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Early Events in Immune Evasion by the Lentivirus Maedi-Visna Occurring within Infected Lymphoid Tissue

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Infections caused by lentiviruses, including human immunodeficiency virus, are characterized by slowly progressive disease in the presence of a virus-specific immune response. The earliest events in the virus-host interaction are likely to be important in determining disease establishment and progression, and the kinetics of these early events following lentiviral infection are described here. Lymphatic cannulation in the sheep has been used to monitor both the virus and the immune response in efferent lymph after infection of the node with maedi-visna virus (MVV). Viral replication and dissemination could be detected and consisted of a wave of MVV-infected cells leaving the node around 9 to 18 days postinfection. No cell-free virus was recovered despite the fact that soluble MVV p25 was detected in lymph plasma. The maximum frequency of MVV-infected cells was only 11 in 10^6 but over the first 20 days of infection amounted to greater than 10^4 virus-infected cells leaving the node. There was a profound increase in the output of activated lymphoblast from the lymph nodes of infected sheep, characterized by an increased percentage of $CD8^+$ lymphoblasts. All of the $CD8^+$ lymphoblasts at the peak of the response expressed both major histocompatibility complex class II DR and DQ molecules but not interleukin-2 receptor (CD25). The in vitro proliferative response of efferent lymph cells exiting the node after challenge with MVV to both recombinant human interleukin-2 and the mitogen concanavalin A was decreased between days 8 and 16 postinfection, and a specific proliferative response to MVV was not detected until after day 15. Despite the high level of $CD8^+$ lymphoblasts in efferent lymph, direct MVV-specific cytotoxic activity was demonstrated in only one of the five MVV-challenged sheep. MVV-specific antibody responses, including neutralization and MVV p25 immune complexes in efferent lymph, were detectable during the major period of virus dissemination. The relationship of these findings to the evasion of the host's acute immune response by MVV is discussed.

Lentiviral infections of humans and animals result in disease characterized by a long incubation period and a slow progression of disease (45). The course of lentiviral infection can be divided into a number of stages: (i) primary exposure to virus leading to the establishment of infection; (ii) a prolonged, largely asymptomatic incubation period of the virus in the host, with gradual development of pathological lesions in a variety of organs and tissues; and (iii) a decline into patent disease and death (40).

Persistent infection with a lentivirus is characterized by continuous viral replication, but this occurs in the face of a vigorous host immune response (24, 45). Studies on the nature of the interactions between the virus and the host immune response are therefore of special interest in understanding the pathogenesis of lentiviral disease. The patho-physiological events occurring immediately postinfection (p.i.) and in the interval between infection and diagnosis remain obscure, yet these early stages of the infective process are likely to have a significant influence on the establishment of infection and hence the progress of disease. In human immunodeficiency virus (HIV), there is evidence that early immune selection of infecting virus subtypes can dramatically alter the time course of disease (64). It is also at this time that any immune mechanisms induced by prior vaccination must act to prevent or clear viral infection.

Lymphoid tissue is the major site of any specific immune reaction and continuing lentiviral replication during disease

progression (10, 50). A unique in vivo model for investigating different phases of the lentivirus-host immune response interaction is provided in the sheep by combining cannulation of the efferent lymphatic vessels (26) with infection of sheep with the ruminant lentivirus maedi-visna virus (MVV). This technique has allowed direct access to the lymphatic output of a node for analysis (65) after acute local infection with MVV in this study.

MVV was the first discovered lentivirus (23, 62), although HIV-1 is now the best-characterized member of this group of viruses. MVV is known to infect cells of the monocyte/macrophage lineage and possibly dendritic cells but not lymphocytes (16, 17, 19, 46). This restriction in tropism makes the study of MVV infection a valuable model for understanding lentiviral pathogenesis in the absence of T-cell infection. Clinical MVV infection consists of a multisystem disease characterized by chronic active inflammation, presenting as interstitial pneumonitis (maedi), demyelinating leukoencephalomyelitis (wasting or visna), and mastitis (7, 23).

In this report, the major features of the acute immune response to MVV infection and viral dissemination are described and the relevance of these data to the failure of the immune system to prevent the establishment of a persistent infection is discussed.

MATERIALS AND METHODS

Animals. Finnish Landrace sheep (1 to 3 years of age) were purchased from the Moredun Research Institute, Edinburgh, Scotland.

Prefemoral or popliteal efferent lymphatic cannulations were performed as described previously (25, 26). Not less

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than 5 days postcannulation, sheep were injected subcutaneously with 2 to 5 ml of mock-infected or virus-infected autologous cell supernatant (10^5 to 10^6 50% tissue culture infective doses [$TCID_{50}$]; see Table 3) at several sites within the drainage area of the cannulated lymph node.

Skin cell lines and virus culture. Autologous skin cell lines for each sheep were derived from skin biopsies taken at least 2 months prior to cannulation. The biopsies and cell lines were prepared by the method of Liu and Karasek (33) as modified by Rheinwald and Green (55).

Ninety percent confluent skin cell monolayers were infected with 0.1 $TCID_{50}$ of MVV strain EV1 61 (EV1) per cell in Dulbecco modified Eagle medium (DMEM) supplemented with 2 mM L-glutamine, 100 U of benzyl-penicillin per ml, 100 U of streptomycin per ml, and 2% fetal calf serum (FCS) (2% DME). When extensive cytopathic effects were seen (usually 5 days p.i.), the virus-containing cell supernatant was harvested, clarified by centrifugation (800 $\times$ g, 20 min), and stored at $-70°C$. The titer of each virus stock was determined on autologous skin cell monolayers, and the concentration was calculated by using the quantal method of Reed and Muench (43). Mock-infected antigen was prepared as described above from day 5 mock-infected skin cell cultures.

Analysis of efferent lymph. Lymph was collected into sterile plastic bottles containing 909 U of benzyl-penicillin and streptomycin and 250 U of sodium heparin (Sigma Chemical Co. Ltd.). Bottles were changed three times daily; short (2-h) day collections were used for fluorescence-activated cell sorting (FACS) analyses, and overnight collections were used for functional analyses. Cells were routinely >99% viable as detected by trypan blue dye exclusion. The volume and cell concentration of each collection were recorded. Cells were resuspended in 10% dimethyl sulfoxide–90% FCS for freezing slowly at $-80°C$ or under liquid nitrogen.

Immunofluorescence analysis of cell phenotype. Ovine $CD4^+$ and $CD8^+$ lymphocytes were identified by using monoclonal antibodies (MAbs) SBU-T4 and SBU-T8, respectively (37). The sheep homologs of major histocompatibility complex (MHC) class II DQ and DR were detected by using the locus-specific anti-α-chain MAbs VPM 36 (DQ) and VPM 38 (DR) (8, 9). Interleukin-2 (IL-2) receptor (IL-2R; CD25) was detected by using IL-A111 (44). VPM 8 (2) recognizes sheep immunoglobulin light chain (IgL). ZG4 (34), an anti-human IgG3, or diluted normal mouse serum was used as a negative control. For two-color analysis, the CD4- and CD8-specific MAbs (both IgG2a) were biotinylated and detected with streptavidin-phycoerythrin (Sero-Tec; Oxford, England). The anti-MHC class II and IL-2R MAbs (all IgG1) were detected by using anti-mouse IgG1-fluorescein isothiocyanate (FITC) (The Binding Site, Birmingham, England). Analysis was carried out on a Becton Dickinson FACScan (Becton Dickinson, Mountain View, Calif.). Ten thousand total efferent lymph cells with erythrocytes or dead cells excluded on the basis of forward scatter (FSC) and 4,000 efferent lymphoblasts (defined by a gate based on their larger size [FSC] and higher side scatter [SSC] properties than those for small lymphocytes [Fig. 1A]) were analyzed per sample, using Consort 30 Version F (Becton Dickinson). Analysis gates were set for strongly positive CD4- and CD8-staining cell populations, and the MHC class II and IL-2R expression on these $CD4^+$ and $CD8^+$ cell populations was assessed.

Quantitation of MVV gag p25. The method is described in reference 54. Briefly, enzyme-linked immunosorbent assay
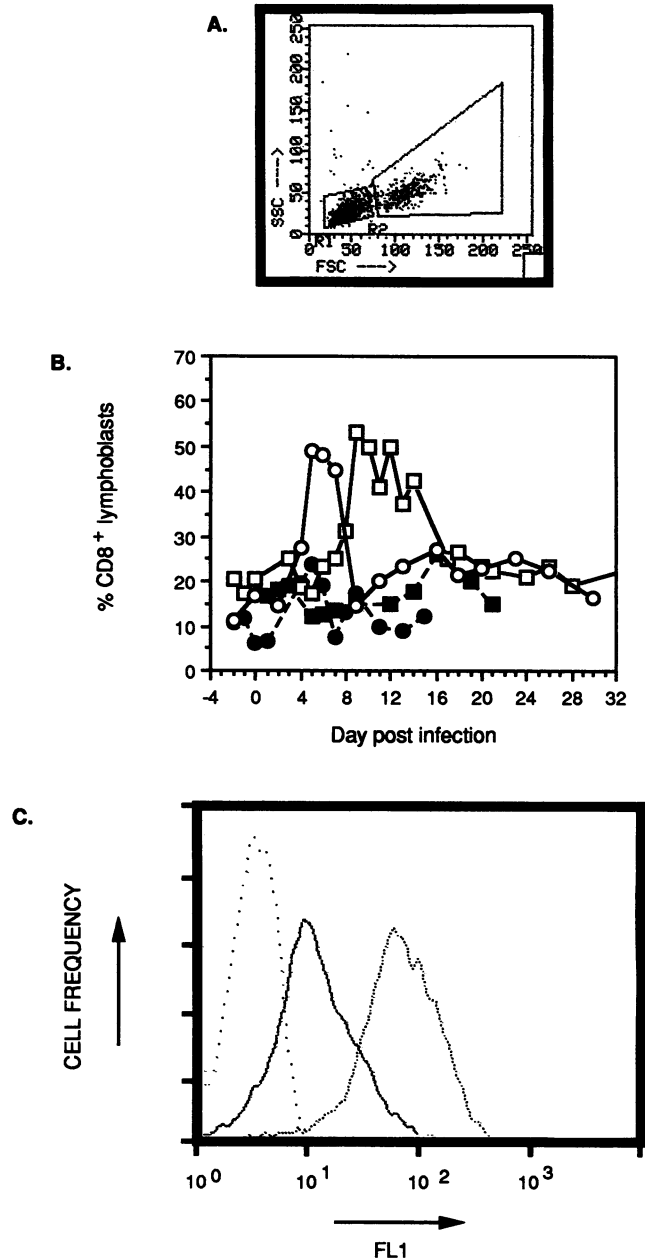

FIG. 1. Major phenotypic change of efferent lymph cells after infection with MVV. (A) FSC and SSC FACS profiles of efferent lymph cells. Sheep 657R was challenged with MVV in the region draining to the prefemoral lymph node, which had been cannulated. The FSC and SSC profiles (both linear scale) of the efferent lymph cells on day 11 p.i. are shown along with representative samples of the lymphoblast collection box (high FSC) and small lymphocyte analysis box (low FSC). (B) Kinetics of $CD8^+$ lymphoblast output of a lymph node after MVV infection. Sheep were challenged in the region draining to the cannulated lymph node on day 0 with MVV (open symbols) or mock-infected control antigen (closed symbols). $CD8^+$ lymphoblasts were determined by immunostaining and FACS analysis. Sheep: □, 657R; ○, 649R; ■, 1060P; ●, 663R. (C) MHC class II expression on efferent lymph $CD8^+$ lymphoblasts. Efferent lymph cells were double stained with SBU-T8 (anti-CD8) and normal mouse serum (· · ·), VPM 38 (anti-DR) (-----), or VPM 36 (anti-DQ) (———). MHC class II expression on $CD8^+$ lymphoblasts is shown for sheep 649R on day 5 p.i., the peak of this sheep's $CD8^+$ lymphoblast response.

(ELISA) plates were coated with affinity-purified rabbit antibody to recombinant MVV p25. Lymph plasma was titrated down the plates, with purified recombinant p25 used as a standard. The plates were then developed with biotinylated rabbit anti-MVV p25, Extravidin-horseradish peroxidase (Sigma), and o-phenylenediamine. This assay could detect a minimum of 150 to 300 pg of MVV p25 per ml.

Quantitation of infectious MVV. Infectious virus was detected by cocultivation of serial dilutions of lymph plasma or cells on heterologous sheep skin fibroblast monolayers in 2% DME. For cell-associated virus, 10^7 cells stored at $-80°C$ were thawed rapidly at $37°C$, washed once in 2% DME, resuspended in 2% DME, counted, and titrated in triplicate in a sixfold-dilution series onto the fibroblast monolayer. Lymph plasma was similarly assayed for infectious MVV from a 1/2 dilution. The monolayers were cultured for 12 to 14 days and then washed in Hanks balanced salt solution, fixed, and stained for MVV *gag* p15 (MAb 415 kindly donated by D. J. Houwers) or *gag* p25 antigen (MAb ID10 [54]) by immunofluorescence. The monolayers were blocked in phosphate-buffered saline (PBS)–10% sheep serum–0.01% Tween 80, incubated with MAb ascites fluid diluted in PBS–2% sheep serum–0.1% Tween 80 (PST), then in sheep anti-mouse Ig-biotin conjugate (Sigma) in PST, and finally in Extravidin-FITC (Serotec) in PST, with washes after each step, and assessed for FITC-positive fluorescence microscopically. Positive controls were 72-h MVV EV1-infected fibroblasts. Negative controls included an irrelevant mouse IgG2a MAb and uninfected fibroblasts stained with MVV-specific MAb.

Detection of viral DNA. Cells (10^7) stored at $-80°C$ were thawed, washed twice in PBS, and resuspended in 400 μl of cell lysis buffer (0.5% sodium dodecyl sulfate [SDS], 50 mM Tris-HCl [pH 8.0], 0.1 M NaCl, 1 mM EDTA). DNA was prepared by standard methods (42), including RNase A and proteinase K digestions. For MVV DNA detection by the polymerase chain reaction (PCR), 500 ng of lymph cell DNA (equivalent to 1.7×10^5 cells) was used as a substrate in an amplification primed with MVV EV1 long terminal repeat (LTR)-specific amplifiers 591H (CTGCAGGTCGACTGT CAGGGCAGAGAACAG, equivalent to positions 8913 to 8933 on the EV1 genome, sense strand [61], plus 5' additions not relevant to this work) and 592H (TCTAGAGTCGA CAGCTGCGAGACCCGCTCCG, equivalent to positions 152 to 133, antisense strand, plus 5' additions not relevant to this work). PCR was carried out in a volume of 100 μl, using 1.6 U of *Taq* polymerase in the buffer conditions of Ohara et al. (49) and 4 cycles of 95°C for 0.6 min, 62°C for 0.6 min, and 72°C for 1 min, followed by 26 cycles of 95°C for 0.6 min, 72°C for 1.5 min, and 72°C for 2 min and a single termination cycle of 72°C for 7.5 min. For the ethidium bromide-stained gel, the reaction mixture was diluted 10-fold, and 2 μl was then amplified through a further 15 cycles with internal 310L (ATGCCCATGATTGAGAATGAC) and 555H (ACAGC CAACTCCTTTATTA) primers as in cycles 5 to 30 above. As a positive control, 500 ng of extrachromosomal DNA from MVV EV1-infected fibroblasts was prepared by the method of Hirt (27) and amplified in the same way. Ten microliters of the PCR products was analyzed by electrophoresis on 1.5% agarose TAE gels, blotted onto nitrocellulose, and hybridized with an EV1 LTR-specific cloned probe, using standard methods (42).

Virus neutralizing antibody. Serial dilutions of heat-inactivated lymph plasma (56°C, 30 min) were incubated overnight at 4°C with 500 $TCID_{50}$ of MVV EV1. The plasma-virus mix was then titrated along a flat-bottom 96-well plate (GIBCO-

BRL, Paisley, Scotland) containing a preformed fibroblast monolayer and incubated for 2 h at 37°C. The virus inoculum was then replaced with 2% DME, and after 7 days of incubation, the cells were fixed, Giemsa stained, and examined microscopically for MVV cytopathic effects. Neutralization titers were defined as the greatest dilution of lymph plasma giving a 50% reduction in virus titer compared with controls (virus incubated with preimmune plasma or no plasma).

Detection of anti-MVV *gag* p25. Purified recombinant p25 (54) was coated onto ELISA plates (Falcon 3912) at 10 μg/ml overnight at 4°C. Plates were washed, blocked with borate-buffered saline–1 mg of bovine serum albumin (BSA) per ml, and then lymph plasma was titrated down the plate in borate-buffered saline–BSA–0.05% Tween 20. After 1 h of incubation, the plates were washed and developed with horseradish peroxidase-conjugated rabbit anti-sheep Ig (Dako Ltd., High Wycombe, England) and o-phenylenediamine. Antibody titer was expressed as the end point titer, taken as the reciprocal of the highest dilution of plasma which gave an optical density twice that of the control (no plasma).

Detection of MVV *gag* p25-sheep Ig immune complexes. MVV p25 in lymph plasma was captured by rabbit anti-MVV p25 as described above, but then the plates were incubated with rabbit anti-sheep Ig conjugated to horse-radish peroxidase and developed as described above. Increasing values of optical density at 492 nm were taken as evidence of immune complex formation.

Proliferation of efferent lymph cells to recombinant human IL-2 and ConA. Efferent lymph cells were washed and then resuspended at 10^6 cells per ml in RPMI 1640 (catalog no. 074-1800; GIBCO-BRL) fully supplemented with 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 200 μg of gentamicin per ml, and 10% FCS (RPMI–10% FCS). Then 10^5 cells per well in 96-well flat-bottom plates were cultured in triplicate with an equal volume of RPMI–10% FCS with final concentrations of recombinant human IL-2 (National Institute of Biological Standards and Control, Potters Bar, England) or concanavalin A (ConA; catalog no. C7275; Sigma) of 20 U/ml or 5 μg/ml, respectively, for 3 days at 37°C in 5% CO_2. Cultures were labeled with 1 μCi of [^{3}H]thymidine (20 to 30 Ci/mmol; catalog no. TRK61; Amersham International PLC) per well and incubated for 5 h at 37°C in 5% CO_2. Cultures were harvested onto Skatron glass fiber filter mats (Skatron Instruments Ltd.) and counted in a LKB Wallac 1218 Rackbeta liquid scintillation counter. Results have been expressed as specific proliferation = (mean cpm of stimulated cultures) − (mean cpm of unstimulated cultures).

Proliferation of efferent lymph cells to MVV antigen. Efferent lymph cells (10^5) in RPMI–10% FCS were plated with 3×10^5 autologous peripheral blood mononuclear cells (PBMC) as antigen-presenting cells and 4 μg of sucrose-purified MVV antigen per ml in triplicate in 96-well flat-bottom plates for 5 days at 37°C in 5% CO_2. Cultures were labeled and harvested as for recombinant human IL-2- and ConA-stimulated cultures, and the results have been expressed as stimulation index = (mean cpm of antigen-stimulated cultures/mean cpm of unstimulated cultures).

Autologous PBMC were collected prior to MVV challenge and cryopreserved. They proliferated in response to ConA but did not proliferate in response to either sucrose-purified MVV antigen or recombinant *gag* p25 MVV antigen (data not shown). MVV antigen was prepared from supernatants of infected cell cultures showing extensive cytopathic ef-

TABLE 1. Summary of the lymphoblast response in efferent lymph to challenge of the lymph node by MVV

Sheep no.	% Lymphoblasts		% CD4+ lymphoblasts		% CD8+ lymphoblasts		Lymphoblast CD4/CD8 ratio		% Ig+ lymphoblasts	
	Day 0[a]	Maximum (day p.i.)[b]	Day 0	Maximum (day p.i.)	Day 0	Maximum (day p.i.)	Day 0	Minimum (day p.i.)[c]	Day 0	Maximum (day p.i.)
EV1 infected										
657R	3.9	19.2 (12)	65.1	66.7 (6–7)	20.5	53.3 (9)	3.2	0.5 (9)	21.2	46.9 (21)
649R	8.9	36.3 (7)	36.4	51.2 (2)	16.8	48.8 (5)	2.2	0.4 (7)	27.0	31.0 (9)
683R	4.0	20.3 (16)	39.5	51.2 (9)	20.6	31.2 (11)	1.9	1.1 (11)	29.1	45.9 (20)
671R	2.8	30.0 (7)	37.2	40.1 (4)	8.9	80.0 (7)	4.2	0.2 (7)	50.1	43.1 (11)
672R	2.8	47.6 (8)	51.5	65.7 (2)	22.6	84.9 (6)	2.3	0.2 (6)	14.1	59.0 (9)
Mock infected										
1060P	4.7	11.7 (5)	64.5	65.0 (16)	18.0	26.0 (16)	3.6	2.5 (19)	10.7	26.8 (19)
663R	4.5	4.6 (8)	45.0	58.0 (15)	11.6	23.7 (5)	7.5	1.7 (5)	31.0	32.1 (11)
647R	3.8	11.0 (2)	50.0	58.2 (6)	6.0	10.0 (3)	8.3	5.3 (3)	16.9	23.5 (5)
EV1 infected, median	3.9	30	39.5	51.2	20.5	53.3	2.3	0.4	27.0	45.9
Mock infected, median	4.5	11.0	50.0	58.2	11.6	23.7	7.5	2.5	16.9	26.8
P[d]	0.551	0.037[e]	0.551	0.766	0.233	0.037[e]	0.074	0.037[e]	0.551	0.074

[a] Values shown preinfection on the day sheep were challenged.

[b] Maximum values seen p.i., with the day p.i. when this occurred in parentheses.

[c] Minimum values seen p.i., with the day p.i. when this occurred in parentheses.

[d] Differences between the groups were analyzed by using Mann-Whitney two-tailed tests.

[e] Differences between groups considered significant if P is <0.05.

fects, which were clarified by centrifugation at 10,000 × g at 4°C for 30 min, and virus was then pelleted at 10,000 × g at 4°C for 16 to 18 h and resuspended in PBS. Pelleted virus was purified on 25 to 60% sucrose gradients at 100,000 × g at 4°C for 16 h, gradient fractions containing reverse transcriptase activity and viral proteins (determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis) were pooled, and the virus was pelleted at 100,000 × g at 4°C for 16 h. Purified virus was stored at −80°C, and optimum concentrations for use were determined by titration in proliferation assays with PBMC from persistently MVV-infected sheep.

Cytotoxic activity of efferent lymph cells. Efferent lymph cells were centrifuged over Lymphoprep (Nycomed AS) at 800 × g for 15 min, and interface cells were washed twice with RPMI-2% FCS before resuspension in RPMI-10% FCS with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; pH 7.2). Cells were made to the required concentration for effector/target ratios of 100:1 to 12:1 before incubation with target cells for 6 h at 37°C in 5% CO₂. Targets were 10^4 autologous or heterologous skin cells either mock infected or infected with 0.25 to 2 TCID₅₀ (depending on virus stock) per cell for 72 h. Cells were labeled with 3 μCi of ^{51}Cr (sodium chromate; 350 to 600 mCi of Cr per mg; Amersham) per 10^4 cells overnight at 37°C in 5% CO₂ before being washed four times with RPMI-2% FCS and were then incubated with efferent lymph effector cells for 6 h. Supernatant (100 μl) was counted in a LKB Wallac 1274 RIA-GAMMA counter, and the results have been expressed as percent specific ^{51}Cr release = (mean experimental ^{51}Cr release − mean spontaneous ^{51}Cr release)/(mean maximum ^{51}Cr release − mean spontaneous ^{51}Cr release) ± (standard deviation of experimental ^{51}Cr release)/(mean maximum ^{51}Cr release − mean spontaneous ^{51}Cr release). All values were obtained from quadruplicate samples, and spontaneous ^{51}Cr release was always less than 35% of the maximum release.

RESULTS

Kinetics and phenotype of cells in efferent lymph after challenge of a lymph node with MVV. Eight peripheral efferent lymphatic cannulations were carried out, five involving prefemoral nodes and three involving popliteal nodes. Challenge was with either mock-infected autologous cell supernatant or MVV (from approximately 1.5 × 10^5 to 16 × 10^5 TCID₅₀). There was no significant difference in the total cell output between MVV-infected and mock-infected sheep (data not shown) with the exception that cell shutdown occurred in three of the five infected animals and never in the mock-infected animals. Cell shutdown of the lymph node (cells are retained within the node) is a normal response to secondary antigenic challenge (39), although it has been shown to occur on primary challenge when other live viruses are used (31, 63). However, in these experiments, the phenomenon was not related to the amount of virus given or the lymph node efferent duct cannulated (data not shown).

Following MVV infection, there was no significant increase in total cell output in the infected group compared with mock-infected controls. The proportion of lymphoblast defined by their FSC/SSC profile on the FACScan (Fig. 1A) was markedly increased in infected sheep (Table 1), with a 4- to 17-fold increase in the percent lymphoblasts from day 0 to the day p.i. showing the maximum percent lymphoblasts (days 7 to 16 p.i.). There was only a 1- to 3-fold increase in mock-challenged sheep (Table 1).

The lymphoblast population comprises mainly CD4+, CD8+, and B-lymphoblast cells. There was no significant difference in the maximum percent CD4+ lymphoblasts in the two groups of animals (P = 0.766; Table 1). However, there was a marked rise in the proportion of CD8+ lymphoblasts exiting the node in response to challenge with virus but not mock-infected antigen (Fig. 1B). These CD8+ cells left the node as a wave, with maximum percentages being seen between days 6 and 11 p.i., depending on the sheep (Table 1). The maximum percent CD8+ lymphoblasts seen varied significantly between MVV-infected and mock-chal-

lenged groups ($P = 0.037$; Table 1). As the percent CD8$^+$ lymphoblasts increased, there was a concomitant fall in percent CD4$^+$ lymphoblasts (data not shown), although later in the response, these figures were also affected by increasing numbers of B lymphoblasts. This is reflected in the CD4/CD8 lymphoblast ratio, which may drop as low as 0.2 from a median starting ratio of 2.3 (Table 1). When the percent CD8$^+$ cells in the small lymphocyte population was studied, none of the changes seen in the lymphoblast population were noted with the percent CD8$^+$ small lymphocytes, remaining constant throughout the course of the cannulation (data not shown).

The large size of the CD8$^+$ lymphoblasts suggested that they were becoming activated. The levels of expression of different MHC class II loci on T lymphocytes are often an indication of the activation state of the cell, with DQ being associated with an activated phenotype (12, 32). To investigate the activation state of lymphocytes exiting peripheral lymph nodes after challenge with MVV, the expression of the sheep DR and DQ MHC class II homologs was studied by double staining with anti-CD4 or anti-CD8. CD8$^+$ lymphoblasts from the preinfected node showed heterogeneous staining intensity (10^0 to 10^3 FL1 intensity) for both DR and DQ MHC class II molecules, and this phenotype was seen throughout the response in mock-infected sheep (data not shown). In response to live viral challenge, the wave of CD8$^+$ lymphoblasts exiting the node appeared to be a homogeneous population in its expression of a high level of DR and a low level of DQ MHC class II molecules (Fig. 1C). This change always occurred in virus-challenged sheep on the day with the maximum percent CD8$^+$ lymphoblasts and then decayed with time to a staining pattern similar to that seen in resting efferent lymphoblasts. Similarly, both MHC class II molecules were expressed with a broad spectrum of staining intensity on CD4$^+$ lymphoblasts preinfection. However, although a change was seen in the MHC class II profile of CD4$^+$ lymphoblasts after challenge, there were no significant differences between MVV- and mock-infected animals (data not shown).

The change in MHC class II profile of the CD8$^+$ lymphoblasts in response to MVV suggested that these cells were in an activated state. It was therefore expected that the lymphoblasts would also express IL-2R on their surfaces. A MAb specific for bovine IL-2R (anti-CD25) (44) which cross-reacts with ovine CD25 became available after these experiments were performed, and so stored cells were used to assess the IL-2R status at the peak of the CD8$^+$ lymphoblast response (day 7 p.i. for sheep 649R; day 11 p.i. for sheep 683R). A proportion of the CD4$^+$ lymphoblast and small lymphocyte populations were positive for IL-2R, while the CD8$^+$ lymphoblasts at the peak of the CD8$^+$ lymphoblast wave were negative (Table 2). There was some loss of CD8$^+$ cells on freezing and resuscitation, but there was no evidence of a selective loss of IL-2R$^+$ CD8$^+$ lymphoblasts on storage of ConA-stimulated lymphoblasts from persistently infected sheep.

The percent γδ T-cell receptor-positive lymphoblasts (T19-positive cells) (36) did not vary after MVV challenge of the node, nor were there significant changes seen in the percent CD4$^+$ and γδ T-cell receptor-positive small lymphocytes (data not shown).

After MVV infection, the increase in percentage of surface Ig (sIg)-positive lymphoblasts between day 0 and the maximum (Table 1) was much less striking than the increase in CD8$^+$ lymphoblasts exiting the node, but the peak number of B lymphoblasts exiting virus-stimulated nodes (median, 4.1

TABLE 2. IL-2R expression of CD4$^+$ and CD8$^+$ lymphocytes at the peak CD8$^+$ lymphoblast output

Sheep no. or sample	Day p.i.	CD4$^+$ % IL-2R$^+$		CD8$^+$ % IL-2R$^+$	
		Small lymphocytes	Lympho-blasts	Small lymphocytes	Lympho-blasts
649R[a]	7	8.5	18.0	0.7	1.3
	32	14.2	14.0	2.8	1.4
683R[a]	−1	17.8	13.6	3.4	1.1
	11	10.7	7.2	2.3	1.7
ConA stimulated[b]					
Fresh		27.3	48.7	1.7	32.9
Stored		36.8	58.8	7.8	43.7

[a] Efferent lymph cells were resuscitated from liquid nitrogen and washed, and the CD4, CD8, and IL-2R expression of cells was determined by FACS analysis. Preinfection samples for sheep 649R were not available, and so a very late sample was used.

[b] Three-day ConA-stimulated (suboptimal concentration, 1.25 μg/ml) efferent lymph cells from a persistently infected sheep were analyzed immediately (fresh) or stored under liquid nitrogen and then resuscitated and analyzed as above.

× 10^6) was significantly higher than for mock-infected sheep (0.8 × 10^6; $P = 0.037$). The number of sIg$^+$ small lymphocytes was also increased but not significantly compared with mock-infected controls (data not shown). The time course of the B-cell changes showed considerable variability between sheep. When the isotype of the sIg on B lymphocytes was examined by two-color FACS to identify sIgM and sIgL or sIgG coexpression, an sIg isotype change from sIgM to sIgG with time was seen both with MVV- and with mock-infected sheep efferent B lymphoblasts (data not shown).

Time course of virus output. Evidence for viral replication within the locally infected node and the relationship of this viral replication to the developing immune response was sought by quantitating the levels of infectious MVV or MVV protein in both the lymph cells and plasma.

From day 6 p.i., MVV p25 antigen was detected in lymph plasma in three of three sheep, with two distinct peaks around days 6 to 10 and from day 22 onwards (Fig. 2A), indicating that MVV replication was occurring. Despite the presence of MVV p25 antigen, no infectious virus was detected in lymph plasma by cocultivation on skin fibroblasts, but because of the nature of the assay, the p25 may be part of a noninfectious virion. Only cell-associated infectious virus could be isolated from four of five MVV-infected sheep (Table 3), and this appeared maximally between days 9 and 14 p.i. (Fig. 2A). No infectious virus was isolated from sheep 671R. This sheep received the lowest dose of infectious virus, but serum MVV-specific antibodies were detected shortly following the cessation of surgical cannulation, indicating that a persistent infection with MVV had been established.

Gene amplification by MVV-specific PCR on DNA isolated from efferent lymph cells from three of three sheep during the period of infectious virus isolation (days 10 to 12) revealed MVV-specific DNA (expected product size of 301 bases) (Fig. 2B and C, lanes 3 to 5). For sheep 649R, MVV proviral DNA was detected in efferent lymph cells before infectious virus was isolated (compare Fig. 2B and C, lanes 4 and 6 to 11, with Fig. 2A). Thus, although proviral DNA detection did not correlate absolutely with infectious virus isolation, MVV-infected cells are present in efferent lymph. The DNA PCR assay is not quantitative, but the requirement for either nested PCR or high numbers of amplification cycles (50 were used) to detect MVV DNA sequences
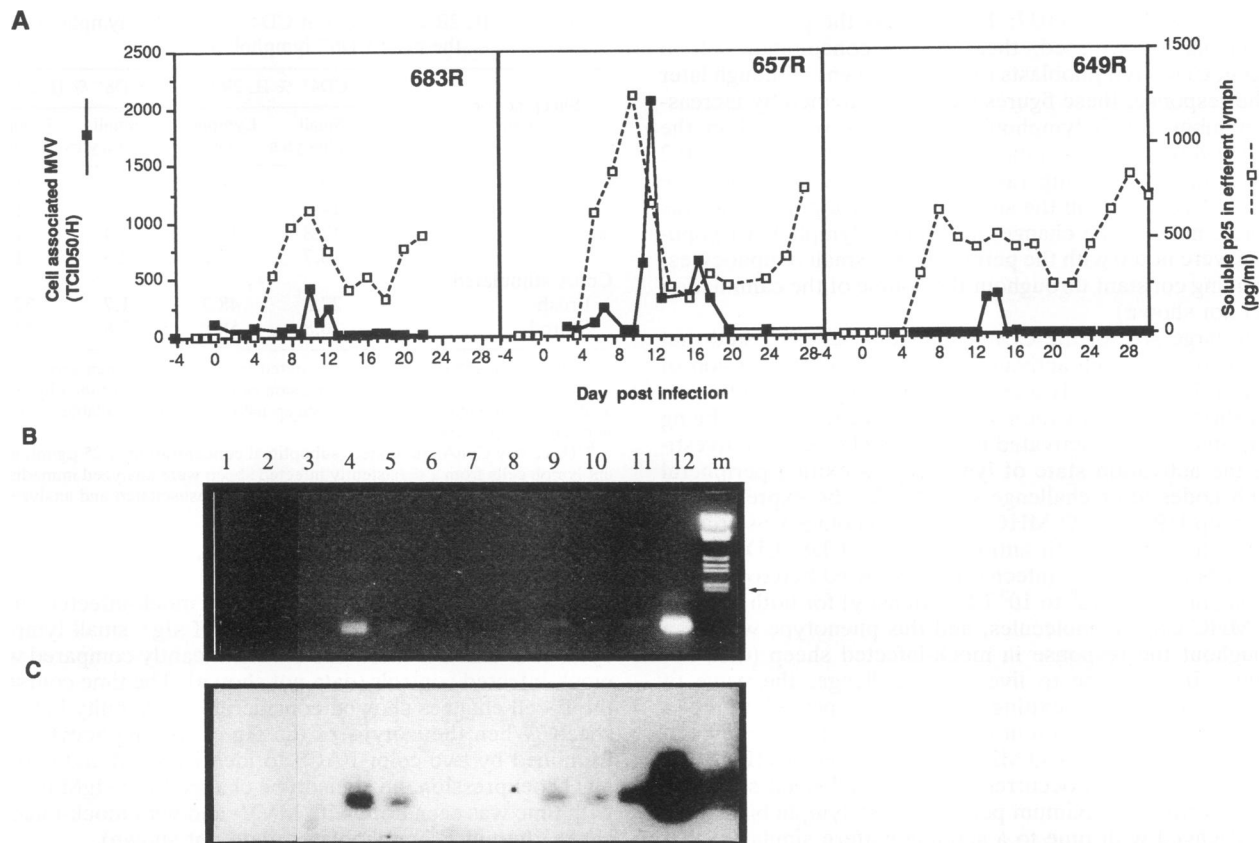
FIG. 2. Virus output in efferent lymph following MVV infection in three sheep. (A) Kinetics of cell-associated infectious virus and soluble MVV p25. Infectious virus was detected by cocultivation of stored efferent lymph plasma or cells from infected sheep on sheep fibroblast monolayers for 12 to 14 days and then subjected to immunofluorescent staining for MVV *gag* p15 in the fixed skin cells. MVV *gag* p25 antigen in lymph plasma was quantitated by capture ELISA on rabbit anti-p25-coated ELISA plates. (B and C) MVV-specific DNA in efferent lymph cells. DNA (500 ng) extracted from efferent lymph cells was amplified by using amplimers specific for the MVV LTR, and the products were run on agarose gels. The molecular weight markers (m) were a *Hin*dIII-*Eco*RI double digest of λ phage DNA. The arrow represents the position of the 831-bp fragment. The expected MVV DNA product size is 301 bases. (B) Ethidium bromide-stained gel; (C) Southern blot of a similar gel probed for MVV DNA sequences. Sources of samples: lanes 1 and 2, uninfected sheep 1060P, days −1 and 10, respectively; lane 3, infected sheep 683R, day 10; lane 4, sheep 649R, day 11; lane 5, sheep 657R, day 12; lanes 6 to 11, sheep 649R, days −1, 0, 6, 9, 12, and 14, respectively; lane 12, positive control DNA from MVV-infected fibroblasts in tissue culture.

suggests that MVV proviral DNA sequences were rare. The frequency of MVV-infected cells in efferent lymph was extremely low even at their peak, the median for four sheep being equal to 11/10^6 cells (Table 3). Despite this low frequency, the total number of infected cells disseminating virus via the efferent lymphatic was sizeable when considered over time, with a median for four sheep of 3.1×10^4

TABLE 3. Frequency and total number of infectious virus-associated cells exiting in efferent lymph following acute MVV infection

Sheep no.	Lymph node efferent duct cannulated	Challenge dose (10^5 TCID$_{50}$)	Day of maximum virus output	Maximum frequency of virus-infected cells/10^6 cells	Total infected cells (10^4 cells/20 days)
657R	Prefemoral	3.2	12	15.3	11.52
649R	Prefemoral	15.0	14	7.35	1.61
683R	Prefemoral	15.0	10	6.1	2.46
671R	Popliteal	1.5		<0.5	0.
672R	Popliteal	16.0	9	55.1	3.73

MVV-infected cells leaving the node during the first 20 days p.i. (Table 3).

Interestingly, the main cell-associated infectious MVV peak occurred just after or coincidentally with the major lymphoblast response in efferent lymph (Table 1), but the low frequency of infected cells has prevented identification of the infected cell type. The peak of these cells was also delayed relative to the first soluble MVV p25 antigen peak in plasma by about 4 days. The second rise in p25 in lymph plasma was not associated with a second wave of infected cells, at least within the time course of the cannulations, indicating that the p25 in lymph plasma may result from virus replication within the node.

MVV-specific immune responses. (i) Antibody. MVV neutralizing activity was not detected in the efferent lymph plasma of three of three MVV-infected sheep before day 10 but thereafter rose steadily in titer with time (Fig. 3A). No neutralizing activity was found in mock-infected sheep lymph plasma. Preincubation of the lymph plasma with polyclonal antibody to sheep Ig inhibited neutralization, indicating that the neutralizing activity was antibody mediated. By Western blotting (immunoblotting), an MVV *gag*
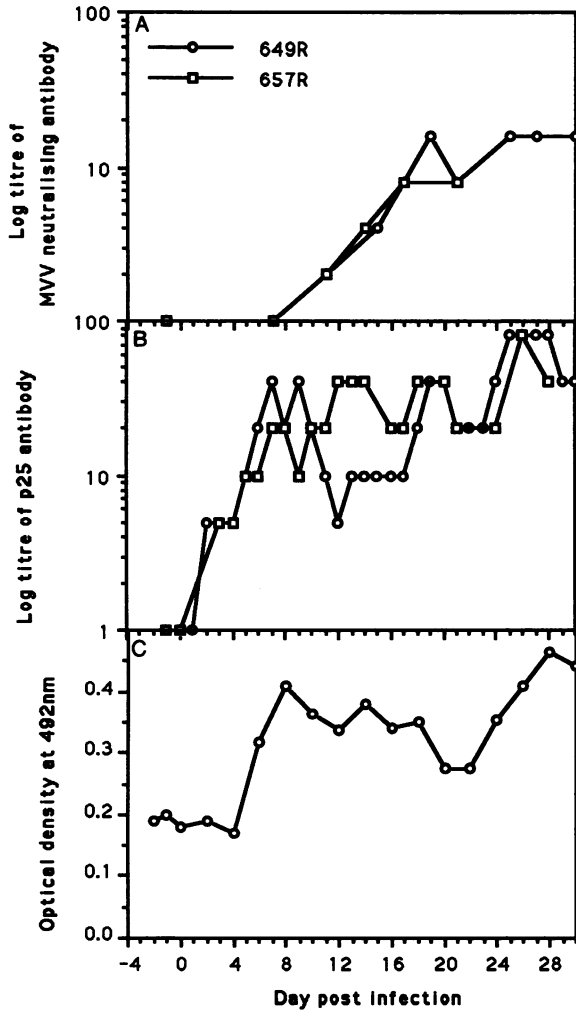
FIG. 3. The time course of the MVV-specific antibody. (A) Neutralizing activity of heat-inactivated lymph plasma was quantitated from the reduction in infectivity of 500 TCID$_{50}$ of MVV EV1 after overnight incubation at 4°C with dilutions of lymph plasma. The titer represents the highest dilution giving 50% inhibition of infectivity. (B) MVV p25-specific antibody. p25-specific antibody in efferent lymph was quantitated by ELISA, using recombinant p25-coated plates. The titer represents the highest dilution with an optical density greater than twice background (no plasma). (C) Soluble MVV p25 immune complexes in the efferent lymph of an infected sheep. These complexes were detected in efferent lymph plasma (sheep 649R) by capture ELISA on anti-p25-coated ELISA plates developed with rabbit anti-sheep Ig antibody.
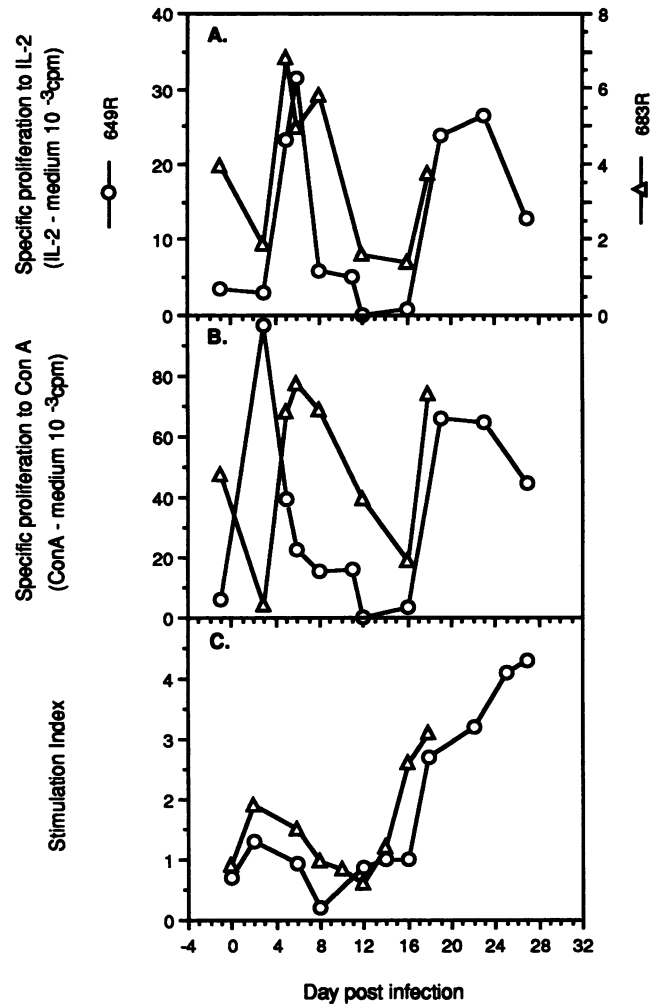

FIG. 4. Proliferation of efferent lymph cells in response to recombinant human IL-2, ConA, and MVV antigen. Efferent lymph cells were set up in culture with 20 U of recombinant human IL-2 per ml (A) or 5 μg of ConA per ml (B) for 3 days or 4 μg of sucrose-purified MVV antigen per ml and autologous PBMC (3:1 PBMC/efferent lymph cells) for 5 days (C). Cellular proliferation was determined by [³H]thymidine incorporation. For 3-day cultures, proliferation is expressed as specific proliferation = (mean cpm of stimulated cultures) − (mean cpm of unstimulated cultures). Unstimulated counts ranged from 82 to 713 cpm. For 5-day cultures, proliferation is expressed as stimulation index = (mean cpm of antigen-stimulated cultures)/(mean cpm of unstimulated cultures), with values for unstimulated cultures always being less than 3,400 cpm. Sheep were infected with MVV in the area draining to the lymph node on day 0. Symbols: O, sheep 649R; △, sheep 683R.

p25-specific antibody response can be detected in efferent lymph as early as day 4 p.i., and an anti-env gp135 response can be detected by day 12, with an isotype change from IgM to IgG1 with time (data not shown). The appearance of neutralizing activity coincided with the detection of IgG1 anti-MVV env gp135 antibody (data not shown).

The anti-MVV p25 antibody response of infected sheep quantitated by ELISA (Fig. 3B) detected specific antibody from day 4 and in two of three sheep showed two peaks of anti-p25 antibody around days 4 to 8 and 12 to 20. It is likely that these two peaks correspond to the IgM and IgG1 isotype responses, respectively. MVV p25 antigen (Fig. 2A) was detected at the same time as anti-p25 antibody in efferent

lymph plasma. During this period, soluble p25 immune complexes were present in efferent lymph plasma (Fig. 3C).

(ii) T lymphocytes (proliferation to IL-2 and mitogen). The functional activation state of efferent lymph cells was assessed by measuring the proliferative capacity of the lymphocytes to respond to two nonspecific stimuli: a cytokine, IL-2, and a mitogen, ConA.

When the response of efferent cells to human recombinant IL-2 was studied, there were major peaks of proliferation in response to IL-2 between days 4 and 8 p.i. (Fig. 4A) and in one sheep a second wave of IL-2 responsiveness between days 19 and 24 p.i. However, these peaks do not correspond

TABLE 4. Cytotoxic activity of efferent lymph cells

Sheep no.	Target cells	% Specific ^{51}Cr release[a] at given days p.i.								
		1-2[b]	3-4	5-6	6-7	10-11	11-12	13-14	15-16	19-20
657R[c]										
	MVV infected									
	Autologous		1.1 ± 2.8		7.4 ± 4.4	20.7 ± 2.3		33.8 ± 2.6		1.5 ± 2.3
	Heterologous		-1.7 ± 0.5		4.4 ± 2.6	1.8 ± 0.9		5.0 ± 0.3		0.7 ± 0.5
	Mock infected									
	Autologous		3.2 ± 0.6		-0.9 ± 0.8	-0.5 ± 1.6		1.1 ± 1.6		0.8 ± 0.9
	Heterologous		-2.2 ± 0.4		3.1 ± 0.1	-1.3 ± 0.4		-1.5 ± 1.8		1.6 ± 1.0
649R[d]										
	MVV infected									
	Autologous	4.2 ± 3.9	14.3 ± 4.8	0.9 ± 3.4			-1.8 ± 0.8		1.2 ± 0.5	
	Heterologous	5.7 ± 3.5	6.2 ± 5.7	14.1 ± 4.2			4.0 ± 1.0		12.2 ± 0.5	
	Mock infected									
	Autologous	0.5 ± 3.0	9.1 ± 1.9	1.5 ± 1.2			-0.4 ± 0.6		0.2 ± 0.6	
	Heterologous	1.4 ± 1.4	0.3 ± 1.1	0.2 ± 2.0			8.2 ± 1.7		-8.1 ± 3.2	

[a] Results shown at effector/target ratio of 50:1.

[b] Effector/target ratio of 15:1.

[c] Assays also performed with cells from days -1 to 0 p.i.; no specific killing of MVV-infected autologous targets (data not shown).

[d] Assays also performed with cells from days -2 to -1, 7 to 8, 9 to 10, and 19 to 20 p.i.; no specific killing of MVV-infected autologous targets (data not shown).

to the peak output of CD8$^+$ or CD4$^+$ lymphoblasts in both sheep. They also do not correspond consistently to any peaks in cell, lymphoblast, or particular small lymphocyte phenotype output (data not shown). The response of efferent lymph cells to mitogenic stimulation was also analyzed (Fig. 4B). Just as with IL-2, there were two waves of proliferation to ConA, with the minimal stimulation being seen between days 10 and 16 p.i. Interestingly, the time p.i. when the efferent lymphocytes were unresponsive to these external stimuli corresponded to the period when maximal numbers of virus-infected cells left the node (Fig. 2).

(iii) T lymphocytes (proliferation to MVV antigen). MVV-specific lymphocyte proliferation could be seen in efferent lymphocytes after day 15 p.i. (Fig. 4C; positive proliferation was taken as stimulation index greater than 2). This proliferation was also seen in response to recombinant *gag* p25 antigen but not ovalbumin or medium alone, and it required the addition to cultures of autologous PBMC (collected preinfection) to act as antigen-presenting cells (data not shown) as there are few professional antigen-presenting cells in efferent lymph (4, 29).

(iv) T lymphocytes (direct lysis of MVV-infected autologous skin cells). Fresh efferent lymphocytes were used in cytotoxicity assays against ^{51}Cr-labeled autologous MVV-infected skin cells. In one sheep (657R) of five tested, specific lysis of MVV-infected autologous skin cells could be seen, and this was between days 10 and 14 p.i. (Table 4). The cytotoxic activity was apparently MHC restricted, as there was no lysis of MVV-infected heterologous cells. This cytotoxicity was during the CD8$^+$ lymphoblast peak of 657R. However, none of the other four MVV-challenged (or mock-infected) sheep ever showed MVV-specific cytotoxicity of autologous cells even at effector/target ratios of 100:1 during the CD8$^+$ lymphoblast response (Table 4, sheep 649R). All cells in virus-infected target cultures expressed *gag* antigen by immunofluorescent staining using an anti-*gag* MAb, and complement lysis of cells could be shown with anti-MVV sheep serum and rabbit complement (data not shown).

DISCUSSION

Lentiviral infections are characterized by a long asymptomatic period, when virus is rarely detected and viral gene expression is highly restricted in the host, and a slow progression of disease in the face of specific B- and T-cell immune responses. There is now evidence that during this time the lymph node is a major reservoir for the virus (10, 50). It is therefore likely that the acute events associated with initial virus infection of a lymph node may be of considerable importance in determining viral persistence and the subsequent course of disease. Therefore, the study within lymphoid tissue of the early T- and B-cell immune response to local lentiviral infection and the correlation of this response to viral replication are potentially of great value in furthering our understanding of the pathogenesis of lentiviral disease. We have shown here that after infection with MVV, sheep mount an antiviral immune response (both T cell and antibody), but despite this, viral replication and dissemination occur.

However, closer study reveals differences in the immune response to MVV (and other lentiviruses) and the immune response induced by other viruses. These include the activation state of the CD8$^+$ lymphoblasts and the ability of the lymph cells to respond to external stimuli during the course of infection.

In most species, activated T lymphocytes have been shown to be lymphoblastoid, to synthesize DNA, and to express MHC class II antigens and IL-2R (11, 12, 18, 21, 32, 60). The CD8$^+$ lymphoblasts, at the peak of the response to MVV, were a homogeneous population expressing MHC class II DR and DQ (DQ at a lower intensity than DR). However, they did not express IL-2R (CD25), while a proportion of the CD4$^+$ lymphoblasts were positive for IL-2R expression. Early after intravenous infection of rhesus monkeys with the simian immunodeficiency virus SIV$_{mac}$, an increase in the number and percentage of CD8$^+$ cells in the peripheral lymph nodes was reported (52). These cells express MHC class II DR but not IL-2R. Similarly, HIV-infected patients exhibit circulating CD8$^+$ DR$^+$ cells negative for IL-2R (51, 59). These cells are defective in their response to anti-CD3 and anti-CD2 or mitogen stimulation, even in the presence of additional IL-2 or IL-4. It is therefore apparent that a common feature of the immune response to lentiviruses (not only immunodeficiency viruses) is the induction of CD8$^+$ lymphocytes in a defective maturation state

(59). This is not seen in the immune response to lymphocytic choriomeningitis virus (LCMV) in mice. In that case, CD8$^+$ lymphocytes are a high percentage of the regional lymph node cells on day 6 p.i., but these cells express IL-2R and will proliferate in response to recombinant IL-2 (35).

In lentiviral infections, the absolute peripheral blood CD8$^+$ lymphocyte numbers do not reflect any measurable virus-specific immune function (48), and it has been postulated that they are immune regulators. We have no evidence from our results for this. The CD8$^+$ lymphoblasts detected in our study do not appear to act as suppressor cells, as the time of their appearance in the efferent lymph does not always correspond to the time at which efferent lymph cells do not respond to cytokine or mitogen stimulation. Nor do the cells have any consistent cytotoxic activity, since in only one of the five sheep was any cell-mediated cytotoxicity demonstrable at this early stage of infection.

The primary B-cell response to MVV was characterized by a significantly increased output of Ig$^+$ lymphoblasts in efferent lymph and the appearance of MVV-specific antibody in lymph plasma by day 4. This is the characteristic time for the humoral response to primary virus challenge. However, MVV neutralizing antibody was not detected until days 12 to 14 p.i., which was when virus was seen disseminating from the lymph node. All detectable virus was cell associated, and neutralizing antibody (assayed on cell-free virus) might not be expected to neutralize virally infected cells. Levels of *gag* p25 antigen in lymph plasma temporarily decreased as initial antibody titers rose. p25 antigen may, in part, represent cell-free virus which has been neutralized by antibody. As we never detected cell-free virus, neutralizing antibody titers may be sufficient to control spread of virus by this route. In SIV and HIV, the presence of neutralizing antibody also correlates with a drop in p24 antigenemia and is associated with a longer survival time (6, 66). However, the rising levels of p25 antigen from day 20 onwards in this study suggest that MVV replication and the number of infected cells within the lymph node are increasing and are not controlled by antibody.

In contrast, viral dissemination from the node was limited in that detectable infectious MVV left the node as a wave of virally infected cells (maximum number of infected cells between days 10 and 14 p.i.), suggesting that immune surveillance is effective apart from this period. What changes therefore occur within the immune system between days 10 to 14 p.i. that allow dissemination of this form of virus? One explanation is that T lymphocytes are unable to respond to virally infected cells at this time. It is during this period that efferent lymph cells do not proliferate to IL-2 and the mitogen ConA, suggesting that T lymphocytes unresponsive to many different stimuli are induced by the virus, allowing its dissemination. Mechanisms which could cause this lack of response include anergy and suppressor lymphocyte activity (38). No assays have been carried out to test whether the decrease in proliferative ability seen here was due to anergy or suppression. However, unresponsive lymphocytes have been recorded in the acute response to cytomegalovirus (5) and Epstein-Barr virus (53), and the latter group has shown that this effect is due to the presence of suppressor cells. It is also well documented in HIV that there are defects in T-cell function early after infection but before the manifestation of clinical disease (22). It is interesting that these viruses all cause persistent infections through latent or restricted replication states in hemopoietic cells. It may well be that the induction of nonfunctional T lymphocytes allows the dissemination of virally infected

cells within the host through the normal lymph/blood transport system.

We have evidence for MVV-specific T-cell responses. Antibody was detected in the lymph plasma from day 4 p.i. onwards, and as this response requires T-cell help, it is likely that MVV-specific T cells are present in the node from this time. However, MVV-specific proliferative responses were not detected in efferent lymph cells until day 15 p.i. In persistently infected sheep, the proliferating cell in this assay was principally CD4$^+$ (54a). The specific T-cell response increased as cell-associated virus exiting the lymph node decreased. Therefore, the increase in MVV-specific CD4$^+$ lymphocytes may be responsible for the reduction of cell-associated virus. A model murine retroviral infection has been shown to require both CD4$^+$ and CD8$^+$ lymphocytes for protection (28).

Direct MVV-specific cytotoxic activity was detected in efferent lymph cells but in only one of five sheep between days 10 and 14 p.i. A similar proportion of HIV patients show cytotoxic T-lymphocyte (CTL) activity in unstimulated peripheral blood lymphocytes (20). However, in HIV infection, there are reports that CD8$^+$ CD57$^+$ lymphocytes produce a soluble factor inhibiting CTL lysis of target cells (1, 58). A similar mechanism operating in sheep could account for the small number of sheep exhibiting direct cytotoxicity in our study. After primary infection with vaccinia virus (30, 31), cytotoxic lymphocytes were detected in efferent lymph at days 4 to 7 p.i. This CTL activity was associated with the lymphoblast population of efferent lymph cells, and in sheep 657R, the cytotoxic activity also corresponded to the lymphoblast wave. In LCMV infection in mice, LCMV-specific CTL appear on day 6 p.i. in the regional lymph node (35), and SIV$_{mac}$ infection of rhesus monkeys also induced CTL in the peripheral lymph nodes in four of eight monkeys; however, the authors do not say at which time these responses were induced (52). The induction of precursor CTL was not assayed in the work presented here.

Interestingly, the CTL activity in sheep 657R was detected during the period when cell-associated virus exited the lymph node, and so this activity had not protected this sheep from viral dissemination. Cell-associated virus was detected by cocultivation of efferent lymph cells on indicator cells. Before cocultivation, MVV may be in a restricted replication state within the host cell and so may not express viral antigen to the immune system. In HIV and SIV, CTL and precursor CTL are present in animals at the same time as virus may be isolated and during disease progression. However, there may be a role for CD8$^+$ cells in reducing viral load, as it has been shown that CTL activity decreases in HIV-infected patients with advanced disease in which virus production is increased (48).

There is evidence that the immune response may activate as well as limit lentiviral infection. The LTR of T-cell-tropic lentiviruses HIV and SIV contain NF-κb-binding elements responsive to cytokine activation of T cells (reviewed in reference 56), and the virulence of one SIV clone is associated with the ability of this clone to directly activate lymphocytes (13). Similarly, the LTR in MVV is known to contain a number of AP1 elements (14) which may be responsive to monokines generated during an immune response. Postinfection inoculation with purified MVV in Freund's adjuvant enhanced the severity of the lesions in MVV infection (47), as did prior vaccination with inactivated caprine arthritis-encephalitis virus (41). Immune activation in vivo generated by purified protein derivative (PPD) also

stimulated MVV replication and dissemination in efferent lymph (our unpublished results). During acute infection of the node by MVV, the developing immune response to the virus may promote viral replication. The timing of both MVV replication (as measured by p25 levels) and late MVV-infected cell dissemination would support this.

By whatever mechanism, MVV-infected cells evaded the host immune response and disseminated from the lymph node. The frequency of these cells was very low, less than 1 in 10^4, but over time, this added up to a considerable number of infectious centers (more than 10^4). A restricted viral replication state is well characterized in persistent MVV in vivo infection (3), and in a lung model of early MVV infection, Geballe et al. (15) believe that a restricted state may be established by day 4 p.i. In this study, we do not know the replication state of the infected cell, as in situ hybridization revealed rare MVV RNA-positive efferent lymph cells (1 to 5 per 10^6 cells) (57), but no MVV gag p15- or p25-positive cells were detected by immunofluorescence during the period when infectious MVV was detected (unpublished results). The lineage of these rare infected cells is unknown. Efferent lymph contains predominantly lymphocytes, but rare macrophage-like cells (by morphology and nonspecific esterase positivity) can be detected at a frequency similar to that of cell-associated virus.

Although here we have studied the acute immune events involved in MVV infection, already some of the pathogenic mechanisms are evident. The continuing virus replication in the presence of MVV-specific antibody leads to immune complex formation, and these complexes may contribute to lesion formation in a variety of organs. Similarly, the infiltration of many MVV-affected tissues by CD8$^+$ lymphocytes may follow the initial activation of this cell population reported here.

ACKNOWLEDGMENTS

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