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Analysis of Antibody Responses to Phenotypically Distinct Lentiviruses

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To define the immune responses against phenotypically and pathogenically distinct lentiviruses, we used an immunoblotting assay to study antibodies to viral proteins of ovine lentivirus (OvLV) in 16 experimentally and 12 naturally infected sheep. Two distinct phenotypes of OvLV were used to experimentally infect lambs: strain 85/34, a "rapid/high" isolate which rapidly induced lysis in infected primary macrophage cultures and replicated to relatively high titers, and strains 84/28 and 85/14, "slow/low" isolates which induced slowly progressive syncytia with minimal lysis in vitro and replicated only to low titers in the same cell type. Serum antibodies against four major viral structural proteins, gp105, p25, p16, and p14, were detected. In a longitudinal study of experimentally infected lambs, the antibody to p25 (major *gag* protein) usually appeared first (average, about 3 weeks postinoculation [p.i.]) and was followed in about 2 weeks by p16, p14, and gp105 almost simultaneously. Six of 16 animals did not develop anti-p14 antibody by the time of necropsy at 9 to 29 weeks p.i. Two of 10 lambs which developed antibody to p14 had the antibody only transiently from 3 to 8 or 13 weeks p.i. and lost it by the time of necropsy at 21 or 22 weeks p.i. In contrast, antibodies to the other three structural proteins remained fairly constant until the time of necropsy. There were differences in the antibody responses of the experimentally infected lambs to the two phenotypes of OvLV. Seven of 10 (70%) lambs which were inoculated with the rapid/high strain developed antibody to p14, whereas only 17% of the lambs inoculated with the slow/low strains had antibody to this protein. In the longitudinal study, no decline was observed in the activity of any specific antibody such as that which occurs with anti-p24 antibody in human immunodeficiency virus infection, except in the case of anti-p14 antibody in two lambs. There were no significant differences in antibody titers against p25, p16, and p14 in final blood samples between rapid/high virus- and slow/low virus-infected groups. However, the rapid/high virus-infected group developed a fivefold-higher geometric mean titer of anti-*env* product (gp105) antibody than did the slow/low virus-infected group ($P \leq 0.1$). Antibody titers to all major structural proteins, except p14, in the naturally infected sheep were markedly lower than those in experimentally induced OvLV infections ($P \leq 0.01$). The failure of the slow/low virus-infected group to develop anti-p14 antibody may suggest diminished viral replication in vivo or a failure of the host to recognize p14 in the slow/low virus-infected group. Since the geometric mean antibody titer to gp105 was threefold higher in lambs with lymphoid interstitial pneumonia than in those without lesions and since no differences were observed in the titers of other antiviral antibodies between these groups, we found no evidence to suggest that levels of such antibodies correlated with protection from OvLV-induced disease.

Ovine lentivirus (OvLV), a member of the *Lentivirinae* subfamily of the family *Retroviridae*, causes multisystemic lesions of sheep, including lymphoid interstitial pneumonia (LIP), lymphocytic mastitis, and nonsuppurative lymphocytic encephalitis (5, 8, 11, 29). Human immunodeficiency virus (HIV), also a lentivirus, shares with OvLV the ability to induce both lymphoproliferative and lymphocytolytic lesions (14). In particular, LIP is frequently reported in pediatric acquired immunodeficiency syndrome (AIDS) patients (18). In sheep, ovine progressive pneumonia (OPP) is commonly associated with naturally acquired OvLV infection and is characterized by LIP and hyperplasia of pulmonary lymph nodes (4–6, 16); experimental induction of LIP by the intratrachial injection of OvLV into neonatal lambs serves as a model of pediatric AIDS victims with LIP (22).

HIV isolates can be divided into two groups, "rapid/high" and "slow/low," according to their character of replication in vitro (9). Rapid/high isolates replicate efficiently and are regularly isolated from AIDS patients. In contrast, slow/low isolates, while usually inducing syncytium formation, replicate poorly and are often isolated from individuals with mild disease or without disease. OvLV shares virological features with HIV: genetically distinct OvLV isolates vary in cytopathic effect in vitro and in the peak titers reached. Rapid/high isolates lyse infected macrophages or fibroblasts within a few days and replicate to high titers (10^5 to 10^6 50% tissue culture infective doses per ml), whereas slow/low isolates produce slowly developing, persistent infections characterized by syncytium formation and replication to low titers (10^3 to 10^4 50% tissue culture infective doses per ml) in the same cell types (20, 33, 34).

An important role of animal models of lentivirus infection is to determine the relevance of in vitro strain differences for in vivo pathogenicity. We previously showed that when phenotypically distinct OvLV strains were injected intratracheally into neonatal lambs, the rapid/high strain, 85/34, caused more frequent and more severe pulmonary lesions than did the slow/low strains, 84/28 and 85/14 (21). In that study, precipitating antibodies were measured consistently, but neutralizing antibodies failed to prevent lesion development in OvLV-infected lambs (21). The further elucidation of humoral immune responses to OvLV infection is of

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interest in clarifying the role of antibodies in lentivirus-infected hosts. In two recent studies, sheep with experimentally and naturally OvLV-induced pulmonary lesions lacked antibodies to p16 and p14 and sometimes p25 (2, 15).

In the present study, to elucidate correlations between immune responses and the pathogenicity of distinct OvLV phenotypes, we tested antiviral antibodies in serially collected serum samples of lambs experimentally infected with rapid/high and slow/low strains. Moreover, terminal antiviral antibody titers in experimental and natural cases of OvLV infection were compared with lung lesions in each animal. Differences were found (i) in the titer of anti-gp105 (*env* product) antibody and in the development of anti-p14 antibody in the group experimentally infected with rapid/high and slow/low OvLV strains, and (ii) in the antibody titers to the three viral proteins in the experimentally infected lambs and naturally infected sheep. These results suggest that heterogeneous immune responses may result from infection with phenotypically distinct lentivirus strains and provide some evidence that humoral immunity is not protective against lentivirus-induced disease.

MATERIALS AND METHODS

Viruses. For the experimental infection of lambs and preparation of antigens for the immunoblotting assay, four distinct strains of OvLV were used. The prototype visnamaedi virus (VMV) OvLV (strain V1514) (30) was grown on Himalayan tahr ovary cells (7) and used as a reference antigen. A rapid/high OvLV strain, 85/34, and two slow/low OvLV strains, 84/28 and 85/14, were cultured in goat synovial membrane cells. These three OvLV strains of two distinct phenotypes were derived from field cases of OPP-affected sheep and plaque cloned within five cell culture passages as described elsewhere (21). The culture fluids containing viruses were stored at −70°C until use.

Animal sources. The procedures and results of experimental OvLV infection of 16 lambs were reported elsewhere (21). Ten lambs were inoculated with rapid/high OvLV strain 85/34, and six lambs were inoculated with slow/low OvLV strains 85/14 and 84/28 (see Fig. 4). Each strain of OvLV was inoculated intratracheally into neonatal lambs confined in pens in separate rooms for each virus strain. Serum samples were collected at approximately 1-week intervals until the time of necropsy. Naturally OPP-affected sheep were obtained from a large sheep flock in Wyoming. All were females greater than 2 years of age. These animals had clinical signs of OPP characterized by emaciation, dyspnea, and mastitis. Serum samples were collected from these naturally infected sheep before they were killed for necropsy.

Lesion grade. Samples of the lungs of all sheep were fixed in 10% buffered Formalin and paraffin embedded. Sections were cut and stained with hematoxylin-eosin in the conventional manner. The extent or histological severity of the lung lesions of LIP was graded from − to ++++ in accordance with previously described criteria (21, 22).

Antigen preparation. OvLV culture fluids of each strain were thawed and clarified at 10,000 × *g* for 10 min. The clarified culture fluids were ultracentrifuged at 72,000 × *g* for 2 h at 4°C. The pellets were suspended at about 1/100 the original volume in a buffer (pH 7.4) containing 10 mM Tris, 100 mM NaCl, and 1 mM EDTA. These concentrated antigens were stored at −70°C until use as the antigens in the immunoblotting assay. For the immunoblotting assay, each antigen was adjusted to a dilution four times lower than the highest dilution which revealed all four virus-specific bands in the assay.

Immunoblotting assay. The immunoblotting assay was performed as reported previously (38) with modifications by using a Mini-Protean II Dual Slab Cell and an Electrophoretic Transfer Cell (Bio-Rad Laboratories, Richmond, Calif.). First, the antigen was fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 4% stacking and 12% resolving gels. Second, the separated polypeptides were electroblotted to a polyvinylidene difluoride (Millipore Corp., Bedford, Mass.) transfer membrane at 4°C for 2 h in transfer buffer (pH 8.3) containing 25 mM Tris, 192 mM glycine, and 20% (vol/vol) methanol at a constant voltage of 200 V. Third, the membrane was incubated for 1 h at room temperature with 10% (vol/vol) nonfat milk (Carnation Co., Los Angeles, Calif.). in 10 mM phosphate buffer (pH 7.2) containing 500 mM NaCl, 1 mM EDTA, and 0.5% (vol/vol) Tween 80 (washing and dilution buffer). Fourth, the membrane was cut into 2-mm-wide strips, and each strip was incubated for 1 h at room temperature with sheep serum diluted 1:200 or 1:400 (for the longitudinal study) or fourfold serially from 1:25 to 1:102,400 (for serum antibody titration). Some strips were incubated under the same conditions with the reference monospecific antiserum: rabbit anti-VMV p16 serum (1:1,000), rabbit anti-VMV p30 serum (1:1,000), or sheep anti-VMV gp135 serum (1:50). These antisera were kindly provided by R. Vigne (Laboratoire de Virologie, Faculté de Médecine Nord, Marseille, France) (39). Next, the strips were incubated for 1 h at room temperature with peroxidase-labeled rabbit anti-sheep immunoglobulin G or peroxidase-labeled goat anti-rabbit immunoglobulin G (heavy- and light-chain specific; Kirkegaard and Perry Laboratories, Gaithersburg, Md.) diluted 1:200 with 10% nonfat milk in washing and dilution buffer. After the primary and secondary incubations, the strips were washed at room temperature six times for 10 min each time with the washing and dilution buffer, and an additional washing was done for 30 min. Finally, the specific immunological reactions were visualized by incubation at room temperature for 3 min with 3,3′,5,5′-tetramethylbenzidine membrane peroxidase substrate (Kirkegaard and Perry Laboratories). In the longitudinal study, seroconversion times for each viral protein were analyzed by Student's *t* test.

To determine the serum antibody titers, we analyzed fourfold serial dilutions of each serum sample in the immunoblotting assay. The antibody titer for each polypeptide was expressed as the reciprocal of the highest dilution of a serum sample which revealed the specific band of the viral polypeptide. The difference in the antibody titer for each group was compared and statistically analyzed by the Wilcoxon rank sum test (35). To evaluate the reproducibility of the assay, we determined titers for 12 serum samples on three separate occasions, and the antibody titers for viral polypeptides p14, p16, and p25 were compared; 82% concordance of the results was found (titers within one fourfold dilution).

RESULTS

The specificity of the polypeptides of three strains of two distinct phenotypes of OvLV recognized by the immunoblotting assay was tested with monospecific antibodies to VMV p16, p30, and gp135 (Fig. 1). All the OvLV strains tested showed identical bands at 16K, 25K, and 105K. This result indicated that these three bands appearing on OvLV antigen strips were virus-specific polypeptides. The large molecule

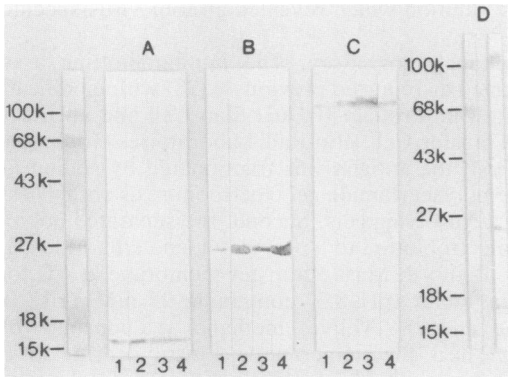

FIG. 1. Specificity of polypeptides detected by an immunoblotting assay. Lanes: 1, VMV; 2, OvLV strain 85/34; 3, OvLV strain 84/28; 4, OvLV strain 85/14. Strips in panels A, B, and C were incubated, respectively, with rabbit anti-VMV p16 serum, rabbit anti-VMV p30 serum, and sheep anti-VMV gp135 serum as primary antisera and with peroxidase-conjugated secondary antisera. In panel D, an antigen strip of OvLV strain 85/34 was incubated with serum from a lamb (86/36) experimentally infected with OvLV strain 85/34. Molecular weight markers (in thousands [k]) are indicated in the strips to the left of panels A and D.

determined with anti-gp135 serum appeared slightly above prestained phosphorylase *b* (100K) but obviously not at the position of 135K. When OvLV antigen strips were incubated with sera from lambs experimentally infected with OvLV, an additional lower-molecular-weight band was apparent. Strip D in Fig. 1 shows the four bands on an antigen strip (molecular weights, 14,000, 16,000, 25,000, and 105,000) of strain 85/34 detected by the serum from an experimentally infected lamb (86/36) at a 1:200 dilution. These bands were considered to represent antibody to viral polypeptides p14, p16, p25, and gp105.

To determine the optimal antigen for the immunoblotting assay, we titrated the serum antibodies in terminal blood samples of lambs experimentally infected with each of the three OvLV strains by using antigens prepared from the three strains (Fig. 2). Antibody titers of lambs (86/37 and 86/13) inoculated with strain 85/34 were highly dependent on the antigen used for the titration. In the sera of the two lambs tested, the antibody titers determined with the homologous antigen were generally higher than those determined with either heterologous antigen (Fig. 2A). However, the antibody titers of the lambs inoculated with the two slow/low strains of OvLV (strains 84/28 and 85/14; lambs 86/34 and 86/46 and lambs 84/29 and 86/28, respectively) showed similar results regardless of the antigen used (Fig. 2B and C). On the basis of these results, the antigen prepared from strain 85/34 was used as the antigen for the following immunoblotting assays.

In a longitudinal study, humoral immune responses to OvLV infection were determined with sera sequentially collected from 16 experimentally infected lambs. Figure 3 presents the kinetics of antibody development in lamb 86/36, which was inoculated with strain 85/34. Anti-p25 antibody appeared 2 weeks postinoculation (p.i.), and anti-p16 and -p14 antibodies appeared 4 weeks p.i. (Fig. 3 and 4). In this lamb, anti-gp105 antibody first appeared at 8 weeks p.i. (not apparent in Fig. 3 until 11 weeks). All the experimentally infected lambs developed anti-p16, -p25, and -gp105 antibodies (Fig. 4), and the activity of these antibodies persisted until the time of necropsy. Some animal variation was observed in the sequence of appearance of antibodies to viral

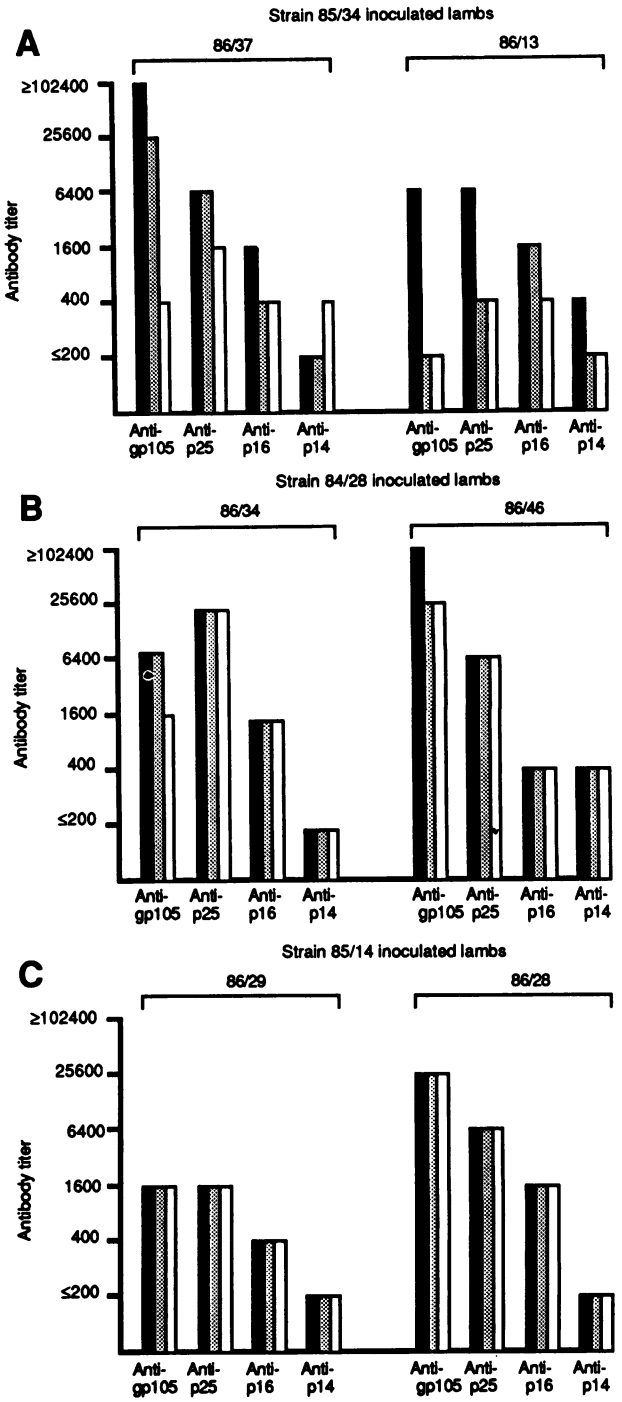

FIG. 2. Comparison of OvLV-infected lamb serum antibody titers for each viral polypeptide with antigens prepared from three different strains of OvLV in an immunoblotting assay. (A) Sera from two OvLV strain 85/34-inoculated lambs (86/37 and 86/13). (B) Sera from two OvLV strain 85/14-inoculated lambs (86/34 and 86/46). (C) Sera from two OvLV strain 84/28-inoculated lambs (86/29 and 86/28). Symbols: ■, ▨, and □, antigen strips prepared from OvLV strains 85/34, 84/28, and 85/14, respectively.

proteins, and no correlation with the viral strain used as the inoculum was apparent. Generally, anti-p25 antibody appeared first and was followed in 2 to 6 weeks by anti-gp105 and anti-p16 antibodies. In the two animals with the most

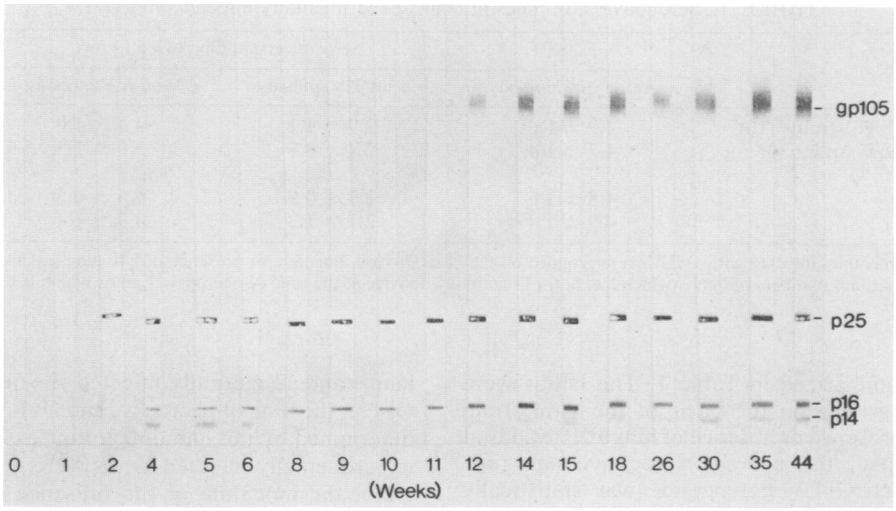

FIG. 3. Pattern of development of antibodies to OvLV structural proteins in a lamb experimentally infected with OvLV strain 85/34.

severe LIP (86/25 and 86/26), most antibodies appeared simultaneously. Anti-p14 antibodies were found in 10 of 16 experimentally infected lambs. Two of these 10 lambs (86/27 and 86/28) had transient activity of anti-p14 antibodies from 3 to 15 weeks or from 3 to 8 weeks p.i., respectively. This activity was detected in the other eight animals throughout the experiment.

The seroconversion times in the 16 experimentally in-

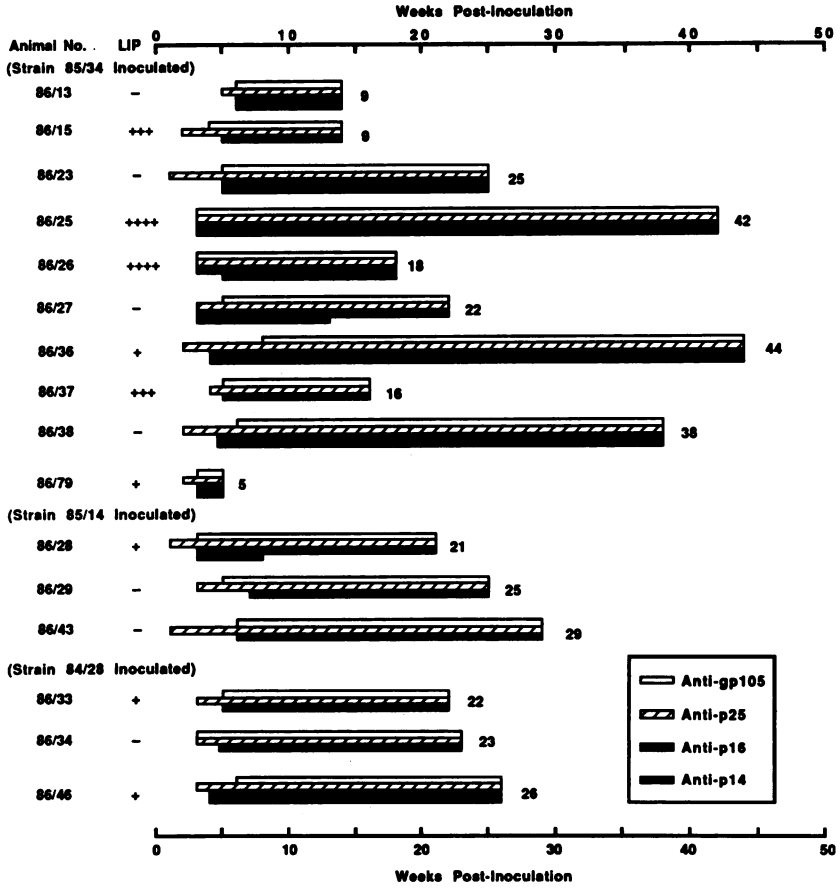

FIG. 4. Longitudinal study of humoral responses to OvLV in lambs experimentally infected with three strains of OvLV of two distinct phenotypes. Strain 85/34 has a rapid/high phenotype, and strains 85/14 and 84/28 have slow/low phenotypes. Lung lesions of LIP are graded from − to +++ in accordance with previously described criteria (22). The numbers to the right of the bars indicate the numbers of weeks p.i. the lambs were killed.

TABLE 1. Seroconversion times in lambs experimentally infected with OvLV

Group (n)[a]	Seroconversion time (wk p.i.) for:			
	Anti-gp105 antibody	Anti-p25 antibody	Anti-p16 antibody	Anti-p14 antibody[b]
Inoculated with rapid/high strains (10)	4.9 ± 1.6	2.9 ± 1.1	4.4 ± 1.0	4.6 ± 1.0 (7/10)
Inoculated with slow/low strains (6)	4.7 ± 1.4	2.4 ± 0.8	5.0 ± 1.3	3.7 ± 0.5 (1/6)
LIP lesion positive (9)	4.8 ± 1.6	2.7 ± 0.9	4.4 ± 0.9	4.1 ± 0.6 (5/9)
LIP lesion negative (7)	4.9 ± 1.4	2.7 ± 1.3	4.9 ± 1.4	4.5 ± 1.3 (3/7)

[a] Sixteen lambs experimentally infected with OvLV were grouped on the basis of viral inoculum or presence of LIP lung lesions.
[b] Numbers in parentheses are numbers of lambs which had anti-p14 antibody activity at the time of necropsy. Lambs which had transient anti-p14 antibody activity were not included.

fected lambs are summarized in Table 1. The lambs were classified into two groups on the basis of the viral strain inoculated and the presence or absence of lung LIP lesions at the time of necropsy; the average seroconversion time (weeks p.i.) for each OvLV polypeptide was statistically analyzed by Student's t test. No significant differences in seroconversion times p.i. were observed whether the lambs were grouped on the basis of the inoculum or LIP lesion development. When the lambs were grouped on the basis of the inoculum, 7 of 10 (70%) of the lambs inoculated with the rapid/high strain had developed anti-p14 antibody at the time of necropsy, whereas 1 of 6 (17%) of the lambs inoculated with the slow/low strains had developed this antibody. There was a significant difference between these two groups (chi-square statistic; $P < 0.05$). However, there was no significant difference in the development of anti-p14 antibody when the lambs were grouped on the basis of the presence of lung LIP lesions at the time of necropsy.

To compare antiviral antibody levels in sheep with experimental and natural OvLV infections, we collected terminal serum samples from 15 of the 16 experimentally infected lambs and 12 naturally infected sheep with clinical signs of OPP at the time of necropsy, and OvLV antibody titers were determined by the immunoblotting assay (Fig. 5). When the experimentally infected lambs were grouped on the basis of either the inoculum or the presence of LIP lesions, there were no significant differences in the antibody titers for p25, p16, and p14 between these two groups. However, the anti-gp105 antibody titer of the group inoculated with the rapid/high strain (geometric mean titer [GMT], 40,637) was fivefold higher than that of the group inoculated with the slow/low strains (GMT, 8,063) ($P \leq 0.1$), and the anti-gp105 antibody titer of the LIP lesion group (GMT, 36,203) was over threefold higher than that of the non-LIP lesion group (GMT, 11,593) ($P \leq 0.1$). The antibody titers to all of the viral polypeptides, except to p14, were significantly lower in the natural cases than in the experimental cases ($P \leq 0.01$). One of the naturally infected sheep which had severe LIP lesions, graded ++++, did not have detectable anti-gp105 antibody activity but did have low titers of anti-p25, -p16, and -p14 antibodies (titer range, 25 to 100).

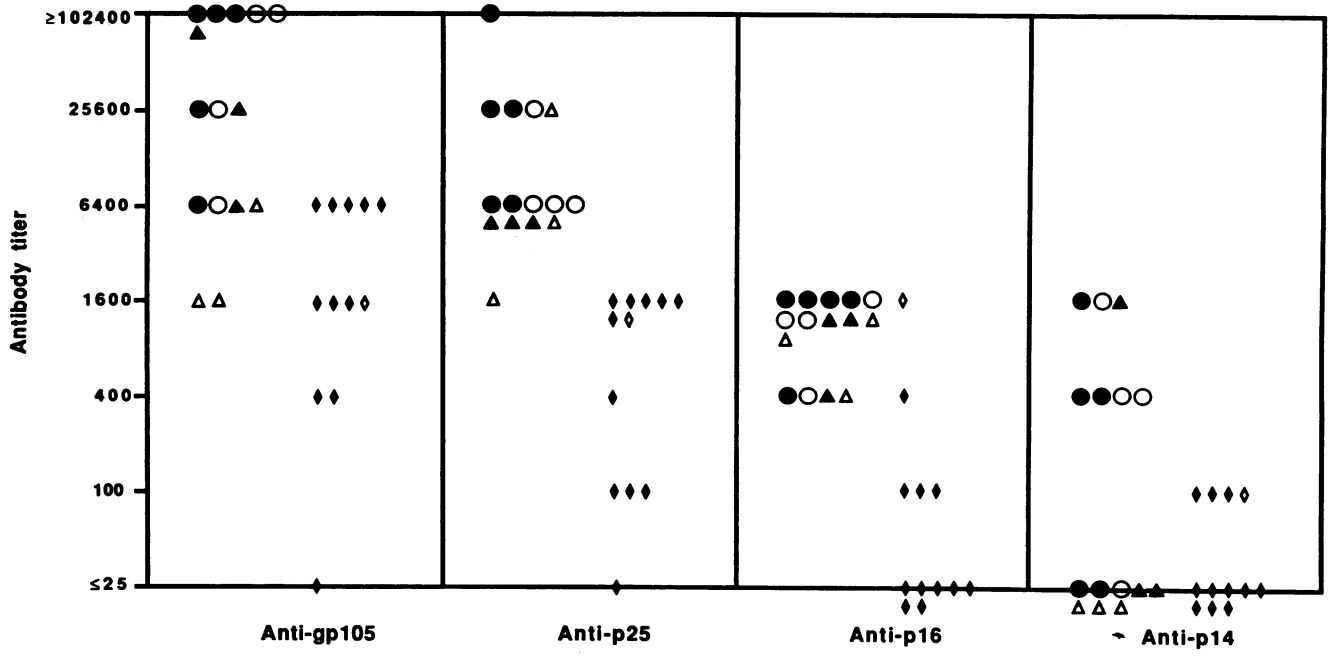

FIG. 5. Serum antibody titration of terminal blood samples collected from 15 lambs experimentally infected with OvLV and 12 sheep with naturally occurring OPP. Symbols: ● and ○, ▲ and △, and ◆ and ◇, lambs experimentally infected with OvLV rapid/high strain and slow/low strains and lambs with naturally occurring OPP, respectively; ●, ▲, and ◆ and ○, △, and ◇, presence and absence of LIP lesions at the time of necropsy, respectively.

DISCUSSION

In the present study, we analyzed the antiviral antibody responses of sheep which were infected with two distinct phenotypes of OvLV or which had clinical signs of OPP in the field. In an immunoblotting assay of sera at predetermined dilutions, four virus-specific bands were recognized in OvLV antigen strips. Three smaller molecules had molecular weights identical to those reported previously (2, 7, 15, 37, 39). However, a broad, higher-molecular-weight band appearing just above the 100K marker was not considered to be the previously reported gp135, and no clear band was consistently observed above this band. Instead, a 105K band was recognized by a monospecific antiserum to VMV gp135 in our study; this band has been recognized by others using monoclonal antibodies raised to envelope glycoprotein gp135 of strain V1514 (37). When four strains of OvLV were incubated with a sheep serum monospecific for gp135, only a 105K band appeared (Fig. 1); therefore, it is likely that the 105K band is a polypeptide that shares homology with gp135 and might result from the degradation of gp135 during the preparation of the antigen. At lower dilutions, some of the serum samples showed inconsistent weak bands at 20K, 35K, and 45K, but the viral specificity of these bands was not determined.

In our study, most lambs experimentally infected with OvLV initially developed anti-p25 antibodies and then developed antibodies against gp105 and p16 almost simultaneously. The average time to anti-p14 antibody development among the lambs which had the antibody was quite similar to that for anti-gp105 and -p16 antibodies. There was a significant difference in the development of anti-p14 antibody when the lambs were grouped according to the viral phenotype inoculated but not according to the presence of LIP lung lesions at the time of necropsy. The function of each structural protein of OvLV has not been elucidated completely, but it is possible to speculate from studies of other retroviruses (26) that p14 is a matrix protein, capsid protein, or protease. Therefore, if the rapid/high strain replicates in vivo in the same manner as it does in vitro, lambs inoculated with the rapid/high strain might be exposed to p14, one of the internal viral proteins, more effectively than might those infected with the slow/low strains.

Previously, no antibody to gp135 was detected in one of two experimentally infected lambs even 150 days p.i. (15). In another report (2), no antibody to glycoprotein (either gp135 or gp105) was observed. Sucrose density gradient-purified viral antigen was used for the assay in that study; therefore, the virus particles might have been disrupted and have lost the envelope antigen during the preparation steps. In the present study, however, all the experimentally infected lambs developed anti-gp105, and the bands were quite intense through the observation period up to 44 weeks p.i. No differences in seroconversion times were observed in these experimentally infected lambs when they were grouped on the basis of either the inoculum or the presence of LIP lesions.

Serum antibodies to p25 were usually detected prior to antibodies to *env* products in the experimentally infected lambs. This result may be characteristic of OvLV infections. Alternatively, the failure to detect anti-*env* product antibodies prior to anti-p25 antibodies may have been related to the inoculum (virus-containing cell culture fluid which may have contained an excess of p25 from disrupted or incompletely formed virions) or to a reduced sensitivity of the assay for envelope glycoproteins relative to other viral proteins, as observed for the anti-*env* product in HIV (3). However, the anti-gp105 antibody titer seemed to vary according to the viral phenotype inoculated or the presence of LIP lesions. Such differences might be related to differences in the replication in vivo of these distinct viral phenotypes. Thus, the OvLV animal lentivirus model may be useful not only for studying the relevance of in vitro strain differences for the assessment of in vivo pathogenicity but also for investigating the immune response to phenotypically distinct lentivirus strains.

The serum antibody titers of terminal blood samples from the 12 sheep with clinical signs of OPP were markedly lower than those in the experimentally infected lambs. This result might have been a consequence of the length of the infection or a decline in all the antibodies to OvLV rather than the avidity of the antibody for a specific antigen, as has been suggested for HIV infections (1, 10, 17, 19, 23, 25, 27, 32, 36). In longitudinal studies of HIV-infected patients, there often was a decline in the reactivity of antibody to the HIV major *gag* protein (p24), whereas the activity against other viral proteins remained constant; this loss of reactivity was associated with the development of the clinical disease form of AIDS or AIDS-related complex or the depletion of CD4 cells (10, 23, 27, 32, 36). Because the anti-p24 antibody reactivity decreased concomitantly with a rise in HIV antigenemia, the loss of the anti-p24 antibody might have been secondary to the formation of antigen-antibody complexes due to increasing amounts of the antigen (10, 23, 27, 32). This phenomenon is quite interesting, not only as an indicator of the onset of AIDS but also for understanding the features of host immune responses and viral replication in vivo. However, there are several lines of evidence indicating restricted viral replication in OvLV-infected animals at local tissue sites of infection (12, 13, 28, 30, 31). Therefore, the lower antibody titers in the natural OPP cases may not have been associated with the formation of immunocomplexes. Alternatively, exposure to a higher titer of virus by an abnormal route of infection in the experimentally infected lambs may have accounted for this difference. Another possibility is the higher susceptibility of neonates to virus infections, as suggested for HIV infections (24). A third possibility is the potential immunosuppression caused by OvLV infections in sheep, but it is unknown if OvLV infections cause general immunosuppression.

The experimentally infected lambs in the present study did not have any changes in antibody activity up to 44 weeks p.i. other than anti-p14 antibodies in two lambs. However, the duration of OvLV infection in the naturally OPP-affected sheep was probably much longer than in the experimentally infected lambs; therefore, there is a possibility that suppression of antibody production might occur during or after a long incubation period. The results obtained in the present study suggest a need for an analysis of immune responses to nonviral immunogens in OvLV-infected sheep to resolve these issues and to define immune parameters important in protection against lentivirus-induced disease.

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