

PERSISTENT EXPRESSION OF Ia ANTIGEN AND VIRAL
GENOME IN VISNA-MAEDI VIRUS-INDUCED
INFLAMMATORY CELLS

Possible Role of Lentivirus-induced Interferon

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Visna and maedi of sheep are classical "slow" virus diseases that have a long incubation period and progressive debilitating clinical courses (1). Histologically, lesions are characterized by infiltration and proliferation of mononuclear cells in an active-chronic inflammatory process in specific organ systems such as the brain, lung, joints, and mammary gland (2). The etiological agents of the disease complex belong to a newly recognized taxonomic group called lentiviruses, which are nononcogenic, replication-competent retroviruses (1). The viruses cause persistent infections in their natural hosts and replicate at a restrictive level indefinitely (3, 4). In nonneural tissues such as the lung, chronic progressive lesions develop, whereas the lesions in the central nervous system (CNS)¹ appear to represent an episodic pathogenesis with repeated episodes of acute lesions leading to repair (5).

Studies on host-virus interactions in vivo (6) have shown that cells of the macrophage lineage are the main virus host cells but that replication is confined to macrophages only in those tissues which develop inflammatory lesions. Thus, the minimally productive virus replication in specific populations of tissue macrophages forms the basis of persistent infection of the animal, "slow" or restricted virus replication in tissue, and lymphoproliferative pathologic responses that occur at the sites of virus replication. The mechanisms of the lesions remain unknown. The recent discovery (preceding article [7]) that an interferon (IFN) is induced during interaction between lymphocytes and lentivirus-infected macrophages provides a potentially important bridge linking the virus-infected

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¹ *Abbreviations used in this paper:* CMV, cytomegalovirus; CNS, central nervous system; DME, Dulbecco's modified Eagle's medium; IFN, interferon; LV, lentivirus; LV-IFN, lentivirus-induced interferon; PBM, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PLPG, periodate-lysine-paraformaldehyde-glutaraldehyde; TrMO, SV40-transformed sheep alveolar macrophages.

macrophage and the lymphoproliferative host response. Since one of the immunological effects of IFN (gamma) is the induction of expression of Ia antigens in macrophages (8, 9), thus causing enhancement of antigen processing and presentation by macrophages (10), we hypothesize that, in the chronic-active visna-maedi virus-induced lesion, IFN induced by the inflammatory cells may cause increased expression of Ia antigens in macrophages. We show in this report that Ia antigens are expressed continuously and at high levels in macrophage-like cells in the inflammatory lesions. We show further that, in interactions between the lentivirus-induced IFN (LV-IFN) and cultured sheep macrophages, the LV-IFN causes expression of Ia antigen in the macrophages, and that persistence of expression requires the continuous presence of LV-IFN in the medium. In addition, the IFN restricted LV replication in cultured macrophages. We suggest that such an IFN, produced in vivo, may play an important role in causing the lymphoproliferative response through its effects on macrophage Ia expression and may curtail virus replication in the macrophages, providing the net effect of "slow replication."

Materials and Methods

Virus. Lentivirus VMA-5 was used in all the experiments in this study. This virus is a field strain of visna virus isolated in Idaho from a sheep with typical inflammatory lesions in the lungs, joints, and brain. VMA-5 virus was cultivated in a cell line of sheep alveolar macrophages transformed by SV40 (11). These cells retain histochemical, functional, and antigenic characteristics of the normal parental alveolar macrophages (11). The titer of supernatant fluids from infected cultures was 5×10^5 infectious doses (TCID₅₀)/ml.

Sheep. Two 3-mo-old Corriedale lambs were anesthetized with halothane, and virus was inoculated once into both the right and left cerebral hemispheres and also deposited in the right anterior lobe of the lung using fiberoptic endoscopy. The animals were killed at 2 wk and 12 wk after inoculation, and perfused through the heart first with phosphate-buffered saline (PBS) and then with periodate-lysine-paraformaldehyde-glutaraldehyde (PLPG) (12) as previously described (6). Small portions of various tissues were post-fixed in PLPG, washed in PBS, embedded in paraffin, which was then serial section cut and heat-baked onto glass slides pretreated with poly-D-lysine. Slides were then processed as described below. Brain tissues from both animals were studied, but all other tissues used in this study were obtained from the sheep killed at 12 wk.

Immunocytochemistry. The antibodies used in this study were a rat monoclonal antibody to sheep Ia antigen (13), and a rabbit polyvalent antiserum to sheep alveolar macrophages, of known specificities (11). This antiserum reacts with monocytes and selected macrophage populations in sections of a variety of fixed normal sheep tissues. Tissue sections or cytocentrifuged cell preparations (see below) were studied using the avidin-biotin-coupled peroxidase complex (12). Tissues or cytopun cell preparations were reacted sequentially with each of the following reagents (a) 2% normal rabbit serum for 20 min; (b) monoclonal rat antibody to sheep Ia antigen or rabbit polyvalent antiserum to sheep macrophages, for 30 min; (c) biotinylated antibody to rat or rabbit immunoglobulins (Vector Laboratories, Inc., Burlingame, CA) diluted 1:100, for 30 min; (d) 0.1% hydrogen peroxide in methanol for 30 min; and (e) avidin-biotinylated horseradish peroxidase complex (Vector Laboratories, Inc.) for 30 min. The reaction was visualized with the chromagen 3,3'-diaminobenzidine tetrahydrochloride, 0.5 mg/ml, in PBS containing 0.01% hydrogen peroxide, for 5 min. Between each of these steps the slides were washed in PBS for 10–20 min. After the label was developed the slides were washed in PBS, dehydrated through 70 and 95% ethanol, and stored at room temperature before processing for in situ hybridization.

In Situ Hybridization. This technique was carried out as previously described (14). Cloned DNA of visna virus and cytomegalovirus (CMV) were separated from the pBR322

and used in this study. In brief, cloned DNA of visna virus was cleaved from the pBR322 with the restriction endonuclease *Sst* I, and the 9.1 kilobase (kb) visna virus fragment was separated by electrophoresis in an agarose gel. The DNA of both viruses was radiolabeled by nick translation (15) using ^{35}S -dATP and ^{35}S -dCTP (Amersham Corp., Arlington Heights, IL). The labeled probe was sized by alkaline-agarose electrophoresis and fragments measuring an average of 70 basepairs were used as previously described (16). The specific activities of the probes were $>5 \times 10^8$ cpm/ μg .

The radiolabeled DNA probes were applied to the tissue sections or cytocentrifuged preparations at a concentration of 0.2 $\mu\text{g}/\mu\text{l}$ on slides pretreated with 0.2 N hydrochloric acid for 20 min, heat (70°C for 30 min), proteinase K (1–5 $\mu\text{g}/\text{ml}$) for 15 min, and acetylation for 10 min. Slides were incubated with the probes for 50 h at room temperature and, after extensive washing, were autoradiographed for 2–10 d. The presence of viral RNA was indicated by silver grains over cells. To control for the specificity of the visna probe the latter was applied to normal uninfected tissue and cultures, and the CMV probe was used on visna-infected material.

In combined immunocytochemistry and in situ hybridization experiments the specimens were first stained immunocytochemically with specific antibodies and then processed for in situ hybridization (14). Since the in situ hybridization signal is reduced after immunocytochemical staining, control slides processed for in situ hybridization alone were always included in this combined procedure.

Spleen and Lymph Node Cultures. Normal spleen and lymph node tissues were obtained from an adult sheep. Cells were teased from both tissues in petri dishes containing $\text{Ca}^{++}/\text{Mg}^{++}$ -free Hanks' salt solution, passed through a metal screen, and layered and centrifuged through a Ficoll-Hypaque gradient (2.4 parts of 9% Ficoll [Sigma Chemical Co., St. Louis, MO] to 1 part of 33.9% Hypaque [Winthrop Laboratories, Sterling Drugs, Inc., New York]). The cells were then resuspended in Dulbecco's modified Eagle's medium (DME) containing 5% lamb serum at a concentration of $5 \times 10^6/\text{ml}$, and 10 ml of the suspension cultures were added to Teflon bottles. These were incubated at 37°C in 5% CO_2/air .

Preparation of LV-IFN. LV-IFN was prepared as described (7). Briefly, macrophage cultures were derived in tissue culture dishes by seeding suspensions of peripheral blood mononuclear cells (PBM) obtained from a normal goat in DME plus 20% heated lamb serum. The cultures were inoculated with a strain of sheep-goat lentivirus (in this case, caprine arthritis encephalitis virus) at a multiplicity of infection (moi) of 2 and incubated for 3 d. The infected cultures were then rinsed and inoculated with fresh PBM, obtained from the same donor, in serum-free DME. The supernatant fluid was harvested 2 d later, clarified by centrifugation at 10,000 g for 15 min, heated at 56°C for 30 min to inactivate the virus, and stored at -70°C . This fluid had an IFN titer of 1:320 when tested in the sheep macrophage-vesicular stomatitis virus assay system.

Transformed Macrophages (TrMO). A cell line of alveolar macrophages from an uninfected sheep was prepared by immortalizing cultures of the macrophages, obtained by bronchoalveolar lavage, with SV40 (11). The cells are trypsin dissociable and maintain macrophage functions. They were cultivated in DME containing 20% lamb serum, either as suspension cultures in Teflon bottles or as monolayer cultures in tissue culture dishes.

Ia Induction Experiments. In these experiments, spleen and lymph node cell cultures in Teflon bottles received four different treatments. Two of the spleen and lymph node cultures were treated with LV-IFN for 24 h, after which VMA-5 virus was added at an moi of 1 to one of these; LV-IFN was maintained in the media of both. The two other cultures were either left untreated or received virus alone without LV-IFN. Cells were harvested at various times after the addition of virus and then centrifuged onto glass slides for immunocytochemistry. The cytospun cells were reacted with the anti-sheep Ia monoclonal antibody and then examined for stained Ia antigen. In similar experiments, monolayers of TrMO cultures (see above) were treated with VMA-5 virus alone, IFN alone, or VMA-5 virus plus LV-IFN, or were left as untreated controls. LV-IFN was left in the culture media during a subsequent 24-h exposure to virus, immediately after which all cultures were processed and examined for Ia expression. We also studied the dynamics of Ia induction in TrMO cultures. In these experiments, cultures were exposed to LV-

IFN for 6, 24, and 48 h and then examined for Ia expression at intervals of 0–72 h after the end of LV-IFN treatment.

Effect of LV-IFN on Replication of Virus in Macrophage Cultures. TrMO were seeded in tissue culture dishes in DME plus 20% lamb serum and, at confluence, some of the cultures were treated with LV-IFN (1:10 dilution of stock LV-IFN). On the next day, treated and untreated cultures were inoculated with virus at an moi of 2 and incubated for 2 h at 37°C. Inocula were then removed and replaced with maintenance medium. LV-IFN was maintained in the treated cultures. Supernatant fluids from both sets of cultures were harvested daily and assayed for infectivity in cultures of synovial membrane cells, as previously described (17).

Results

Viral RNA Expression in Macrophages in Different Tissues. To investigate the pathogenesis of the lymphoproliferative response in visna lesions, we first defined the tissues and cell populations that supported viral replication. Viral RNA was detected using in situ hybridization, and, in double-labeling experiments using combined in situ hybridization and immunocytochemistry, it was possible to localize viral RNA in macrophages identified with the antimacrophage serum (6).

Viral RNA was detected in the lung, spleen, and lymph nodes but not in the liver or brain. Large numbers of macrophages containing many copies (100–500 copies per cell) of viral RNA were detected in spleen and mediastinal and cervical lymph nodes; much smaller numbers of infected cells were found in the mesenteric lymph node. The distribution of virus-infected macrophages was as previously described (6) in that they were found prominently in the marginal zones surrounding the uninfected germinal centers (see Fig. 3). Large numbers of cells containing viral RNA were also detected in the lung in the interstitial spaces between alveoli and within areas of peribronchial inflammation. We confirmed that viral infection was restricted to macrophages, as reported previously (6), and that these are found in the lung, mediastinal and cervical lymph nodes, and spleen, but not in the brain (Table I). In infected tissues only a very small

TABLE I
*Percent of Viral RNA-containing Cells Identified as Macrophages or Ia⁺
Cells in Different Tissues*

Tissue	Viral RNA ⁺ /Mac- rophage ⁺ cells	Viral RNA ⁺ /Ia ⁺ cells
	%	
Lung	28.2*	33
Mediastinal lymph node	88	7.7
Spleen	85	5.6
Brain	0	0

Sections of tissues were stained with either antimacrophage or anti-Ia antibodies and then treated with radiolabeled viral nucleic acid probe. 200 cells were counted on each slide and the percent of cells that both contained viral RNA and were labeled with the appropriate antibody was determined. The macrophage data was obtained from Gendelman et al. (6); their figures were confirmed in this study.

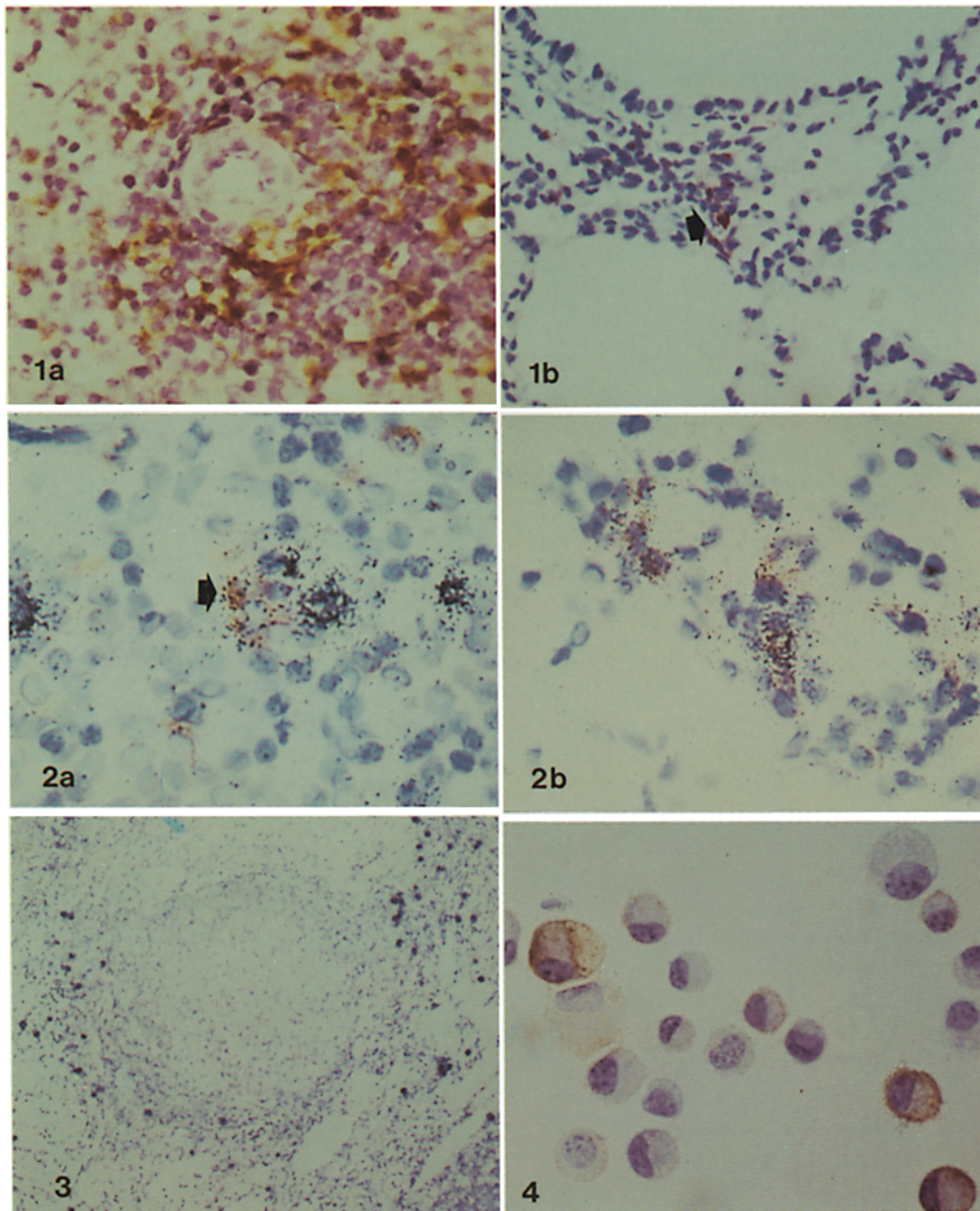
* The 72% of cells that were not labeled with the antimacrophage serum morphologically resembled pulmonary macrophages (6).

percentage (~1%) of the total number of immunocytochemically identified macrophages contain viral RNA (6). Sections of infected tissues treated with the CMV probe and uninfected tissues treated with the visna probe did not show hybridization.

Correlation Between Viral Replication and the Inflammatory Response. The above data reiterated our previous observations (6) that viral replication is associated almost exclusively with infection in selected macrophage populations. Having established this pattern of infection we next asked whether viral replication occurred only in tissues containing inflammatory lesions. The lymph nodes showing the greatest lymphoproliferative response were the mediastinal and cervical lymph nodes, which drain the lung and the brain, respectively. Both of these nodes showed gross hypertrophy (10–15 times normal size) and also contained very large amounts of viral RNA in macrophages. By contrast, the mesenteric lymph node was not hyperplastic, did not show a lymphoproliferative response, and contained very few infected cells. The spleen showed a considerable inflammatory response and also contained many infected cells. The liver contained neither viral RNA nor inflammatory lesions. The two target organs, the lung and the brain, both had inflammatory lesions consisting of a mononuclear cell infiltrate. However, the lung contained many infected macrophages whereas we did not detect viral RNA in the brains of these animals at this point after virus inoculation. Thus, with the exception of the brain, viral RNA was confined to macrophages only in tissues that developed inflammatory lesions.

Increased Ia Antigen Expression in Infected Tissues. The above observations indicate that the specific infection in populations of macrophages was one determinant of the lymphoproliferative response. We then asked how this semi-permissive infection, confined to macrophages, could cause lymphoproliferation. One way to reconcile these observations was the possibility of interference with normal immunological functions of the macrophages. Using antibodies to sheep Ia antigen, we examined tissue sections to determine whether there was increased expression of this antigen in macrophages.

Ia antigens were detected in all tissues except liver. Very few Ia⁺ cells were seen in the brain, and only an occasional Ia⁺ cell was detected in the inflammatory lesions. Such cells were extremely rare despite the presence of a very marked inflammatory reaction in the brain. In the lung, a large number of Ia⁺ cells were seen in the mononuclear inflammatory cells accumulating around bronchi, as well as in the interstitial spaces in the lung where macrophages had begun to accumulate (Fig. 1). The greatest amount of Ia immunoreactivity, as assessed by the size of the areas of tissue sections stained and by the intensity of staining, was in the spleen and mediastinal and cervical lymph nodes, while a much smaller amount of staining was seen in the mesenteric lymph node and the normal lymph node and spleen controls. Ia antigen staining always showed the same pattern in lymph node and spleen, irrespective of the amount of staining, and intense staining of large cells was noted in a circular distribution around the germinal centers (Fig. 1). Fewer Ia⁺ cells were distributed throughout the sinusoidal areas of the spleen and lymph nodes. The distribution of Ia staining in the marginal areas outside the germinal centers very closely resembled that seen with the antimacrophage serum. At higher magnification these were large cells with



FIGURES 1-4. *Visna virus-Ia-macrophage interactions*: All tissues (Figs. 1-3) were fixed in PLPG and embedded in paraffin before immunocytochemical staining and/or in situ hybridization. All sections or cultures were counterstained with hematoxylin. (1) The distribution of Ia antigens, after labeling with monoclonal antibody to sheep Ia antigen, in sections of visna-infected spleen (a) (original magnification, $\times 300$) and lung (b) ($\times 250$). (a) Ia antigens are seen within and surrounding a follicle. (b) A lesser degree of Ia antigen staining is seen within an area of interstitial mononuclear cell infiltration (arrow). (2) Viral RNA in infected macrophages that have also been labeled with monoclonal anti-sheep Ia antibody, in sections of mediastinal lymph node (a) ($\times 500$) and lung (b) ($\times 312$). (a) A number of unstained cells contain viral RNA and one Ia⁺ cell (arrow) also contains viral RNA. (b) Several cells express both Ia antigens and viral RNA. (3) A section of spleen with cells containing viral RNA distributed in a circular pattern around a follicle but not within the germinal center ($\times 70$). (4) Cultures of TrMO show labeling with anti-sheep Ia antibody after exposure to LV-IFN ($\times 400$). Note that three cells in the field show Ia labeling.

extensive cytoplasm, resembling macrophages, not lymphocytes. Most of the Ia⁺ cells in the perigerminal center areas, therefore, were almost certainly macrophages.

These observations clearly show that there was a persistent and increased expression of Ia antigens in the inflammatory lesions in tissues containing viral RNA, and that there was a correlation between the amounts of viral RNA and Ia antigen expressed by infected tissues.

Double-Labeling Experiments to Identify Ia⁺ Cells Containing Viral RNA. We next asked whether the Ia⁺ cells themselves were infected with virus. We used the combined in situ hybridization and immunocytochemical procedure to determine the proportion of Ia⁺ cells containing viral RNA. The results are summarized in Table I, which also compares the numbers of viral RNA-positive/macrophage-positive cells with those of the RNA⁺/Ia⁺ cells. Neither viral RNA⁺/Ia⁺ cells nor infected macrophages were detected in the brain. However, RNA⁺/Ia⁺ cells were detected in the reactive lymphoid tissues, and larger numbers were present in the lung. One-third of the cells containing viral RNA expressed Ia antigen in the lung (Fig. 2) and these were located in both the inflammatory cell exudates and the pulmonary interstitial areas.

By contrast, a smaller proportion (5.6–7.7%) of virally infected spleen and lymph node cells were Ia⁺ (Fig. 2); these were located in a circular distribution surrounding, but never within, the germinal centers. Since viral replication is virtually restricted to macrophages, it is clear that the RNA⁺/Ia⁺ cells represented a small subpopulation of Ia⁺ macrophages that were infected with virus. This population is estimated to be ~0.07% of the total number of macrophages identified in tissue sections stained with the antimacrophage serum.

Induction of Ia Antigens in Spleen and Lymph Node In Vitro by LV-IFN. Having demonstrated increased and persistent expression of Ia antigens in the inflammatory lesions, and virus infection in some of these cells, we addressed the question of how these Ia antigens might be induced in vivo. We designed in vitro experiments to test two possible mechanisms: a direct infection of macrophages or induction of Ia by LV-IFN.

Since the spleen and lymph nodes contained the greatest amounts of Ia antigens, cultures of normal sheep spleen and lymph nodes were treated with virus and/or LV-IFN and examined immunocytochemically to detect increases in their Ia expression. At day 1 in spleen cultures exposed to LV-IFN alone (Tables II and III), there was a 70% increase in the percentage of Ia⁺ cells compared with untreated control cultures and cultures treated with virus alone. Treatment of cultures with LV-IFN plus virus also caused a marked increase in Ia antigen expression, and the increases were still present at day 5. There was no noticeable difference in expression of Ia in cells in infected cultures compared with uninfected control cultures. The results in lymph node cells showed a very similar increase in Ia expression with LV-IFN. The intensity of the surface Ia staining was also increased in cultures showing an increase in the overall percentage of Ia⁺ cells. It was clear that Ia antigens could be increased by LV-IFN but not by virus infection alone.

Ia Induction by LV-IFN in TrMO Cultures. Although we demonstrated a clear increase in Ia antigen expression in spleen and lymph node cells by LV-IFN, we

TABLE II
Ia Expression in Splenocytes After Exposure to Virus and/or LV-IFN

Time after virus exposure*	Percent of cells expressing Ia antigen in cultures of:			
	Splenocytes (control)	Splenocytes + LV-IFN	Splenocytes + virus	Splenocytes + virus + LV-IFN
<i>d</i>			%	
1	27	46	28.5	55.5
5	22	58.5	20.5	48

Two of the splenocyte cultures were treated with IFN for 24 h and then visna virus was added to one while maintaining IFN in the media of both. Other cultures were only exposed to virus (moi of 1); control cells received neither virus nor IFN. 200 cells per slide were counted in duplicate experiments and the percent of cells labeling with anti-Ia antibody was determined.

* The percent of control cells expressing Ia was 22% immediately after cell dissociation at day 0.

TABLE III
Ia Expression in Lymph Node Cells After Exposure to Virus and/or LV-IFN

Time after virus exposure*	Percent of cells expressing Ia antigen in cultures of:			
	Lymph node (control)	Lymph node + LV-IFN	Lymph node + virus	Lymph node + virus + LV-IFN
<i>d</i>			%	
1	26.5	59	29	50.5
3	25.5	49.5	25.5	45
6	26	58	18	40

Dissociated lymph nodes were treated as described in text and in legend to Table II. Results were determined as described in legend to Table II.

* The percent of control cells expressing Ia was 26% immediately after dissociation at day 0.

could not identify with certainty the cell type in which the antigen had been induced because of the pluripotentiality of spleen and lymph node cells to express Ia. To determine whether macrophages would express Ia antigens after infection or after treatment with LV-IFN, we prepared four cultures of TrMO in Teflon bottles and made the following inoculations: (a) no treatment; (b) virus at an moi of 3; (c) LV-IFN (1:10 with stock); (d) virus plus LV-IFN at the same concentrations used in b and c. There was no constitutive expression of Ia antigen in the macrophages, and infection of the macrophages with virus alone did not result in Ia expression. However, cultures that had received LV-IFN with or without infectious virus showed high levels of Ia expression, with 17.5% of the total cells expressing Ia antigens. Since these cultures are heterogeneous (11) it was not surprising that only a proportion of the macrophages expressed Ia antigens after LV-IFN treatment.

Dynamic Studies of Ia Induction by LV-IFN in TrMO Cultures. The above experiments showed that expression of Ia antigen by the TrMO was contingent upon the presence of LV-IFN. To determine the dynamics of Ia induction in these cells, TrMO cultures were exposed to LV-IFN for varying periods at 37°C and then examined for Ia expression 0–72 h after removal of the LV-IFN. After each treatment period, the cells were removed, washed by centrifugation, resus-

TABLE IV
Dynamic Studies of LV-IFN-induced Ia Antigen Expression in Transformed Macrophage Cultures

Duration of LV-IFN exposure	Percent of cells expressing Ia antigens after LV-IFN treatment				
	Time after end of treatment				
	0 h	6 h	24 h	48 h	72 h
6 h	0	0	5	2	<1
24 h	9	7.5	4.5	3	<1
48 h	11	5	4	1	<1
14 d	5	ND*	ND	ND	ND

Cultures of TrMO in Teflon bottles were exposed to LV-IFN for 6, 24, and 48 h and then examined for Ia expression at intervals after the end of the treatment. Results were determined as described in legend to Table II. Untreated control cultures remained negative for Ia expression throughout the experiment.

* Not done.

pendent in fresh medium, and reincubated at 37°C. The results (Table IV) showed: (a) Ia antigens did not appear until 6–24 h after LV-IFN exposure; (b) irrespective of the duration of LV-IFN treatment, Ia antigens were rapidly depleted in culture, with <1% of the total cells being Ia⁺ after 72 h; (c) increasing the time of exposure to LV-IFN slightly increased the percentage of cells expressing Ia; and (d) maintaining LV-IFN in the cultures for longer periods increased the total duration of Ia expression. When LV-IFN was maintained in the cultures for 2 wk, 5% of the total cells were found to be Ia⁺, indicating that Ia expression required the continuous presence of LV-IFN in the medium. Since the TrMO population was heterogeneous and dividing, it is possible that this continuous Ia expression represented transient asynchronous expression of Ia by different cells.

Effects of LV-IFN on Lentivirus Replication in Macrophages. Having shown that LV-IFN could induce Ia antigen expression in macrophage cultures, we asked whether the IFN would also cause reduction in virus replication in the macrophages (Fig. 5). Infected cultures treated with LV-IFN produced ~1,000-fold less infectious virus than untreated controls.

Discussion

We have shown in this report that the lymphoproliferative inflammatory response caused by visna virus in sheep was associated with a persistently high level of transcription of the viral genome in macrophages, accompanied by an intense level of expression of Ia antigen in infected and uninfected macrophage-like cells. In a previous study (6) we showed that replication of this virus was confined to cells of the macrophage lineage, with no involvement of lymphocytes. The mechanism of lymphoproliferation represented a paradox. Our finding that these lentiviruses induce an IFN during the interaction of infected macrophages and lymphocytes and that the IFN in turn caused expression of Ia antigen in infected and uninfected macrophages, provides a fresh perspective for evaluating the pathogenesis of the persistent virus-induced inflammation.

The combined immunocytochemical and in situ hybridization procedure pro-

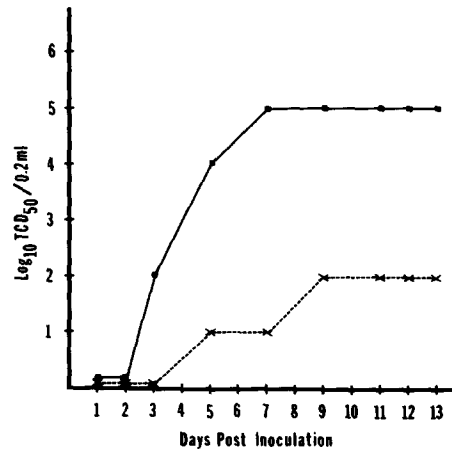


FIGURE 5. Infectivity titers in TrMO cultures inoculated with VMA-5 at an moi of 2 and maintained with (---) and without (—) LV-IFN.

vided an excellent means to identify viral RNA in cells expressing Ia antigens. Since viral RNA was found only in macrophages, the small number of cells containing viral RNA and Ia antigen must have been infected macrophages expressing Ia antigen. By implication, if the same cell that is presenting viral antigen to helper T cells is also infected with virus, a potentially aberrant host immune response could result, leading possibly to lymphoproliferation and failure to clear the infection. Infection of Ia⁺ macrophages has also been reported (18) in mice infected with lactic dehydrogenase virus, and similar abnormal host responses have also been implicated in this infection. Large numbers of such infected Ia⁺ cells may not be required to maintain this aberrant response. A greater percentage of RNA⁺/Ia⁺ cells was detected in the lung than in the mediastinal lymph node and spleen. Although the reasons for this discrepancy are not clear, it almost certainly relates to the different status of the two tissues, in that the lung is a primary target organ for the disease whereas the other tissues are reactive. Virus replication, however, remained restricted in all of these organs.

The amounts of Ia antigen and viral RNA expression were closely correlated in the lung, lymph nodes, and spleen, which were tissues with ongoing inflammatory lesions. Our in vitro data showed that Ia was induced in macrophages by LV-IFN but not by virus infection alone, and that Ia expression diminished in the absence of LV-IFN in the medium. Therefore, persistent expression of Ia antigen required the continuous presence of IFN. Extrapolation of these data to events in the animal suggests that virus infection alone is not sufficient to induce Ia expression in macrophages. We speculate that IFN must be induced locally to cause expression of this antigen. This in turn could generate the ongoing inflammatory induction of T lymphocyte populations. Although LV-IFN has not been isolated from infected tissues (7), both infected macrophages and lymphocytes, which are required for its production (7), are present within the inflammatory lesion.

It was clear from our studies that viral RNA expression in macrophages

correlates with both inflammation and high expression levels of Ia antigens. However, virus-cell reactions in the brain were different from those in the lung and lymph nodes. Encephalitis at 2 and 12 wk after intracranial virus inoculation was associated with neither viral RNA nor Ia antigen expression. Other investigators (19) have examined visna virus expression in brain and detected small amounts of viral RNA 6 wk after inoculation. Differences in virus strains and breeds of sheep may account for these discrepancies. In our study it is possible that the paucity of viral RNA in CNS cells may reflect a greater resistance of these cells to infection and/or a unique response of CNS tissues to fixation or other laboratory procedures that may have occluded access of the probe to the RNA. These possibilities are being examined in current experiments. Previous studies have shown that the visna brain lesions are immunopathologic, since they may be prevented by immunosuppression (20) and their induction is associated with a virus-specific cellular immune response (21). We have also shown (5) that cell-free virus can be isolated from brain tissue homogenates soon after inoculation but not at 3 mo and that lesions proceed to scarification, instead of chronic inflammation as found in the lung (5). We interpret the inflammatory brain lesions that lacked both Ia and virus RNA at and beyond 2 wk postinoculation as being reparative rather than chronic, in keeping with an episodic "hit-and-run" pathogenesis. Persistence or lack of expression of Ia and viral RNA may thus help determine whether lesions become chronic and indolent, as in the lung, or episodic, as in the brain.

The ability of LV-IFN to inhibit virus replication in TrMO may be an *in vitro* correlate of virus replication *in vivo* and may be implicated in the mechanism of restricted virus replication in tissue macrophages. IFN-mediated restriction of replication of retroviruses occurs at a late stage of the virus life cycle, between assembly and maturation of the infectious virion (22). Whether this also applies to the replication of the lentiviruses is not known. The precise stage of the virus life cycle in TrMO blocked by LV-IFN has not been identified, but our results may provide a model system for further dissection of the semi-abortive replicative cycle of the virus in tissue macrophages.

Although LV-IFN has not been identified directly in tissue extracts, the manner of restriction of virus replication, the persistence of Ia antigen expression, and the local presence of the cells required for virus production provide strong circumstantial evidence for a role of LV-IFN *in vivo*. Our studies suggest that LV-IFN induced *in vivo* during replication of lentiviruses may have both beneficial and deleterious effects on the host. Whereas LV-IFN may restrict virus replication and thus slow the course of the infection, it may also induce Ia antigens in infected macrophage target cells and potentiate immunopathologic disease. Such a pathogenesis may apply to the lymphadenopathy seen in humans with acquired immune deficiency syndrome (AIDS), in which the replication of lentivirus HTLV-III is minimal, lymphoproliferation is high, and IFN levels in serum are clearly detectable (23–25).

Summary

In this study we investigated the pathogenesis of the lymphoproliferative response in the chronic-active visna maedi virus-induced inflammatory lesions.

Viral RNA expression was confined to macrophages, but only in tissues showing inflammatory lesions. A persistent and high level of Ia antigen expression was seen in macrophage-like cells in the inflammatory lesions, and the amounts of viral RNA and Ia expression were closely correlated. A small subpopulation of macrophages contained both viral RNA and Ia antigen, and these were found in greatest number in the lung. In vitro experiments showed that a lentivirus-induced interferon (LV-IFN) could induce Ia antigens in normal sheep spleen and lymph node cells as well as in a transformed sheep macrophage cell line. Ia antigen expression in macrophages was transient in the absence of a continuing IFN stimulus and persisted for at least 2 wk in the presence of LV-IFN. LV-IFN also restricted viral replication in macrophages. It is suggested that LV-IFN induced by the inflammatory cells in visna-maedi lesions may induce Ia antigen expression in macrophages, thereby indirectly causing the lymphoproliferative response and restricted virus replication.

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