/ml) and H₂O₂ (0.002%, v/v) as the chromogen-substrate and NiCl₂ (0.04%, w/v) to enhance the colour. The reaction was stopped with distilled water.

**Lymphoproliferation assays.** PBMC were isolated from heparinized blood by centrifugation over Lymphopaque (Nycomed) as previously described (Collen & Doel, 1990). PBMC were aspirated, pelleted and resuspended in erythrocyte lysis buffer (ammonium chloride (155 mm), EDTA (0.1 mM) and sodium bicarbonate (10 mm), pH 7.2) for 5 min on ice. The cells were washed three times in ice-cold Eagle’s medium and resuspended at 10⁶ cells/ml in complete RPMI medium [RPMI 1640 medium containing heat-inactivated fetal calf serum (5%, v/v), L-glutamine (2 mm), sodium pyruvate (1 mm), Hepes (20 mm), 2-mercaptoethanol (0.05 mm), penicillin (100 IU/ml) and streptomycin (100 µg/ml)]. Aliquots (100 µl) of cells were dispensed into 96-well round-bottomed microtitre plates together with 100 µl of various concentrations of purified FMDV (12, 1-2, 0.12, 0.012 and 0.0012 µg/ml final concentration), r-VPI (10, 1, 0.1, 0.01 and 0.001 µg/ml final concentration) or mitogen [ConA (Sigma C2010), 5 µg/ml; PHA-P (Sigma L9132), 2 µg/ml]. Cultures were incubated in a humidified atmosphere for 5 days at 37 °C, pulsed with tritiated [*methyl-³H]*thymidine (0.2 µCi per well) and cultured for a further 18 h. Labelled cells were harvested onto glassfibre filtermats using a Skatron harvester and the incorporation of thymidine determined using a scintillation counter (LKB Wallac; 1205 BetaPlate). The mean of triplicate cultures was expressed as stimulation index (S.I.; sample c.p.m. divided by background c.p.m.).

**Cytotoxicity analysis.** The monoclonal antibodies used in this study have been described elsewhere (Howard et al., 1991). PBMC were suspened in FACS diluent [PBS containing azide (0.1%, w/v) and BSA (5%, w/v)] and labelled with IL-A43 (anti-CD2)+CC15 (anti-WC1) to stain total T cells, IL-A12 to stain CD4⁺ T cells, IL-A17 to stain CD8⁺ T cells, IL-A29 (anti-WC1) to stain gamma/delta T cells, CC21 (anti-WC3, putative anti-CD21) to stain B cells, IL-A30 to stain IgM⁺ B cells or IL-A46 to stain monocytes. Cells and antibodies were incubated for 20 min at 4 °C and washed three times with FACS diluent. Labelled cells were incubated with goat anti-mouse IgG-phycoerythrin conjugate (Southern Biochemicals; 5 mg/ml) for 15 min at 4 °C, washed and resuspended in PBS containing paraformaldehyde (1%, v/v) overnight at 4 °C. Fixed cells were diluted with PBS and analysed by flow cytometry (FACScan, Becton Dickinson). Data was processed using Lysys II software (Hewlett Packard).

**Results**

**Specificity of the cellular immune response to FMDV**

The specificity of the proliferative cellular immune response to FMDV was examined using PBMC from a bovine which had been multiply vaccinated with FMDV O1. PBMC from animal RU68 were cultured with different concentrations of O1, A24, C1 and Asial. In certain cases, different isolates, e.g. O1 and C3, were used for in vitro stimulation and cattle vaccination due to the availability of these vaccines (provided by the International Vaccine Bank, Pirbright, UK). Proliferative responses titrated with decreasing virus concentration (data not shown) were serotype cross-reactive (Fig. 1a). The magnitudes of responses varied from week to week although the responses to the homologous virus were always greater than those to heterologous virus and the relative degree of cross-reaction was in general agreement between assays. Fig. 1(b) shows the mean of normalized values for animal RU68 where the homologous response is given a value of 100%. The percentage relative cross-reactivity was 68.6% ± 11.1% for A24, 45.2% ± 11.1% for C1 and 56.6% ± 5.6% for Asial. In other similar experiments utilizing animals vaccinated against different serotypes of FMDV the extent of cross-reactivity was seen to vary with the serotype of the vaccine and between individual animals (data not shown).

**Immune responses of vaccinated and infected cattle**

Infection has been reported to induce higher titres of neutralizing antibody and longer lasting protection than vaccination. To examine whether this could be due to a more vigorous cellular immune response to virus by infected animals, cattle were vaccinated or infected and then challenged. The cellular and humoral immune responses of both groups of animals were systematically analysed over the time-course of the experiment. Protection was observed after challenge of vaccinated and infected animals.

Virus-specific antibody was induced by infection (SC79, SC80 and SC81) and vaccination (SC85, SC86 and SC87) of cattle and responses were of a greater magnitude but less sustained in the infected compared to the vaccinated cattle (Fig. 2). However, the highest antibody titres did not correlate with the strongest proliferative responses (data not shown).

The optimum dose of virus for stimulation of lymphoproliferative responses was found to be 1.2 µg/ml for O1, A24 and C3 and 12 µg/ml for Asial. Responses analyzed.
Fig. 1. Cross-reactive cellular immune response of PBMC from cattle multiply vaccinated with O1 Lausanne (three independent experiments). Proliferative response in vitro to FMDV serotypes O1BFS 1860, A24 Cruzeiro, C1 Noville and Asial Iran 1/73 using PBMC from animal RU68 multiply vaccinated with O1 Lausanne (see Methods). Isolated PBMC were stimulated in vitro with the optimal antigen concentration for each FMDV serotype. The experiments were performed over consecutive weeks on days 12, 19 and 33 post-tertiary immunization. (a) Experiment 1 (filled bars), experiment 2 (empty bars) and experiment 3 (double-hatched bars). The background c.p.m. were 917, 1294 and 901, respectively. (b) Normalized proliferation indices of the mean of the experiments described in (a). PBMC (10^6/well) were cultured with 1 μg/ml of purified FMDV serotypes and proliferation was measured by [3H]TDTR incorporation after 6 days. Proliferation is expressed as a stimulation index (S.I.; c.p.m. in the presence of antigen/c.p.m. with medium alone). Standard deviations were < 20%.

Fig. 2. ELISA antibody responses of cattle infected or vaccinated with O1 Lausanne. (a) Primary infected animals (10^5 TCID on the tongue): SC79 (C), SC80 (O) and SC81 (■). (b) Primary vaccinated animals (inactivated virus emulsified with incomplete oil adjuvant and applied subcutaneously): SC85 (C), SC86 (O) and SC87 (■). Serum antibody titres were determined against the homologous FMDV serotype by direct ELISA (see Methods). The titres were expressed as the reciprocal of the serum dilution with an absorbance of 0.3 after subtracting that of the corresponding control serum.
were compared at 1.2 μg/ml. Immediately after infection (Fig. 3) or vaccination (Fig. 4) proliferative responses were significant but of low magnitude. The three vaccinated cattle presented highly variable responses (data not shown) and a weak serotype cross-reactive response between O1 and Asia1 was seen with vaccinated and infected cattle (Figs 3a, d and 4a, d). Serotype cross-reactivity between all of the viruses used became apparent after challenge (Figs 3 and 4). In some assays the response of vaccinates to heterologous virus was higher than the responses to homologous virus despite the same antigen stocks being used throughout the experiments. These results were observed very randomly in 2/6 animals. Data is shown for animals SC80 (infected) and animal SC86 (vaccinated) where the degree of serotype cross-reactivity observed after challenge was O1 > C3 = Asia1 > A24 and O1 > C3 > A24 > Asia1, respectively.

**Phenotypic analysis of vaccinated and infected cattle**

Cellular proliferation against purified virus did not distinguish infected from vaccinated cattle. To examine whether infection might differ from vaccination with respect to the lymphocyte subsets which were activated, circulating lymphocyte populations were examined by fluorometric cytometry. Table 1 shows the mean of the cell subset percentages present in peripheral blood from cattle primary infected (SC79, SC80 and SC81) or vaccinated (SC85, SC86 and SC87) with FMDV O1 Lausanne and then challenged with live virus. PBMC were isolated from peripheral blood before and after the secondary immunization at regular intervals. PBMC were stained as described in Methods. Total and CD8+ T cells of primary infected animals showed, after challenge with live virus, a greater increase than did primary vaccinated animals. However, there was no apparent difference in the numbers of circulating CD4+ T cells for either group. The increase in T cell numbers following challenge was as follows: 17.4% vs 9.4% total T cells; 6.5% vs 3.4% CD8+ T cells; 5.6% vs 5.1% CD4+ T cells for infected and vaccinated cattle, respectively.

**Cellular and humoral responses induced by recombinant VP1**

Serological responses to VP1 have been extensively studied for more than a decade. To examine the cellular
response, recombinant VP1 was cloned, expressed and purified. FMDV 1D genomic DNA, amplified by PCR, was ligated into pGEX-2T expression vector. The new construct, pGEX-1D, encoded a fusion protein consisting of VP1 linked to GST (F-VP1). The antigens used for the immunological studies were F-VP1 and the cleaved product VP1 (r-VP1). Both antigens were purified by electro-elution from Coomassie blue stained SDS–PAGE gel bands and used to immunize groups of cattle. Cellular and humoral responses were analysed.

To determine whether the recombinant products could be recognized by FMDV immune cattle, purified r-VP1 and F-VP1 were used to stimulate PBMC from vaccinated-challenged cattle SC86 and SC87 (data not shown). The optimum dose for stimulation of proliferation was 1-2 μg/ml and the response was dose-dependent. On this basis, 1 μg/ml of recombinant protein was used to stimulate proliferation in subsequent experiments.

All of the animals immunized with r-VP1 failed to develop a significant anti-viral antibody response and there was no protection at challenge (data not shown). Moreover, after a single immunization with 0.25 mg r-VP1 in oil, no anti-VP1 antibody was detected and only low titres were detected after a second dose (data not shown). Similarly, peripheral T cells taken from animals immunized once or twice with r-VP1 or F-VP1 failed to respond to recombinant proteins or purified virus in vitro (data not shown).

Since vaccinated-challenged cattle were capable of responding to r-VP1 and F-VP1 (data not shown), T cell recognition of VP1 was examined using cattle multiply vaccinated against O1, A24, C1 and Asial. All of the animals responded heterotypically to FMDV O1 (Fig. 5) and the O1 vaccinates also responded to r-VP1 and F-VP1. However, none of the animals immunized with A24, C1 or Asial vaccines showed any response to r-
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Fig. 5. Serotype-specificity of the proliferative response to VP1 and F-VP1 recombinant proteins. Proliferative responses to recombinant proteins (VP1, F-VP1, GST and ASF fusion protein) and FMDV O1 serotype of PBMC from cattle multiply vaccinated (see Methods) with O1 Lausanne (animals RU68 and RV95), A24 Cruzeiro (animals RV96 and RU70), C1 Oberbayern (animals RV93 and RV94) and Asial India (animals RV91 and RV92). Proliferative responses of PBMC from animals: (a) RU68, RV96, RV93 and RV91; (b) RV95, RU70, RV94 and RV92. From left to right: FMDV O1 (double-hatched bars), VP1 (filled bars), F-VP1 (hatched bars), GST (empty bars) and ASF fusion protein (dotted bars). Background c.p.m. values were 954, 1142, 2715, 821, 610, 3109, 582 and 538 for animals RU68, RV96, RV93, RV91, RV95, RU70, RV94 and RV92, respectively. PBMC (10^5/well) were cultured with purified FMDV or recombinant proteins (1 μg/ml), and proliferation was measured by [3H]Tdr incorporation after 6 days. Proliferation is expressed as a stimulation index (S.I.). Standard deviations were < 20%.

VP1. The responses to the control antigens GST and F-ASF were background for all of the animals.

Discussion

Humoral immunity to foot-and-mouth disease has been extensively studied in both small animal models and in a natural host, cattle, although there has been a lack of systematic investigation of the role of T cells in the immune process due to a long-standing perception that antibody alone is sufficient for protection. Protection can be transferred with immune antibodies (McCullough et al., 1986) or B cells (Borca et al., 1986) but not T cells (Borca et al., 1986; Knudsen et al., 1979) even though FMDV is a T cell-dependent antigen (Collen et al., 1989). In cattle, the level of antibody induced by inactivated virus vaccine has been correlated with protection (Pay & Hingley, 1992). Here we report the results of a longitudinal study of cellular and humoral immune responses to FMDV and the subunit antigen, VP1, in cattle. Given that both infection and vaccination confer protection and that protection induced by infection is widely considered to be of longer duration (Cunliffe, 1962; Garland, 1974), both routes of immunity were included in the experimental design to permit a comparison of vaccination versus infection and primary versus secondary exposure to FMDV.

A serotype-specific humoral response developed following infection or vaccination. As observed by Meloen (1979a, b), the magnitude of the response was greater for infected than for vaccinated animals. The titre of antibody induced by infection gradually declined over the first 2 months post-infection although in other studies neutralizing antibody has been observed to remain high for at least 18 months (Cunliffe, 1962; Garland, 1974; Gomes et al., 1972). The titre of antibody induced by vaccine was lower but more sustained, which probably reflects the influence of adjuvant.

After priming cattle by infection or vaccination, proliferative T cell activity was of a low magnitude and showed only limited serotype cross-reactivity for the serotypes studied. However, we did not examine the cross-reactivity of C3 with O1 before challenge. The magnitude of these responses and the level of serotype cross-reactivity were both greatly increased after challenge and were in general agreement with previous studies (Collen & Doel, 1990; van Lierop et al., 1992). However, neither the magnitude nor the dynamics of
proliferative responses for infected and vaccinated cattle showed sufficient differences to be useful to classify animals in either of these two groups.

The similarity in proliferative responses probably reflects the use of purified virus as the in vitro stimulant since in principle this form of antigen contains only structural proteins which would be present in both vaccine and infectious virus. Cytofluorometric analysis of circulating lymphocytes suggests that infection may preferentially activate CD8+ T cells as would be predicted for proteins resulting from cytosolic replication and endogenous processing (Townsend & Bodmer, 1989). Thus, subsequent studies need to examine and contrast the role of structural and nonstructural proteins in the repertoire of infected versus vaccinated animals.

The low primary cellular immune response to virus, relative to boosting after challenge, was in contrast to the vigorous development of antibody during the initial priming response. One possibility is that the initial response to infection and vaccination may be biased towards the activation of Th2 cells. If this is indeed the case, then it should be possible to monitor such an event by determining the cytokine messages present in circulating T cells during this period. Although we do not have any cytokine data to support this, an increase in IL-4 and IL-10 versus IFN-γ and IL-12 in sera would support the tendency for a Th2 response.

Finally, almost all of the work on VP1 to date has concentrated on the antibody response to this protein rather than the cellular immune response. Virus-derived VP1 was shown to induce neutralizing antibodies in pigs (Laporte et al., 1973) and guinea-pigs (Bachrach et al., 1975), although protection was achieved only after multiple immunization. While others have achieved protection of cattle (Kleid et al., 1981, 1984; Yansura et al., 1983; Shire et al., 1984; McKercher et al., 1985) and pigs (Kleid et al., 1981; Broekhuijsen et al., 1987) with recombinant VP1, our results showed only low neutralizing antibody titres after two immunizations of either r-VP1 or F-VP1, and a complete lack of protection. Similarly, poor results were originally reported by Kleid et al. (1981) using whole VP1 and protection was subsequently achieved using only the FMDV loop region of VP1. We and others have shown that the T cell response to this region is poor in cattle (Collen et al., 1991; Glass et al., 1991; van Lierop et al., 1994) and is restricted by MHC class II molecules (Glass et al., 1991). Indeed, van Lierop et al. (1994) have shown that the FMDV loop region is not a source of T cell determinants after in vitro cathepsin D treatment and thus, albeit indirectly, this region is unlikely to be a good source of T cell determinants in vivo. Thus, it still remains to define a suitable antigenic region capable of potentiating virus protection in a wide range of cattle populations.

References


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