Ovine Lentivirus Lymphoid Interstitial Pneumonia

Rapid Induction in Neonatal Lambs

MICHAEL D. LAIRMORE, DVM, RAUL H. ROSADIO, MS, DVM, and JAMES C. DeMARTINI, DVM, PhD

From the Department of Pathology, Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, Colorado

For examination of the characteristics of lentivirus-induced pulmonary disease in an animal model, neonatal lambs were given intratracheal injections of high- and low-passage ovine lentivirus (OvLV) isolates. In 6 of 6 lambs inoculated with low-passage OvLV or OvLV from lung lavage fluid, lesions of lymphoid interstitial pneumonia (LIP) developed. In none of 7 lambs inoculated with a high-passage OvLV or 4 control lambs inoculated with medium alone or ultrafiltered lung fluid did lung lesions develop. Systemic distribution of lentivirus was greater and development of lentivirus antibody was more rapid in lambs inoculated with low-passage OvLV, compared with lambs inoculated with high-passage OvLV. The number of lymphocytes in bronchoalveolar lavage samples was increased in lambs with lymphoid interstitial pneumonia. The development of lymphoid interstitial pneumonia was markedly accelerated, in comparison with previous reports of experimentally induced lentivirus pneumonia in sheep. In lentivirus-inoculated lambs pulmonary lesions developed comparable to lymphoid interstitial pneumonia associated with the acquired immunodeficiency syndrome and other human benign lymphoid disorders of the lung. Similarities between the disease manifestations and virologic properties of OvLV and human T-cell lymphotropic virus III argue for the relevance of OvLV-induced disease as a model for human retrovirus diseases. The ability of OvLV to cause accelerated pulmonary disease in neonates may be due to age-related susceptibility factors that enhance the pathogenicity of lentiviruses. (Am J Pathol 1986, 125:173–181)

LENTIVIRUSES are a subfamily of retroviruses that include visna/maedi virus, caprine arthritis encephalitis virus, and ovine progressive pneumonia virus. The ovine lentiviurses (OvLV) are naturally transmitted through ingestion of milk or exposure to respiratory secretions, and cause persistent infections in sheep that result in lymphoid interstitial pneumonia, regional lymphadenomegaly, and less frequently nonsuppurative encephalitis and erosive polyarthritis.[1–4] The clinical disease often terminates with progressive emaciation, dyspnea, hypergammaglobulinemia and development of opportunistic infections.[4,5] Ovine progressive pneumonia virus infection is common among adult sheep in the United States, where the pneumonic lesion is the primary manifestation of infection.[6,7]

Lentiviruses are thought to replicate slowly in their hosts, and this seems true, judging from results obtained in studies of spontaneously diseased animals and animals with experimentally induced infections with reports of incubation periods of lentivirus-induced disease from a few months to a few years.[8–11] However, most previous experimental infections were performed in older lambs or adult sheep with the use of unnatural routes of inoculation. Under these conditions lentivirus replication may not be facilitated. Without a consistent and temporally convenient model of lentivirus-induced lymphoid interstitial pneumonia, the predominant clinical and pathologic manifestation of infection, investigations concerning pathogenic mechanisms of OvLV have been limited. To examine the role of lentivirus in the neonate and to develop a convenient, reproducible model of lymphoid interstitial pneumonia,

Dr. Lairmore is supported by an award from the National Cancer Institute (1T15C50940801). The work is supported in part by a grant from the United States Agency for International Development Title XII Small Ruminant Collaborative Research Program (AID/DSAN/XII-6-0Q49).

Accepted for publication May 27, 1986.

Address reprint requests to Michael D. Lairmore, DVM, Colorado State University, Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO 80523.

we studied neonatal lambs given intratracheal injections of OvLV.

Visna virus, the prototype of the ovine lentiviruses, has been shown recently to share morphologic similarities and extensive genome sequence homology with human T-cell lymphotropic virus III (HTLV-III/LAV), the etiologic agent of acquired immunodeficiency syndrome (AIDS).[12,13] Visna virus infections of sheep cause demyelinating meningoencephalitis, thought to be the result of an immunopathologic mechanism directed at cellular and/or viral antigenic proteins.[10] Investigations concerning mechanisms of lentivirus persistence have demonstrated *in vivo* restriction of visna virus gene expression in sheep alveolar macrophages[14] and bone marrow stem cells.[15] HTLV-III/LAV induces a persistent infection, which causes lymphoproliferative and lymphocytolytic lesions, replicates in the central nervous system, and has been associated with neurologic disease.[16] Lymphoid interstitial pneumonia has been reported recently in a high proportion of children with AIDS[17-19] and as an infrequent lesion in adults with AIDS.[20,21] Similarities between the disease manifestations and virologic properties of OvLV and HTLV-III/LAV argue for the relevance of OvLV-induced disease as a model of human retrovirus diseases.

We report here that OvLV injected intratracheally into neonatal lambs induces an accelerated form of lymphoid interstitial pneumonia. The association of OvLV, like HTLV-III/LAV, with accelerated pulmonary disease in neonates suggests that age-related susceptibility factors enhance the pathogenicity of lentiviruses and suggests an additional target tissue for human retrovirus infections.

Materials and Methods

Lambs

Newborn crossbred lambs were obtained prior to suckling to prevent colostral transmission of OvLV. The lambs were maintained on bovine colostrum and commercial milk replacer as described by Houwers et al.[1]

OvLV Source and Inoculation of Lambs

OvLVs used for inoculations were isolated from naturally occurring cases of ovine progressive pneumonia or from lambs with experimental lentivirus-induced lymphoid interstitial pneumonia. The virus isolates were grown and maintained in goat synovial membrane (GSM) cells as described by Querat et al.[22] The isolates were titered in GSM cells in a syncytia-induction endpoint dilution assay. Low-passage lentivirus isolates were defined as isolates which, following initial isolation, were passed less than 10 times in GSM cells. All low-passage isolates were lytic in cell culture except one (inoculated in Lamb 41, Table 1) which produced a persistent infection in GSM cells, characterized by cycles of syncytia formation following trypsinization. A high-passage isolate, originally isolated by Dr. William Hadlow from a sheep with progressive pneumonia, was kindly provided by Dr. Ashley Haase.[23] The high-passage isolate was maintained in sheep choroid plexus cells and GSM cells, was plaque-cloned, and was rapidly lytic in infected cell cultures.

The lambs were divided into three groups (Table 1). Group 1 lambs were inoculated intratracheally with 5–10 ml of cell culture medium containing 1.0×10^4–3.1×10^6 $TCID_{50}$ low-passage OvLV (each lamb inoculated with an isolate from separate sources; Lambs 5, 41, 79, 70, and 72) or lung lavage fluid containing 1.5×10^6 $TCID_{50}$ OvLV (Lamb 34). Group 2 lambs were inoculated with the same volume of tissue culture medium containing 5.7×10^6 $TCID_{50}$ high-passage OvLV (same isolate inoculated in Lambs 17, 18, and 28 through 31). Group 3 were control lambs inoculated with medium alone (Dulbecco's modified Eagle's medium, DMEM, containing 5% fetal bovine serum and 50 μg/ml gentamicin antibiotic; Lambs 32 and 33) or ultrafiltered lung lavage fluid (molecular weight filter limit, 50,000 daltons, to eliminate retroviruses; Lambs 23 and 25).

Lentivirus Isolation

Tissues and cells for virus reisolation were collected at the time of death and cocultured with GSM cells. Cultures were monitored for evidence of cytopathic effects and passed at least three times at weekly intervals before being considered negative. Positive cultures and original inoculates were confirmed as lentivirus by immunoblotting with polyvalent lentivirus antiserum.[22]

Serology

Serum samples were collected approximately biweekly from all lambs. The agar gel immunodiffusion test, with cell-culture–derived ovine progressive pneumonia virus as antigen, was used for detection of lentivirus-specific antibody.[24]

Gross and Microscopic Pathology

Lambs were anesthetized by intravenous inoculation with xylazine and ketamine and killed by electrocution. Selected organs were excised and weighed. Tissues were fixed in 10% buffered neutral formalin solution, sectioned at 5 μ, and stained with hematoxylin and eosin (H&E). Selected lung sections were also stained with

Table 1—Lentivirus Infection of Neonatal Lambs

Lamb	Inoculum* TCID50	OvLV antibody†	Sacrifice age	Lesions‡		
				Lung LIP	Lymph node hyperplasia	Dyspnea§
Group 1 (OvLV)						
5	1.5×10^6‖	+ 4 weeks	4 weeks	+ +	+	+
79	3.1×10^6‖	–	4 weeks	+	–	–
70	3.1×10^6‖	+ 7 weeks	13 weeks	+ + + +	+ + + +	+ + + +
34	1.5×10^6¶	+ 4 weeks	14 weeks	+ + +	+ + +	+ + +
72	1.0×10^4‖	+ 14 weeks	14 weeks	+	+ +	–
41	1.5×10^6‖	+ 4 weeks	26 weeks	+ + +	+ +	+ +
Group 2 (OvLV)						
17	5.7×10^6	–	1 week	–	–	–
18	5.7×10^6	–	3 weeks	–	–	–
27	5.7×10^6	–	5 weeks	–	–	–
28	5.7×10^6	–	8 weeks	–	–	–
30	5.7×10^6	–	11 weeks	–	–	–
29	5.7×10^6	+ 12 weeks	24 weeks	–	–	–
31	5.7×10^6	–	28 weeks	–	–	–
Group 3 (controls)						
32	–**	–	3 weeks	–	–	–
33	–**	–	7 weeks	–	–	–
23	–††	–	21 weeks	–	–	–
25	–††	–	26 weeks	–	–	–

* Inoculum: OvLV isolates as described in Materials and Methods. Group 1, low-passage OvLV isolates and OvLV from lung lavage fluid; Group 2, high-passage OvLV; Group 3, medium and lung lavage fluid without OvLV.

† Agar gel immunodiffusion detection of serum antibody to OvLV glycoproteins; age at which reaction was first detected.

‡ Lymphoid interstitial pneumonia ranked in severity from + (least severe) to + + + + (most severe) as described in Materials and Methods and Table 2. Pulmonary lymph node (caudal mediastinal lymph node) hyperplasia ranked in severity: +, few lymphoid germinal centers per 4× magnification; + + + +, many lymphoid germinal centers per 4× magnification.

§ Dyspnea assessed daily throughout the experiment defined as increased respiratory rate and exercise intolerance, compared with that of control lambs: +, mild increase in respiratory rate and little exercise intolerance, + + + +, rapid respiration without exercise and severe dyspnea with exercise.

‖ Low-passage OvLV isolates.

¶ OvLV from lung lavage fluid.

** Medium.

†† Lung lavage fluid without OvLV.

periodic acid–Schiff, Masson's trichrome, Congo red, and Verhoff–van Gieson stains.

After bronchopulmonary lavage (see below) the right lung lobes were excised and insufflated with 10% buffered neutral formalin solution by gravity flow until distended, held for a minimum of 48 hours, and then prepared for histologic examination as described above. Transverse sections from each lung lobe were examined histologically for the presence and severity of lymphoid interstitial pneumonia (LIP). LIP was defined as a pulmonary lesion consisting of two principal components: 1) thickening of alveolar septa by interstitial accumulations of lymphocytes, plasma cells, and mononuclear phagocytes and the presence of leukocytes and fibrin in alveolar lumens and 2) prominent peribronchiolar or parenchymal lymphoid follicles. The severity of the first component was ranked from least to most severe as follows: +, multifocal interstitial leukocytes with no alveolar exudate; + +, multifocal interstitial leukocytes with few leukocytes with alveoli; + + +, multifocal to confluent areas of alveolar septal thickening and leukocytes and fibrin within alveoli; + + + +, confluent areas of alveolar septal thickening with numerous leukocytes and abundant fibrin within alveoli. The severity of the second component was ranked from least to most severe by the number of lymphoid follicles per microscopic field under a 4× objective as follows: +, 0–2 follicles; + +, 3–4 follicles; + + +, 5–6 follicles; and + + + +, 7 follicles. Other tissues examined histologically included transverse sections from the hilus of each cranial mediastinal lymph node and sections of most major organ systems.

Bronchopulmonary Lavage

Immediately after death the lungs were excised; and a sterile solution of phosphate buffered saline (pH 7.4) containing antibiotics was instilled intrabronchially by gravity flow into the right lung lobes. The lavage fluid was clarified by passage through gauze, centrifuged at 200 *g* for 10 minutes, and resuspended in DMEM. The lavage cells were either placed in coculture or cytocentrifuged onto glass slides. The proportion of viable cells, assessed by trypan blue dye exclusion, was determined

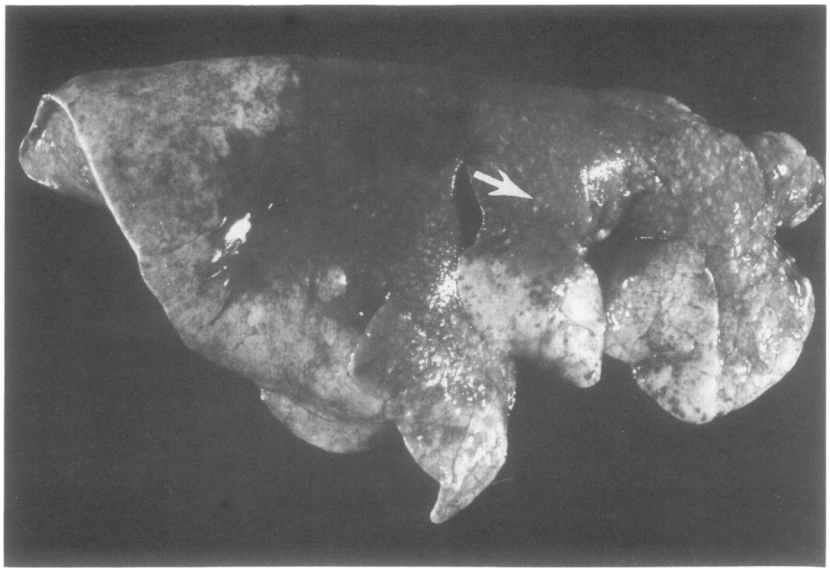

Figure 1—Gross appearance of the right lung of a lentivirus-inoculated lamb (70, Group 1) with severe lymphoid interstitial pneumonia. Note subpleural foci (*arrow*: lymphoid follicles) and confluent central discoloration of the middle and diaphragmatic lobes.

by hemacytometer counts. The cytocentrifuge preparations were stained by the Wright's method for morphologic identification.

Results

Clinical signs of respiratory disease were present in lambs inoculated with low-passage OvLV (Table 1). These symptoms were first noted at approximately 3 weeks of age, as evidenced by increased resting respiratory rates, compared with those of control lambs. Clinical signs progressed over time to severe exercise intolerance and occasional coughing. Lambs in Group 1 were sacrificed at either 4 weeks (for assessment of early lesions of lymphoid interstitial pneumonia; Lambs 5 and 79) or after repeated episodes of dyspnea (Lambs 34, 41, and 70). Lamb 72, inoculated with lower $TCID_{50}$ of low-passage lentivirus, did not develop clinical signs and was sacrificed at 14 weeks. Lambs inoculated with the high-passage lentivirus (Group 2) or control lambs (Group 3) failed to show clinical signs throughout the study. Lambs in Group 1 also had decreased weight gains (0.15 kg/day versus 0.24 kg/day in control Lambs 33, 23, and 25). Febrile episodes corresponded to occasional periods of anorexia. Joint swelling was noted in 1 lamb (41).

Gross lesions were limited to the lungs and pulmonary lymph nodes of lambs in Group 1 (Table 1). The lungs were increased in consistency and failed to collapse properly. These lungs also had multifocal red to brown discolorations on the pleural surfaces, and numerous minute, gray, well-demarcated subpleural foci (Figure 1). On sectioned surface, these areas consisted of firm tan to brown tissue with 1.0–3.0 mm tan foci. The average lung weight, expressed as percentage of body weight (%BW), of lambs 79, 70, 72, and 41 was 2.8%, compared with 1.5% in control Lambs 33 and 23. The middle and cranial lung lobes of 1 lamb (34) contained focal abscesses and focal consolidation of lung parenchyma. *Pasteurella hemolyticum* was cultured from the abscesses. Selected lung samples were negative when cultured for *Mycoplasma* species and failed to react with antibody to respiratory syncytial virus in an immunofluorescence assay.

Lambs in Group 1 had enlarged pulmonary lymph nodes 0.04%BW (Lambs 70, 72, and 41) versus 0.02%BW in a control Lamb of similar age (23). Cortical lymphoid follicles in these lymph nodes were increased in number and size. Thymuses from the experimental lambs with lung lesions were often small, compared with those of controls. The thymus weights of Lambs 70, 72, and 41 averaged 0.098%BW, compared with 0.15%BW of control Lambs 33 and 23. Lambs inoculated with the high-passage OvLV, tissue culture medium, or lung lavage fluid without OvLV had no gross lesions.

Lung lesions were consistent with those of lymphoid interstitial pneumonia (Figure 2). The severity of lymphoid interstitial pneumonia correlated with the severity of dyspnea (Table 1). Moderate to severe peribronchial, peribronchiolar, and perivascular hyperplastic lymphoid follicles were present in 4 of 6 lambs (Table 2). The degree of lymphoid follicular hyperplasia related directly to interstitial inflammatory cell infiltrates, which consisted of lymphocytes, plasma cells, and macrophages. Alveolar walls were focally disrupted, which resulted in short and incomplete separations between adjacent alveoli. Lymphoid follicles within the lung con-

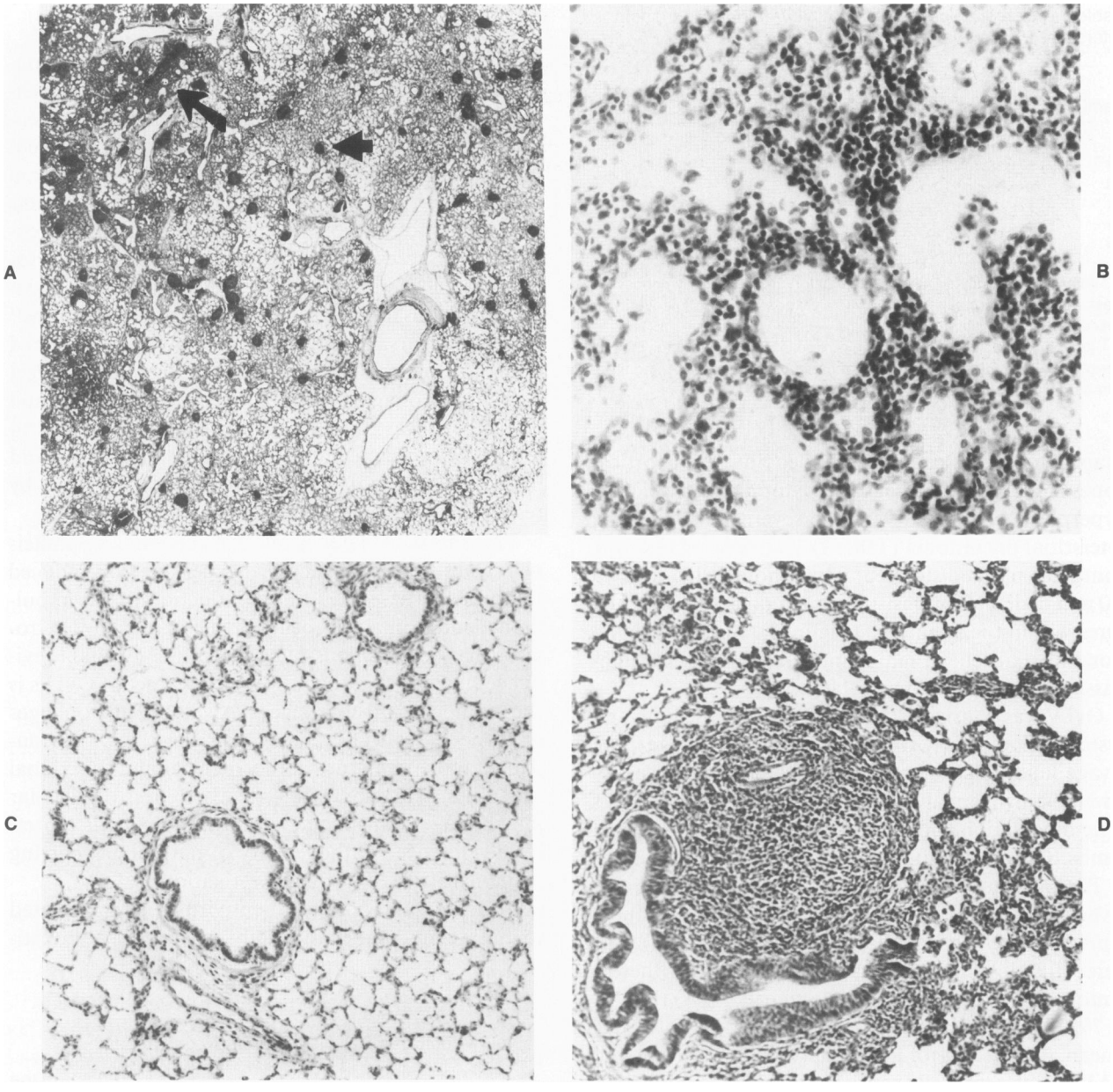

Figure 2A—Section of the insufflated right middle lung lobe of a lentivirus-inoculateed lamb (70, Group 1) with multifocal lymphoid follicles (*short arrow*) adjacent to confluent interstitial pneumonitis (*long arrow*). (H&E, ×2) **B**—Thickened interstitium due to polymorphic leukocytic infiltrates of an OvLV-inoculated lamb. (70, Group 1). (H&E, ×240) **C**—Normal lung of a control lamb. (33, Group 3) **D**—Hyperplastic lymphoid follicle which compresses the adjacent bronchiole and arteriole. Adjacent interstitium is thickened with polymorphic leukocytic infiltrates (70, Group 1).

sisted of well-demarcated aggregates of lymphocytes often with germinal centers of large lymphoblasts with numerous mitotic figures surrounded by rims of small to medium lymphocytes. Alveoli in consolidated areas contained increased numbers of macrophages, lymphocytes, and fibrin. Early changes of lymphoid interstitial pneumonia from Lambs 5 and 79 killed at 4 weeks after inoculation consisted of lymphoid follicles with prominent germinal centers. Pneumonitis lesions were less evident in these 2 lambs, consisting of mild to moderate, multifocal interstitial thickening and alveoli containing predominantly macrophages with minimal amounts of fibrin. The lamb inoculated with 10^4 $TCID_{50}$ of low-passage lentivirus (72) contained lesions confined to hyperplastic peribronchiolar and perivascular lymphoid follicles without significant degrees of interstitial infiltrates (Figure 3).

The caudal mediastinal (pulmonary) lymph nodes of lambs in Group 1 contained varying degrees of lymphoid follicular hyperplasia. Enlarged and irregularly

Table 2—Microscopic Features of OvLV-Induced Lymphoid Interstitial Pneumonia in Neonatal Lambs

Lamb	Interstitial inflammation*	Lymphoid follicles†		
		Peribronchiolar	Perivascular	Subpleural
5	+	+ +	+ +	+
79	+/−	−	−	+
70	+ + + +	+ + + +	+ + +	+ + +
34	+ +	+ + +	+ +	+ +
41	+ +	+ + +	+ +	+ + +
72	+/−	+	+	+

* Interstitial leukocytes mixed inflammatory infiltrates of lymphocytes, plasma cells, macrophages, and alveolar exudate ranked in severity as described in Materials and Methods.

† Lymphoid follicles within the lung ranked in severity: +, least in number per 4× magnification; + + + +, most in number per 4× magnification as described in Materials and Methods.

shaped germinal centers extended into paracortical zones (Figure 3). The degree of lymph node follicular hyperplasia correlated with the severity of lymphoid interstitial pneumonia (Table 1). Lamb 41 of Group 1 contained marked subpleural lymphoid follicles 4.0 to 8.0 mm in diameter consisting of lymph node-like structures within the subpleura (Figure 3). Microscopic lesions were absent in lambs inoculated with the high-passage OvLV and control lambs.

OvLV was consistently reisolated from the lung and systemic tissues of Group 1 lambs (low passage/lung lavage fluid OvLV-inoculated; Table 3). OvLV was recovered only from alveolar leukocytes through 8 weeks after inoculation in the high-passage OvLV-inoculated lambs. OvLV was not recovered from control lambs.

The percentage of lymphocytes in bronchoalveolar lavage (BAL) samples from OvLV-inoculated Group 1 lambs was greater than that of controls (Figure 3). The percent lymphocytes in lavage samples in 5 of these 6 lambs ranged from 12% to 33% (mean, 20%) (Lambs 5, 79, 72, 34, and 41), compared with a range of 4–8% (mean, 6%) in control Lambs 32 and 33. Control lamb BAL samples typically consisted of 90% macrophages, 4–8% lymphocytes, and 1–3% neutrophils and eosinophils. The total leukocyte count of BAL cells from the low-passage OvLV-inoculated lambs averaged 7.5 $\times 10^5$ cells/ml (200 ml recovered of a total lavage volume of 250–300 ml). Control lamb BAL cell recovery averaged 0.5 $\times 10^5$ cells/ml (similar recovery and lavage volumes). BAL samples from the lambs of the high-passage group did not differ from the control lambs.

Five of 6 Group 1 lambs produced antibody against OvLV between 4 and 14 weeks after inoculation (Table 1). In only 1 of 7 lambs from the high-passage group had detectable OvLV antibody developed prior to death. Control lambs were negative for OvLV antibody throughout the study.

Discussion

The development of lymphoid interstitial pneumonia in the OvLV-inoculated lambs was markedly accelerated in comparison with previous reports of experimentally induced lentivirus pneumonia in sheep.[9–11] Recent transmission studies report prevalence rates of lymphoid interstitial pneumonia ranging from no lesions produced[25] to 54% of inoculated sheep having pneumonitis.[26] Successful transmission studies of lentivirus-induced lymphoid interstitial pneumonia report incubation periods to onset of clinical signs from 8 months[11] to several years.[26] Adult animals were not inoculated with our isolates of lentiviruses, and therefore a direct comparison of age susceptibility between neonates and adult sheep was not made in our study. There are no reports of experimentally induced lentivirus lymphoid interstitial pneumonia in lambs exposed to the virus by respiratory routes as neonates.

This shortened latency interval in neonates parallels neonatal susceptibility in other experimentally induced retrovirus diseases.[27,28] Rapid induction of sheep pulmonary adenomatosis (believed to be caused by a retrovirus) has been demonstrated in lambs.[29] Available evidence suggests that the primary cell target of OvLVs is the monocyte/macrophage.[15] Alveolar macrophages from neonates of several species have been demonstrated to be immature in biochemical and functional capacities.[30] The increased permissiveness of alveolar macrophages in neonatal lambs presumably is central to the enhanced pathogenicity of naturally occurring ovine lentiviruses in this investigation.

The strain and dosage of lentivirus used was related to disease outcome in the lambs in the present investi-

Table 3—Lentivirus Reisolation From Neonatal Lambs

	Low-passage/lung lavage OvLV-inoculated lambs (positive/no. tested)	High-passage OvLV-inoculated lambs (positive/no. tested)
BAL leukocytes*	4/4	5/7
Blood leukocytes†	2/4	0/5
Lung	5/6	3/5
Spleen	3/6	0/5
Pharyngeal lymph node	3/4	0/2
Mesenteric lymph node	3/4	0/3
Choroid plexus	2/3	0/3
Bone marrow	2/3	0/3

* Bronchoalveolar leukocytes seeded in coculture at 1 $\times 10^6$ cells per 35 mm tissue culture flask containing GSM cells. BAL procedure and virus isolation as in Materials and Methods.

† Blood mononuclear cells prepared from buffy coat samples after erythrocyte lysis in ammonium chloride solution.

Control lambs (Group 3) negative for lentivirus from similar tissues/cells.

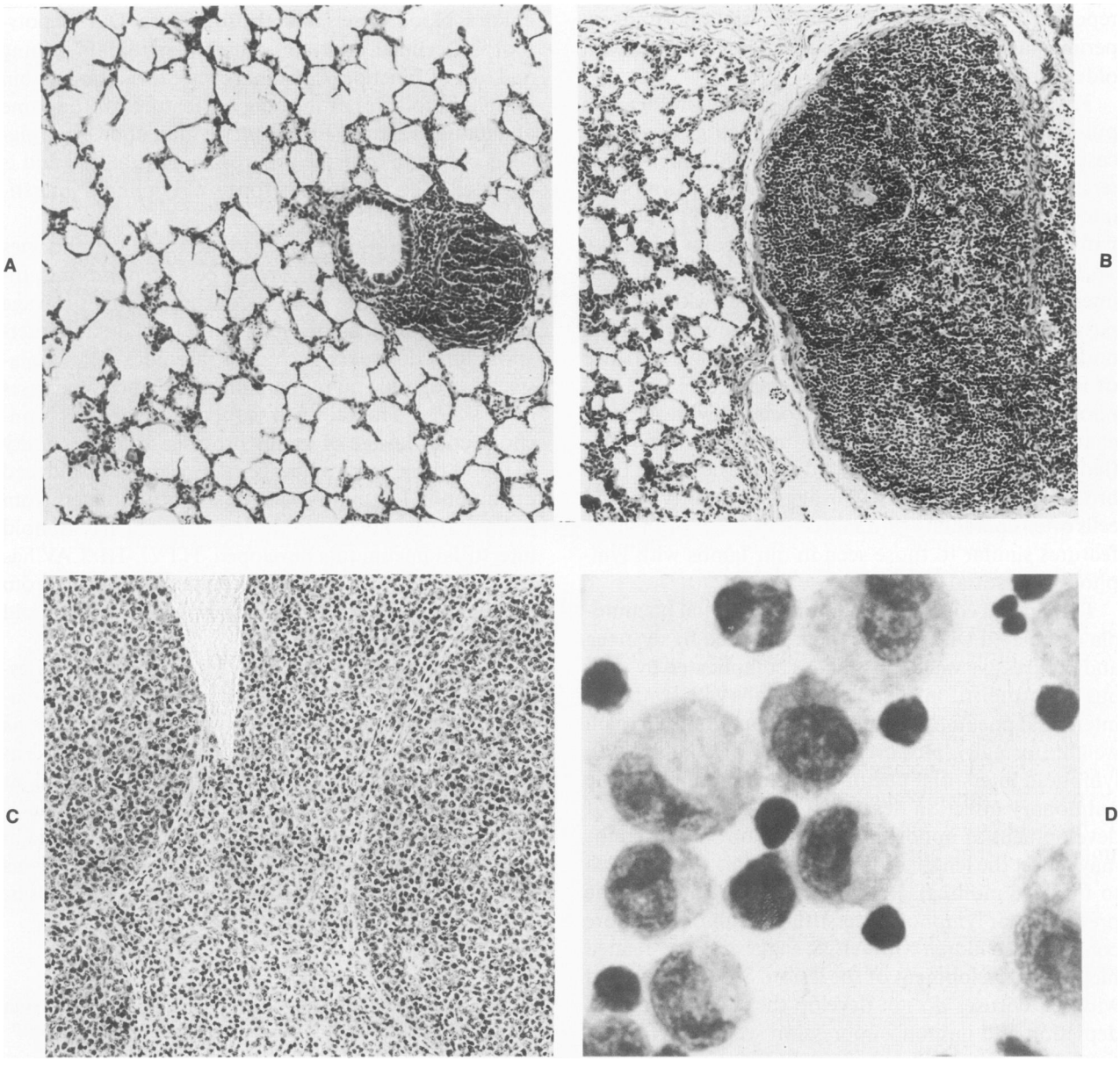

Figure 3A—Germinal center within a peribronchiolar lymphoid follicle (Lamb 79, Group 1) with minimal interstitial leukocytic infiltrates. (H&E, ×85) **B**—Prominent subpleural lymphoid nodule of a lamb with lymphoid interstitial pneumonia (Lamb 41, Group 1). (H&E, ×85) **C**—Irregular, enlarged germinal centers within the pulmonary lymph node (Lamb 72, Group 1) of a lamb with lymphoid interstitial pneumonia. (H&E, ×240) **D**—Cytocentrifuge preparation of cells obtained by bronchoalveolar lavage from a lamb with lymphoid interstitial pneumonia (Lamb 41, Group 1). Note the proportion of lymphocytes. (Wright's stain, ×1400)

gation. The most severe lesions were within lambs inoculated with medium containing 10^6 $TCID_{50}$ of low-passage OvLV or OvLV directly from lung lavage fluid. Lamb 72 (Group 1), inoculated with only 10^4 $TCID_{50}$ lentivirus showed a milder pneumonitis and less lymphoid follicle hyperplasia, suggesting that dosage of lentivirus inoculated was a factor in disease outcome. The high-passage lytic OvLV isolate failed to produce lesions through 28 weeks after inoculation. This isolate may have lost pathogenicity in repeated cultures or may have shown a slower progression of lesions if the lambs were examined later than 28 weeks. Decreased pathogenicity of high passage lentiviruses has been previously reported by Sihvonen.[11]

Group 1 lambs had a greater degree of systemic viral distribution, compared with high-passage OvLV-inoculated lambs, as indicated by coculture of tissues. More lambs seroconverted in this group suggesting that low passage OvLV or lung lavage OvLV may result in greater viral replication than the high passage OvLV isolate. In addition, age related factors may have enhanced OvLV replication in these lambs. Georgsson et al[31]

reported enhanced visna virus reisolation from experimental inoculation of ovine fetuses, compared with older lambs and adult sheep.

The OvLV-inoculated lambs had lymphoproliferative pulmonary lesions with similar morphology as natural cases of ovine progressive pneumonia and lymphoid interstitial pneumonia associated with AIDS. Lymphoid interstitial pneumonia associated with AIDS is accompanied by pulmonary lymph node follicular hyperplasia,[17] unlike many other forms of human lymphoid interstitial pneumonia. Lambs inoculated with our low-passage lentiviruses also contained pulmonary lymph node follicular hyperplasia, but did not have evidence of further systemic lymphoproliferative disease. Lymphoid interstitial pneumonia associated with AIDS is characterized by polymorphic interstitial infiltrates (lymphocytes, plasma cells, and monocytes) with peribronchiolar and perivascular aggregates of lymphoid cells often containing germinal centers,[17,20] morphologic features similar to those seen in our lambs with lymphoid interstitial pneumonia.

The clinical course of lymphoid interstitial pneumonia associated with AIDS is characterized by dyspnea and progressive weight loss and is complicated by various opportunistic infections. Our lambs with lymphoid interstitial pneumonia were dyspneic and failed to gain weight normally. However, in only 1 of 6 lambs with lymphoid interstitial pneumonia did an opportunistic pulmonary infection develop (Lamb 34). The lack of development of opportunistic infections in our lambs may reflect the length of time the animals were allowed to live (1–6 months). Recent reports indicate that the prevalence of lymphoid interstitial pneumonia is more common in children with AIDS, suggesting age-related factors in development of the lesion. Sheep inoculated with lentiviruses do not develop the severe lymphoid depletion and immunosuppression seen in AIDS victims. However, hypergammaglobulinemia, decreased lymphocyte-generated interleukin-2, and decreased concanavalin A-induced suppressor cell activities have been reported in naturally occurring ovine progressive pneumonia.[5,32,33] The immunologic status of neonatal lambs inoculated with lentivirus is currently being investigated. The pathogenesis of lymphoid interstitial pneumonia in humans and sheep is not clear, but a pulmonary lymphoproliferative response associated with similar etiologic agents with concurrent immunologic dysregulation is common to both lesions.

Lymphoid interstitial pneumonia of the lambs of this report also shares features with human lymphoid interstitial pneumonias of unknown etiologic associations. The pattern of lymphoid interstitial pneumonia induced by OvLV is lymphangitic in pattern within the lung with lymphoid follicles adjacent to bronchi, bronchioles, blood vessels, and the subpleura. Diffuse polymorphic cellular infiltrates cause interstitial thickening and loss of function. Germinal center formation within lymphoid follicles of the lung are features of the ovine and human lesion.[34] Pulmonary lymph node hyperplasia is common to ovine progressive pneumonia and is reported in certain human forms of lymphoid interstitial pneumonia.[34]

The lambs of this report had increased lymphocytes within bronchoalveolar lavage cell preparations. Increased lymphocytes within bronchopulmonary lavage samples has been associated with several human interstitial lung disorders, including sarcoidosis,[35] neoplasia,[36] iodiopathic pulmonary fibrosis,[37] and some cases of AIDS.[38,39] The ability to sequentially perform bronchoalveolar lavage of lambs provides an opportunity to follow the kinetics of lavage cells in lentivirus-induced pneumonia. Lentivirus was consistently isolated from pulmonary leukocytes of the lambs in which lymphoid interstitial pneumonia developed. HTVL-III/LAV has been isolated from bronchoalveolar lavage fluid from a patient with AIDS-related complex with lymphoid interstitial pneumonia.[40]

The ability to produce an accelerated disease manifestation of a "slow" virus in lambs provides the opportunity for further study of the pathogenic mechanisms of lymphoproliferation in the lung, co-factors governing disease expression and potential therapies that might abate debilitating lymphoid expansion within the lung. Lentivirus-induced lymphoid interstitial pneumonia in neonatal lambs will allow investigation of mechanisms of age-related resistance to this group of retroviruses.

References

1. Houwers D, Konig C, de Boer G, Schaabe J: Maedi-Visna control in sheep: I. Artificial rearing of colostrum-deprived lambs. Vet Microbiol 1983, 8:179–185
2. Oliver R, Gorham J, Parish S, Hadlow W, Narayan O: Ovine progressive pneumonia: Pathologic and virologic studies on the naturally occurring disease. Am J Vet Res 1981, 42:1554–1559
3. Ellis J, DeMartini J: Immunomorphologic and morphometric changes in pulmonary lymph nodes of sheep with progressive pneumonia. Vet Pathol 1985, 22:32–41
4. Cutlip R, Jackson T, Lehmkuhl H: Lesions of ovine progressive pneumonia: Interstitial pneumonitis and encephalitis. Am J Vet Res 1979, 40:1370–1374
5. Molitor T, Light M, Schipper I: Elevated concentrations in serum immunoglobulins due to infection by ovine progressive pneumonia virus. Am J Vet Res 1979, 40:69–72
6. Cutlip R, Jackson T, Laird G: Prevalence of ovine progressive pneumonia in a sampling of cull sheep from western and midwestern United States. Am J Vet Res 1977, 38: 2091–2093
7. Huffman E, Kirk J, Winward L, Gorham J: Serologic prevalence of ovine progressive pneumonia in a western range flock of sheep. J Am Vet Med Assoc 1981, 178: 708–710
8. Haase A, Stowring L, Narayan O, Griffin D, Price D: Slow

persistent infection caused by visna virus: Role of host restriction. Science 1977, 195:175–177
9. Sigurdsson B, Palsson P, Tryggvadottir A: Transmission experiments with maedi. J Infect Dis 1953, 93:166–175
10. Petersson G, Nathanson N, Georgsson G, Panitch H, Palsson P: Pathogenesis of visna: I. Sequential virologic, serologic, and pathologic studies. Lab Invest 1976, 35:402–412
11. Sihvonen L, Estola T, Tuomi J: Experimental maedi infection in sheep: I. Detection of virus, clinical course, histopathology. Acta Vet Scand 1980, 21:113–123
12. Gonda M, Wong-Staal F, Gallo R, Clements J, Narayan O, Gilden R: Sequence homology and morphologic similarity of HTLV-III and visna virus: A pathogenic lentivirus. Science 1985, 227:173–177
13. Sonigo P, Alizon M, Staskus K, Klatzmann D, Cole S, Danos O, Retzel E, Tiollais P, Haase A, Wain-Hobson S: Nucleotide sequence of the visna lentivirus: Relationship to the AIDS virus. Cell 1985, 42:369–382
14. Geballe A, Ventura P, Stowring L, Haase A: Quantitative analysis of visna virus replication in vivo. Virology 1985, 141:148–154
15. Gendelman H, Narayan O, Molineaux S, Clements J, Ghotbi Z: Slow, persistent replication of lentiviruses: Role of tissue macrophages and macrophage precursors in bone marrow. Proc Natl Acad Sci 1985, 82:7086–7090
16. Ho D, Rota T, Shooley R, Kaplan J, Allan J, Groopman J, Resnick L, Felsenstein D, Andrews C, Hirsch M: Isolation of HTLV III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. N Engl J Med 1985, 313:1493–1498
17. Joshi V, Oleske J, Minnefor B, Saad S, Klein K, Singh R, Zabala M, Dadzie C, Simpser M, Rapkin R: Pathologic pulmonary findings in children with the acquired immunodeficiency syndrome: A study of ten cases. Hum Pathol 1985, 16:241–246
18. Parks W, Scott G: Pediatric AIDS: A disease spectrum causally associated with HTLV-III infection. Cancer Res (Suppl) 1985, 45:4659–4661s
19. Scott G, Buck B, Leterman J, Bloom F, Parks W: Acquired immunodeficiency syndrome in infants. N Engl J Med 1984, 310:76–81
20. Grieco M, Chino-acharya P: Lymphocytic interstitial pneumonia associated with the acquired immune deficiency syndrome. Am Rev Respir Dis 1985, 131: 952–955
21. Marchevsky A, Rosen M, Chrystal G, Kleinerman J: Pulmonary complications of the acquired immunodeficiency syndrome: A clinicopathologic study of 70 cases. Human Pathol 1985, 16:659–670
22. Querat G, Barban V, Sauze N, Filippi P, Vigne R, Russo P, Vitu C: Highly lytic and persistent lentiviruses naturally present in sheep with progressive pneumonia are genetically distinct. J Virol 1984, 52:672–679
23. Haase A, Levinson W: Inhibition of RNA slow viruses by thiosemicarbazones. Biochem Biophys Res Commun 1973, 51:875–880
24. Cutlip R, Jackson T, Laird G: Immunodiffusion test for ovine progressive pneumonia. Am J Vet Res 1977, 38:1081–1084
25. Larsen H, Hyllseth B, Krogsrud J: Experimental maedi virus infection in sheep: Cellular and humoral immune response during three years following intranasal inoculation. Am J Vet Res 1982, 43:384–389
26. Cutlip R, Lehmkuhl H, Brogden K, McClurkin A: Vasculitis associated with ovine progressive pneumonia virus infection in sheep. Am J Vet Res 1985, 46:61–64
27. Hoover E, Rojko J, Wilson P, Olsen R: Determinants of susceptibility and resistance to feline leukemia virus infection: I. Role of macrophages. J Natl Cancer Inst 1981, 67:889–898
28. Mogesen S: Macrophages and age-dependent resistance to hepatitis induced by herpes simplex virus type 2 in mice. Infec Immun 1978, 19:46–50
29. Sharp J, Angus K, Gray E, Scott F: Rapid transmission of sheep pulmonary adenomatosis (jaagsiekte) in young lambs. Arch Virol 1983, 78:89–95
30. Nerurkar L, Zeligs B, Bellanti J: Biochemical studies of respiratory enzymes of rabbit alveolar macrophage during development. Fed Proc 1976, 35:716–721
31. Georgsson G, Petursson G, Miller A, Nathanson N, Palsson P: Experimental visna in foetal icelandic sheep. J Comp Pathol 1978, 88:597–605
32. Ellis J, DeMartini J: Ovine interleukin-2: Partial purification and assay in normal sheep and sheep with ovine progressive pneumonia. Vet Immunol Immunopathol 1985, 8:15–25
33. Ellis J, DeMartini J: Evidence of decreased concanavalin A induced suppressor cell activity in the peripheral blood and pulmonary lymph nodes of sheep with ovine progressive pneumonia. Vet Immunol Immunopathol 1985, 8:93–106
34. Kradin R, Mark E: Benign lymphoid disorders of the lung, with a theory regarding their development. Hum Pathol 1983, 14:857–867
35. Saltini C, Crystal R: Pulmonary sarcoidosis: Pathogenesis, staging and therapy. Int Arch Allergy Appl Immunol (Suppl) 1985, 76:92–100
36. Fedullo A, Ettensohn D: Bronchoalveolar lavage in lymphangitic spread of adenocarcinoma to the lung. Chest 1985, 87:129–131
37. Martin W, Williams D, Dines D, Sanderson D: Interstitial lung disease: Assessment by bronchoalveolar lavage. Am J Pathol 1979, 97:149–206
38. Venet A, Sandron D, Clavel F, Israel-Biet D, Dennewald G, Reynaud P, Dufat R: Bronchoalveolar lavage in acquired immunodeficiency syndrome: A useful procedure for opportunistic infection diagnosis and immunological studies (Abstr). Am Rev Respir Dis 1984, 129:A187
39. Hopewell P, Luce J: Pulmonary involvement in the acquired immunodeficiency syndrome. Chest 1985, 87:104–112
40. Zaza J, Brun-Vezinet F, Venet A, Rouzioux C, Traversat J, Israel-Biet B, Barre-Sinoussi F, Godeau P: Lymphadenopathy-associated virus isolated from bronchoalveolar lavage fluid in AIDS-related complex with lymphoid interstitial pneumonitis. N Engl J Med 1985, 313:183

Acknowledgments

The authors thank Drs. Gary Cockerell, Edward Hoover, and Peter Gasper for excellent reviews of the manuscript; Dr. Michael Sharp and Helena Russell for technical assistance during the investigation; and Susan Jones, Esta Amen, Sharon Robinson, and Charles Kerlee for preparation of the manuscript.